academicJournals

Vol. 13(27), pp. 2696-2703, 2 July, 2014
DOI: 10.5897/AJB2014.13750
Article Number: 106134B45706
ISSN 1684-5315

http://www.academicjournals.org/AJB

African Journal of Biotechnology

Full Length Research Paper

In vitro multiplication of banana (Musa sp.) cv. Grand Naine

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Received 21 February, 2014; Accepted 16 June, 2014

A micropropagation method is described for banana (*Musa Spp.*) Cv. Grand Naine. Suckers were surface sterilized with $HgCl_2$ (0.1%) for 6 min which gave minimum contamination with maximum culture establishment. Of various treatment combinations, Murashige and Skoog (MS) medium + BAP 4.00 mg/l with IAA 2.00 mg/l resulted in maximum establishment of cultures in lesser time. MS medium + BAP 4.00 mg/l + IAA 2.00 mg/l gave maximum multiple shoots. Maximum rooting was obtained on MS medium (half strength) supplemented with IBA 1.00 mg/l and activated charcoal 200 mg/l.

Key words: Grand naine, micro propagation, murashige and skoog medium, *Musa* spp. and suckers.

INTRODUCTION

Banana is a perennial herbaceous monocot which belongs to *Musa* genus of the *Musaceae* family. It can be cultivated under sub-tropical conditions if the planting time is regulated in such a manner that bunches are initiated in summer, shot in autumn and mature in winter (Simmonds, 1996). It is believed to be one of the oldest fruits which have originated from Malaysia through a complex hybridization process (Novak, 1992). Cultivated banana is a triploid derived from two diploid species that is, *Musa acuminata (*Malaysia) and *Musa balbsiana* (India) (Georget et al., 2000). Banana plantlets produced through micro propagation method have been found to establish faster, healthier, stronger, shorter production cycle and higher yield than those produced through conventional methods (Ortiz and Vuylsteke, 1996) as millions of plants can be grown from a small or even a microscopic piece of plant tissue within a year (Mantell et al., 1985) and plants multiplication can be continued throughout the year irrespective of the season (Razdan, 1993). As regards yield performance in banana, tissue cultured plants have been reported to produce 39% higher yield than plants from sword suckers (Pradeep et al., 1992). The rapid proliferation obtained in tissue culture allows nurserymen to meet an unexpected demand for a particular cultivar; a million or more plants can be produced in a year from a single meristem tip. Another advantage of micropropagation is the elimination of pest and pathogen pressure during the production cycle, assuming that the initial stock plant is free of diseases (Faccioli and Marani, 1998).

In order to achieve the above objectives, several workers have developed tissue culture protocol and

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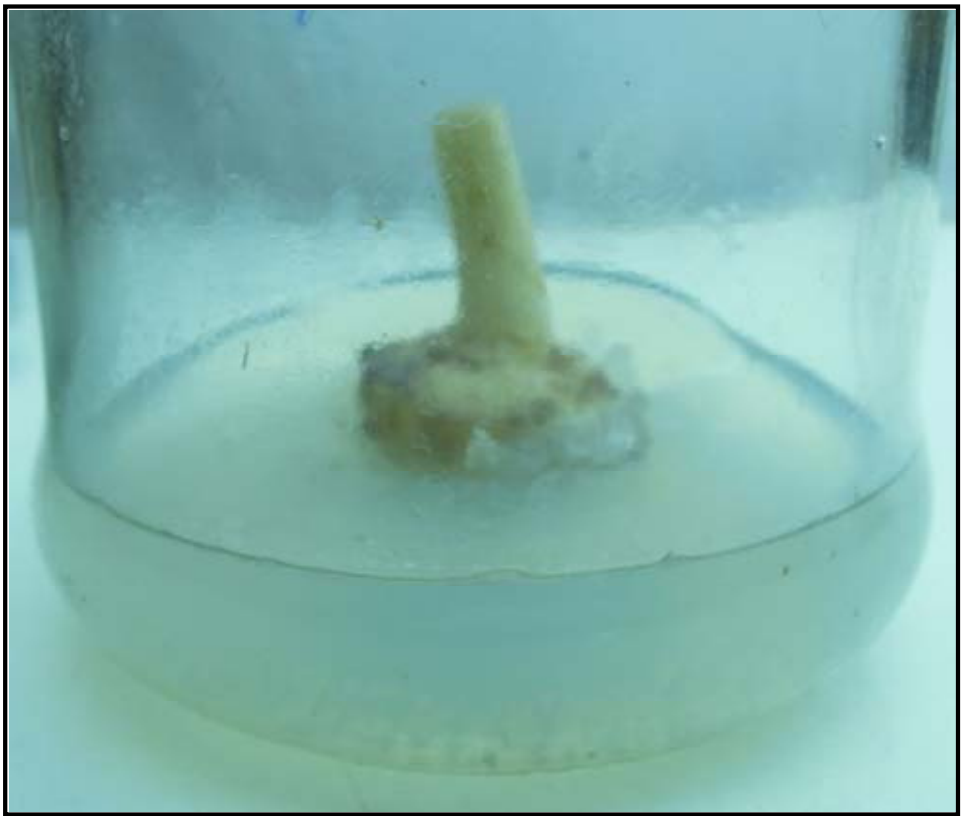

Figure 1. Culture initiation of banana explants cv. Grand Naine.

ascertained field performance of *in vitro* developed banana plantlets (Jalil et al., 2006; Resmi and Nair, 2007; Shirani et al., 2009). However, it is very important to standardize the technique under sub-Himalayan mountainous regions of Jammu because the performance of plant tissue is dependant on a number of factors which is intimately connected with the physiological state of the donor and the explants.

