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Full Length Research Paper

Initiation, proliferation and development of micro-propagation system for mass scale production of banana through meristem culture

Aamir Ali, Anum Sajid[1], Naima Huma Naveed[1], Abdul Majid[1], Asif Saleem[1], Umair A. Khan[1], Fisal Iqbal Jafery[1] and Shagufta Naz[2]*

[1]Department of Biological Sciences, University of Sargodha.
[2]Lahore College For Women University, Lahore.

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Explants were taken from field grown plants in 2010. The shoot apical meristem of different sizes was cultured on Murashige and skoog's (MS) medium supplemented with different concentrations and combinations of 6-benzylamino-purine (BAP), kinetin (Kin) and α- naphthaleneacetic acid (NAA) either alone or in combination with each other under different temperature conditions ranging from 23 to 27°C. Shoot formation response from shoot apical meristem showed that MS medium containing 1.0 mg/l BAP showed best response for shoot formation. For shoot multiplication, MS medium containing 1.0 mg/l BAP + 0.25 mg/l kin provided the best multiplication response which was 8 shoot per culture vial within 21.6 days after inoculation into shoot multiplication medium. Shoot formation and multiplication response was also affected by temperature variations. The best results were obtained at 27°C ± 1°C. By increase or decrease in temperature, the rate of *in vitro* response was also decreased. For rooting of well developed *in vitro* shoots MS medium supplemented with 1.0 mg/l Indole-3- butyric acid (IBA)+ 0.5 mg/l NAA showed 3.6 roots per plant after 6.8 days of inoculation into rooting medium with an average root length of 2.4 cm. 100% hardening response was obtained in Peat moss after 21 days of transplantation in glass house. The experiments were designed in completely randomized pattern.

Key words: Murashige and skoog's medium, proliferation, banana, micro propagation system.

INTRODUCTION

Banana is prized for its nutritive value with high carbohydrates (22.2%), fibre (0.84%) and protein (1.1%) with less fat (0.2%) and water (75.7%). The world production of banana is about 95 million tons per year; these are grown in 132 countries worldwide (UNIDO, 2008). In Pakistan, it is grown on 30,000 hectares with total production of 10, 3000 tonnes/year (UNIDO, 2008). Sindh covers about 91% of total area under banana cultivation of the country. Cultivation of banana needs specific environmental and soil conditions. They are sterile and parthenocarpic; so use of conventional methods for breeding improvement of banana is difficult to practice (Suprasanna et al., 2008). Bananas are triploid and are are vegetatively propagated through suckers which are the rhizome cut off from the mother plant.

Non-professional cultivation practices, pest epidemic and viral diseases particularly bunchy top virus drastically affect the yield and quality of our crop. Our annual production of this crop is very low as banana is mostly propagated through Suckers. The process is rather very slow as the rate of multiplication of suckers is at the pace of 15 to 20 per year depending on clone, agro climatic conditions and cultural methods.

Therefore, mass scale propagation of new elite variety of banana or a superior hybrid evolved by banana breeders is serious bottleneck for banana breeder. To have substantial sufficient planting material for cultivation in acreage with the newly introduced clone/hybrid, it will take years when suckers are used for propagation. Moreover, if propagated by vegetative method, the plant once infected by pathogen can transfer it generation after

*Corresponding author. E-mail: aamirali73@hotmail.com.Tel: +92-333-4722410.

generation and the entire population may be infected within few years. To better improve plant propagation capacity for our producers, new high value commodities of this plant are needed to be developed not only to fulfill the domestic demand but also to have export quality planting material. Micro-propagation is one of the advanced techniques for mass scale production of virus free, high yielding and premium quality plating material. In recent years, this technique is used for rapid clonal multiplication of several economic plants, restoration of vigor and yield loss due to infection. Using this technique, the disease free stock can be provided to the growers in order to achieve high yield and quality of field crops. By keeping in view this background information, present research work was undertaken to standardize efficient protocols for mass scale propagation of banana using *in vitro* techniques to enhance yield per hectare of the crop.

MATERIALS AND METHODS

Meristem of banana was excised from field growing plants. As apical meristem of banana is wrapped deep in covering of rolled leaves sheath and is naturally sterilized. Therefore, it is not necessary to disinfect them. However, to prevent any contamination, surface sterilization was carried out. For sterilization, explants were first washed with running tap water then treated with house hold detergent for five minutes. This was followed by second washing with tap water to remove all the traces of detergent. The explant was then treated with 1% sodium hypochlorite solution for 15 min. After discarding sodium hypochlorite, the explants were washed three times with sterilized distilled water to remove all the traces of sodium hypochlorite. The sterilized explants were then inoculated by proper dissecting and sizing the meristem (0.5 to 1.0 cm) on MS (Murashige and Skoog, 1962) medium supplemented with different concentrations of BAP either alone or in combination with Kin or NAA. For multiplication of induced shoots, hormonal concentration was changed and shoot multiplication response was observed. For *in vitro* rooting MS medium containing different concentrations of NAA and IBA (ranging from 0.5 to 2.0 mg/l each) was used either alone or in combination with each other. 3% sucrose was used in all the media.

The pH of the medium was adjusted to 5.70 to 5.74 with 0.1 N solution of NaOH or HCl. MS medium was used both in solid and liquid farms. For solidification, 0.6% agar was used. In case of liquid medium, autoclaved cotton was used to support the plant tissues. The medium was autoclaved at 121°C and 15 lbs/Inch^2 pressure for 15 min. Cultures were maintained under fluorescent light having 3000 lux light intensity. To determine the optimum temperature different conditions ranging from 23°C ± 1°C to 30°C ± 1°C were tested under different light and dark conditions in each 24 h cycle. After 3 to 4 week of shoot formation, actively growing cultures were transferred to fresh medium in jars for further growth and proliferation. First sub-culturing was done after two week and rest sub-culturing after four week. During each sub-culturing all dead or dis-coloured shoots were removed. The shoots which were obtained from shoot apical meristem were further sub-cultured for *in vitro* shoot multiplication on MS medium supplemented with different types of hormones. Hardening was carried out in glass house under natural light conditions in sterilized medium. The effect of different temperature conditions ranging from 23 ± 1 to 30 ± 1°C was also studied to determine the optimum temperature for growth of *in vitro* plants.

RESULTS AND DISCUSSION

The result of the present study demonstrates the effect of phytohormones for shoot formation and multiplication. Among the various phytohormones, mainly two cytokinins that is, BAP and Kin were used in MS medium either alone or in combination with each other or with NAA. It was observed that when 1.0 mg/l of BAP was added in MS basal medium, maximum shoot formation response was obtained which was 100% within 10.6 days of inoculation (Table 1, Figures 1, 2a and b). Altvorst et al. (1992) also reported that in this medium, adventitious shoots developed after 2 weeks from basal part of leaf explants. However, various workers have supported the use of auxin and cytokinins for *in vitro* propagation of plants (Arinaitwe et al., 2000; Wojtania and Gabrysweska, 2001; Shabbir et al., 2009). Addition of kin alone in the medium produced less regeneration response and produced less shoots. At 1.5 mg/l of kinetin, 80% shoot formation response was obtained within 14 days but when BAP was added with kin, high frequency of regeneration was obtained, particularly if there was a great deal of enhancement of multiple shoot formation using the combination of the aforementioned two hormones (Table 1). It was found in the present study that the presence of cytokinin in the media not only determined the regeneration response but also affected the mode of regeneration. At 1.0 mg/l of BAP with 0.5 mg/l of kin maximum shoot formation response was 96% shoots within 14 days of meristem inoculation (Table 2). Akbar and Roy (2006) reported that the combination 0.5 mg/l BA + 0.5 mg/l Kinetin+ 0.5 mg/l NAA was found to be the best for shoot formation from shoot apical meristem.

Many other scientists have also reported that *in vitro* shoot formation response from shoot apical meristem can be obtained in MS medium containing a combination of BAP with kin (Geeta and Padmanabhan, 2001; Dhillon et al., 2002). 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 (Hager et al., 1991). Combination of BAP with NAA was also tested in the present study to check their effect for *in vitro* shoot formation. It was observed that when 0.25 mg/l NAA was added in MS medium along with 0.5 mg/l BAP, 84% shoot formation response was obtained within 13.8 days of meristem inoculation. Plants raised through pre-existing shoot meristem could be highly useful for clonal propagation and developing disease free plants. *In vitro* micro-propagation of shoot meristem resulting in multiple shoot formation without change in parent stock also helps in shortening the time span and space problem in such breeding programmes (Ali, 2008). The optimum temperature for *in vitro* shoot formation from apical meristem was found to be 27 ± 1°C. By increasing or

Table 1. Effect of different hormones on shoot formation from shoot apical meristem.