MATERIALS AND METHODS

The explant materials of Grand Naine cultivar of banana were obtained from plants grown at the Research Orchard of Division of Biotechnology, Punjab Agriculture University Ludhiana. The suckers were used as explant. The suckers were excised and surface sterilized by using different combinations and concentration of mercuric chloride, sodium hypochlorite and ethanol, individually or in combination which was followed by thorough rinsing with sterile distilled water.

Suckers were cultured on agar gelled MS medium with full strength salts supplemented with specific concentration of growth regulators (BAP, IAA and NAA) singly or in combination and 3% sucrose was used for culture establishment and shoot multiplication and MS half strength salts containing various concentration of auxins (IBA/NAA) were used for adventitious root formation. All the cultures were incubated in culture room at a temperature of 26 ± 2°C with relative humidity of 55 ± 5% and were exposed to 16 h photo period provided by 40 W cool white fluorescent tubes kept 50 cm above bench surface. Data recorded for different parameters were subjected to completely randomized design (CRD). Statistical analysis based on mean values per treatment was made using analysis of variance (ANOVA) technique of CRD. Number of treatments used varied according to the combinations of hormones used and number of replications was three. The software used for data analysis was O.P. State.

RESULTS

Surface sterilization

The study demonstrated the feasibility of propagating banana *in vitro* (Figure 1). The effect of different treatments of sterilants used either singly or in combination for the surface sterilization of explants is given in Table 1. Mercuric chloride (0.1%) for 6 min gave the best sterilization of explants, recording less (5%) contamination and the highest establishment (95%). Sterilization of explants with sodium hypochlorite alone was found to be ineffective resulting in very high contamination (55 to 100%) and also low survival percentage (0 to 15). On the other hand, considerable reduction in the rate of contamination (10%) with 85% survival of explants was obtained by treating the explants with sodium hypochlorite (5 %) for 10 min after rapid rinsing with ethanol (70%) for 30 s and mercuric chloride (0.1 %) for 6 min.

Table 1. Standardization of surface sterilization treatments for banana explants cv. Grand naine.

Sterilant	Duration	Contamination (%)	Death of culture (%)	Culture establishment (%)
Mercuric chloride (0.1%)	4 min	40(39.21)	30 (32.97)	30 (32.97)
Mercuric chloride (0.1%)	6 min	5 (12.64)	0 (0.00)	95 (77.53)
Mercuric chloride (0.1%)	8 min	50 (44.98)	25 (29.80)	25 (29.80)
Mercuric chloride (0.1%) + ethanol (70%)	4 min + 30 s	40 (39.15)	25 (29.88)	35 (36.14)
Mercuric chloride (0.1%) + ethanol (70%)	6 min + 30 s	15 (22.44)	10 (17.78)	75 (60.10)
Mercuric chloride (0.1%) + ethanol (70%)	8 min + 30 s	45 (42.04)	25 (29.82)	30 (32.88)
Sodium hypochlorite (5%)	5 min	100 (90.00)	0 (0.00)	0 (0.00)
Sodium hypochlorite (5%)	10 min	55 (47.87)	30 (32.88)	15 (22.65)
Sodium hypochlorite (5%) + Ethanol (70%) + Mercuric chloride (0.1%)	5 min + 30 s + 4 min	35 (36.14)	20 (26.35)	45 (42.05)
Sodium hypochlorite (5%) + Ethanol (70%) + Mercuric chloride (0.1%)	5 min + 30 s + 6 min	10 (17.78)	10 (18.30)	80 (63.61)
Sodium hypochlorite (5%) + Ethanol (70%) + Mercuric chloride (0.1%)	5 min + 30 s + 8 min	30 (32.97)	25 (29.86)	45 (42.04)
Sodium hypochlorite (5%) + Ethanol (70%) + Mercuric chloride (0.1%)	10 min + 30 s + 4 min	25 (29.78)	10 (18.06)	65 (53.83)
Sodium hypochlorite (5%) + Ethanol (70%) + Mercuric chloride (0.1%)	10 min + 30 s + 6 min	10 (17.23)	5 (12.37)	85 (67.24)
Sodium hypochlorite (5%) + Ethanol (70%) + Mercuric chloride (0.1%)	10 min + 30 s + 8 min	30 (32.88)	15 (22.65)	55 (47.95)
SE (m) ±		3.34	2.84	3.31
CD (0.05)		9.72	8.27	9.62

Values in bracket indicate that they are transformed values. Data recorded for different parameters were subjected to completely randomized design (CRD). Statistical analysis by (ANOVA) technique of CRD software used for data analysis is O.P. State.