S/N	Code	Media (MS)	Composition (mg /l)	Number of test tube inoculated	Day of shoot formation	Number of test tube showing shoot formation	Frequency of shoot formation (%)
1	MS1	Basal		5	17.6±0.536bc	3.0±0.282de	60
2	MS2	BAP	1.0	5	10.6±0.536fg	5.0±0.282a	100
	MS3	BAP	1.5	5	9.4±0.357g	4±0.4abcd	88
	MS4	BAP	2.0	5	13.6±0.536e	3.8±0.334bcde	76
	MS5	BAP	2.5	5	14.2±0.657e	4.2±0.334abc	84
3	MS6	Kin	1.0	5	14±0.565e	4±0.282 abcd	76
	MS7	Kin	1.5	5	14±0.489e	3.8±0.178bcde	80
	MS8	Kin	2.0	5	18±0.565b	3.6±0.219bcde	72
	MS9	Kin	2.5	5	21±0.565a	2.8±0.438e	56
4	MS10	BAP+Kin	0.5+0.5	5	14±0.4e	4±0.282 abcd	80
	MS11	BAP+Kin	1.0+0.5	5	14±0.282e	4.6±0.219ab	96
	MS12	BAP+Kin	2.0+0.5	5	11.6±0.456f	4.6±0.456ab	92
	MS13	BAP+Kin	2.5+0.5	5	15±0.282de	3.2±0.334cde	64
5	MS14	BAP+NAA	0.5+0.25	5	13.8±0.521e	4.2±0.178abc	84
	MS15	BAP+NAA	1.0+0.25	5	11.2±0.521f	4±0.282 abcd	80
	MS16	BAP+NAA	2.0+0.5	5	14.2±0.438e	3.8±0.178bcde	76
	MS17	BAP+NAA	3.0+0.5	5	16.2±0.334cd	3.2±0.334cde	64
LSD (0.05)					1.49	0.928	

Means followed by different letters in the same column differ significantly at *P=0.05* according to Duncan's new multiple range tests.

Figure 1. Initiation of shoot formation on MS medium containing 1.0 mg/l BAP (3X).

decreasing the temperature, the rate of shoot formation decreased. The regenerated shoots obtained were further multiplied by sub-culturing on fresh medium and maintained for one year (Figure 5a, b c and d). Maintenance and multiplication of germplasm stock in reduced space for several months was also reported by Engelman (1995).

In the present study, it was observed that the removal

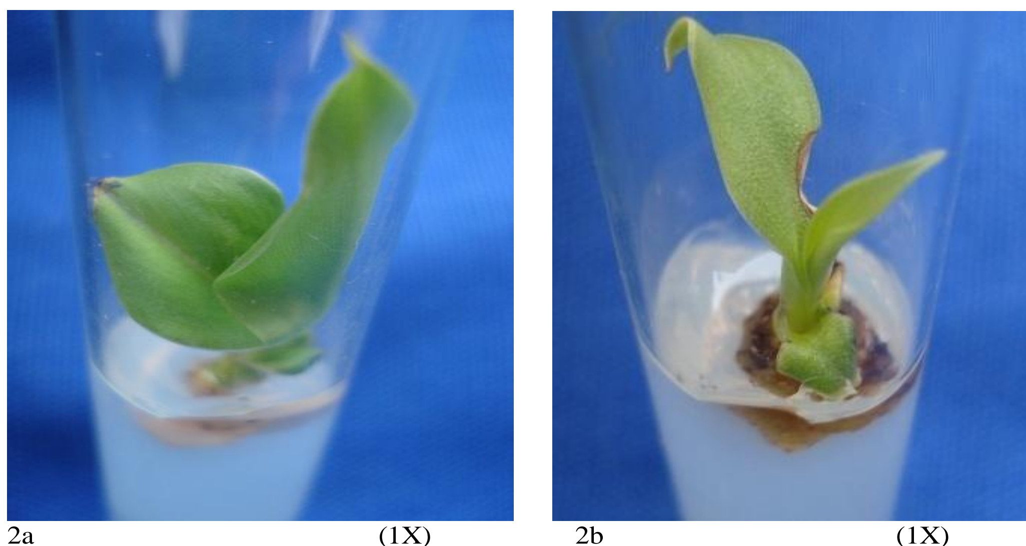

Figure 2. Shoot formed on MS medium containing 1.0 mg/l BAP; a - after 10 days of meristem inoculation; b - after 14 days of meristem inoculation.

Table 2. Effect of different concentrations of BAP on *in vitro* shoot multiplication.

S/N	Code	Media (MS)	Composition (mg/l)	Number of test tube inoculated	Day for shoot multiplication	Number of shoot formed per culture vial	Average shoot length (cm)
1	M1	Basal		5	23.8±0.521c	2.4±0.219e	2.4±0.357abc
2	M2	BAP	0.5	5	17.4±0.456g	2.2±0.178e	3.4±0.219bc
	M3	BAP	1.0	5	19.8±0.521f	4.0±0.282cd	1.6±0.219c
	M4	BAP	1.5	5	20.2±0.521ef	5.8±0.334b	3.4±0.536a
	M5	BAP	2.0	5	16.4±0.456g	4.8±0.178c	2.6±0.357abc
3	M6	BAP+Kin	0.25+0.25	5	24.2±0.334bc	3.0±0.282de	1.8±0.178c
	M7	BAP+Kin	0.5+0.25	5	25.8±0.334ab	3.0±0.4de	2.6±0.357abc
	M8	BAP+Kin	1.0+0.25	5	21.6±0.669de	8.0±0.282a	3.4±0.219a
	M9	BAP+Kin	2.0+0.25	5	22±0.565d	4.4±0.219c	2.2±0.334bc
4	M10	BAP+NAA	0.5+0.25	5	24±0.565bc	4.0±0.282cd	1.6±0.219c
	M11	BAP+NAA	1.0+0.25	5	24.8±0.769bc	3.0±0.282de	2.4±0.219abc
	M12	BAP+NAA	2.0+0.25	5	26.6±0.357a	4.2±0.334c	3.0±0.282a
	M13	BAP+NAA	3.0+0.25	5	23.8±0.593c	3.8±0.334cd	1.8±0.334c
LSD (0.05)					1.67	0.904	0.983

Means followed by different letters in the same column differ significantly at P=0.05 according to Duncan's new multiple range tests.

of kin from shoot induction medium enhanced shoots multiplication response. It was also noted that the best shoot multiplication response was obtained with MS medium containing BAP and kinetin at concentration of 1.0 mg/l and 0.25 mg/l, respectively (Medium M_8, Table 2). Using this shoot multiplication medium, 8 shoots per culture vial were obtained within 21.6 days of inoculation as shown in Table 2. Proliferation of shoot started and during secondary proliferation stage, lateral shoots developed from the base of newly initiated shoot. As a result, a dense mass of shoots (25 to 30) was developed in each culture jar (Table 2, Figure 3). After 21 days, these bunches were further sub-divided in bunches containing 4 to 5 shoots and were transferred into fresh medium in jars. In this way, shoot multiplication was maintained for several passages by regular transfer to

Figure 3. Initiation of shoot multiplication on MS medium containing 1.0 mg/l BAP + 0.25 mg/l Kin. (12 days after inoculation on shoot multiplication medium) (1.5X).

Figure 4. Initiation of root formation in *in vitro* developed shoots on MS medium containing 1.0 mg/l IBA + 0.5 mg/l NAA. (7 days after inoculation on rooting medium) (1.0X).

fresh medium (Figure 3). It was also observed that shoot multiplication response was enhanced in liquid medium while solid medium delayed shoot multiplication response. While Rahman et al. (2002) reported that MS medium supplemented with 5 mg/l of BAP and Kin produced the highest number of shoots per explants of cv. Sabri.

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. (2000). Addition of NAA with BAP did not show any support to shoot multiplication. From the data presented in Table 2, it is evident that rate of shoot multiplication increased by decreasing the concentration of BAP.

Rooting of regenerated shoots.

For *in vitro* root induction, full and half strength MS medium supplemented with 20 different concentrations and combinations was used. Frequency of root formation was different in all the media. Best root induction response was obtained on MS medium containing 1.0 mg/l NAA with 0.5 mg/l IBA (Medium M_{19}, Table 3 and Figure 4). At this concentration, 100% shoots formed roots within seven days of inoculation with 3.6 roots pershoot having an average length of 2.4 cm. Half strength MS medium supplemented with the same auxins used for full strength MS medium were also tested (Table 3 and Figure 4). No significant effect of half strength MS

Table 3. Effect of different concentration of NAA for rooting of *in vitro* developed shoots.

S/N	Code	Media (MS)	Composition (mg/l)	Number of test tube inoculated	Day for root formation	Number of roots /plant	Average root length (cm)
1	MR1	Basal		5	17.2±0.178a	2.0±0.282d	2.0±0.4d
2	MR2	½ +NAA	0.5	5	10.6±0.606efghi	2.4±0.219cd	3.2±0.178abc
	MR3	½ +NAA	1.0	5	11.2±0.438cdefg	3.0±0.4bcd	2.4±0.357cd
	MR4	½ +NAA	1.5	5	11.8±0.657bcde	3.2±0.334bc	2.8±0.178abcd
	MR5	½ +NAA	2.0	5	12.8±0.334b	2.8±0.334bcd	3.0±0.282abcd
3	MR6	NAA	0.5	5	11.4±0.536cdefg	2.4±0.456d	3.6±0.219ab
	MR7	NAA	1.0	5	8.4±0.219jk	3.6±0.219ab	2.8±0.334abcd
	MR8	NAA	1.5	5	11.0±0.282defgh	3.0±0.282bcd	2.6±0.219bcd
	MR9	NAA	2.0	5	11.2±0.178cdefg	2.8±0.334bcd	2.4±0.219cd
4	MR10	½ +IBA	0.5	5	9.8±0.334hi	2.4±0.219cd	2.1±0.089cd
	MR11	½ +IBA	1.0	5	12.0±0.282bcd	4.4±0.219a	3.0±0.282abcd
	MR12	½ +IBA	1.5	5	10.4±0.536fghi	3.6±0.456ab	3.6±0.357ab
	MR13	½ +IBA	2.0	5	11.4±0.219cdefg	3.2± 0.334bc	3.8±0.334a
5	MR14	IBA	0.5	5	11.6±0.219bcdef	2.6±0.219bcd	3.2±0.334abc
	MR15	IBA	1.0	5	7.6±0.357ki	3.2±0.178bc	2.2±0.178cd
	MR16	IBA	1.5	5	9.4±0.219ij	3.0±0.282bcd	3.6±0.456ab
	MR17	IBA	2.0	5	10.2±0.438ghi	2.4±0.219cd	2.8±0.334abcd
6	MR18	NAA+IBA	0.5+0.5	5	12.4±0.219bc	2.4±0.219cd	2.6±0.219bcd
	MR19	NAA+IBA	1.0+0.5	5	6.8±0.334i	3.6±0.357ab	2.4±0.456cd
	MR20	NAA+IBA	1.5+0.5	5	9.4±0.357ij	3.0±0.282bcd	2.8±0.334abcd
	MR21	NAA+IBA	2.0+0.5	5	9.6±0.357ij	2.8±0.334bcd	3.2±0.334abc
LSD (0.05)					1.17	0.959	0.961

Means followed by different letters in the same column differ significantly at *P=0.05* according to Duncan's new multiple range tests.

medium was found for root initiation and development. Pruski et al. (2005) also reported best rooting response in combination of IBA and NAA. However, De Langhe (1985) and Novak et al. (1990) used half strength MS + 1.0 mg/l IBA, whereas Cronauer and Krikorian (1984) used auxin-free MS for rooting of banana microshoots. On the other hand, Banerjee et al. (1986) and Azad and Amin (2001) obtained rooted banana shoots in half strength MS agar-gelled medium supplemented with 0.2 mg/l IBA. Akbar and Roy (2006) reported 1.0 mg/l IBA for best rooting response of *in vitro* cultured plants.

Hardening of *in vitro* raised plants

For hardening, *in vitro* raised plants were shifted in the glass house in three different medium compositions. 100% hardening response was obtained in a Peat moss after 24.2 days of transplantation in glass house.

Pure peat moss and pure sand when used alone did not give good results (Table 4). This may be due to the reason that pure sand has very poor water holding capacity which affects the relative humidity of the plant whereas in pure peat moss there again becomes the problem of relative humidity as it has very high water holding capacity.

Ali et al. (2004) reported best hardening response of *Mentha arvensis* in a mixture of sand + soil + peat at 1:1:1 but in our study, this mixture produced only79.2% hardening response.

This difference may be due to the difference in the humidity requirement of the plants.

Table 4. Hardening of *in vitro* developed Plants.

S/N	Medium composition	Number of plants shifted	Days for hardening	Number of plants hardened	% of hardened plants
1	Autoclaved Sand	5	28±0.282a	3±0.282c	60
2	Peat Moss	5	24.2±0.521b	5±0a	100
3	Sand + Peat Moss	5	22.2±657c	4.98±0.0178a	99.6
4	Sand+ Soil+ Peat Moss	5	27.6±0.669a	3.96±0.0357b	79.2
LSD (0.05)			1.860	0.529	

Means followed by different letters in the same column differ significantly at P=0.05 according to Duncan's new multiple range tests.

5a 5b 5c 5d

Figure 5. Different steps of micropropagation in banana. a – Shoot initiation from shoot apical meristem. b- *In vitro* shoot multiplication. **c** – Rooting of *in vitro* developed shoots. d – Well developed *in vitro* rooted plants ready for shifting in to hardening.

Abbreviations

MS, Murashige and skoog's medium; **Kin,** kinetin; **BAP,** 6-benzylamino-purine; **NAA,** α- naphthaleneacetic acid; **IBA,** indole-3- butyric acid.

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