Culture establishment

The result on number of days taken for establishment and per culture establishment obtained under each treatment are presented in Table 2. Figure 2 indicates that out of various treatments tried, the vegetative bud explants registered per cent establishment on MS medium supplemented with BAP (2.00, 4.00 and 6.00 mg/l) or BAP (2.00, 4.00 and 6.00 mg/l) in combination with IAA (2.00 mg/l). The time taken for culture establishment was less on the medium containing BAP in combination with IAA as compared to other treatments containing BAP alone or in combination with kinetin. The explants recorded least time (14.33 days) for culture establishment on MS medium supplemented with 4.00 mg/l BAP and 2.00 mg/l IAA. On the other hand, the treatment containing MS basal medium took significantly more time (25.00 days) for culture establishment.

Culture proliferation

In order to fix the optimum growth regulator concentration for shoot proliferation, the vegetative buds established on

Table 2. Effect of cytokinins and auxin on culture establishment of banana explants cv. Grand Naine.

Treatment	Time taken for culture establishment (days)	Culture establishment (%)
MS medium + BAP (2.00 mg/l)	18.00	85.33 (67.46)
MS medium + BAP (4.00 mg/l)	16.66	100.00 (90.00)
MS medium + BAP (6 .00 mg/l)	18.00	63.33 (52.73)
MS medium + BAP (2.00 mg/l) + IAA (2.00 mg/l)	15.00	87.66 (69.42)
MS medium + BAP (4.00 mg/l) + IAA (2.00 mg/l)	14.33	100.00 (90.00)
MS medium + BAP (6.00 mg/l) + IAA (2.00 mg/l)	16.33	76.00 (60.67)
MS medium + BAP (4.00 mg/l) + NAA (2.00 mg/l)	16.33	100.00 (90.00)
MS medium + BAP 4.00 mg/l + NAA 2.00 mg/l + IAA(2.00 mg/l)	14.66	100.00 (90.00)
MS Basal medium	25.00	41.00 (39.78)
SE (m) ±	0.14	0.06
CD (0.05)	0.44	0.20

Values in bracket indicate that they are transformed values. Data recorded for different parameters were subjected to completely randomized design (CRD). Statistical analysis by (ANOVA) technique of CRD software used for data analysis is O.P. State.

Figure 2. Culture establishment of banana explants cv. Grand Naine.

MS medium supplemented with BAP (4.00 mg/l) and IAA (2.00 mg/l) were re-cultured on the medium containing different concentration of growth regulators and the results on the frequency of explant showing multiple shoots, number of shoots per explant as well as length of longest shoot are presented in Table 3 and Figure 3. The frequency of the cultures showing multiple shoots ranged from 86.50 to 99% in treatment containing BAP (2.00, 4.00 and 6.00 mg/l) in combination with 2.00 mg/l IAA in which number of shoots ranged from 8.86 to 10.66 per culture. Further, the treatment combination of 4.00 mg/l BAPand 2.00 mg/l IAA gave maximum number of shoots (10.66) followed by 2.00 mg/l BAP and 2.00 mg/l IAA and were significantly superior to all other treatments.

Table 3. Effect of cytokinins and auxins on shoot proliferation of banana explants cv. Grand naine.

Treatment	Culture showing multiple shoot (%)	Number of shoots per culture	Length of longest shoot (cm)	Number of leaves on longest shoot
MS medium + BAP (2.00 mg/l)	56.00 (48.43)	4.66	8.10	7.33
MS medium + BAP (4.00 mg/l)	67.50 (55.23)	5.33	10.00	8.66
MS medium + BAP (6.00 mg/l)	52.50 (46.42)	3.33	7.30	6.00
MS medium + BAP (2.00 mg/l) + IAA (2.00 mg/l)	90.50 (72.16)	9.33	16.40	12.00
MS medium + BAP (4.00 mg/l) + IAA (2.00 mg/l)	99.00 (85.36)	10.66	18.30	13.00
MS medium + BAP (6.00 mg/l) + IAA (2.00 mg/l)	86.00 (68.03)	8.86	15.00	10.33
MS medium + BAP 2.00 mg/l + NAA 1.00 mg/l + IAA 2.00 mg/l	46.50 (42.97)	3.00	6.20	5.33
MS medium + BAP 4.00 mg/l + NAA 2.00 mg/l + IAA (2.00 mg/l)	77.50 (61.72)	8.00	14.30	9.33
MS medium + BAP 6.00 mg/l + NAA 3.00 m g/l + IAA 2.00 mg/l	39.50 (38.89)	2.00	4.50	4.00
SE (m) ±	1.48	0.17	0.84	0.22
CD (0.05)	4.44	0.52	2.51	0.67

Values in bracket indicate that they are transformed values. Data recorded for different parameters were subjected to completely randomized design (CRD). Statistical analysis by (ANOVA) technique of CRD software used for data analysis is O.P. State.

Figure 3. Culture proliferation of banana explants cv. Grand Naine.

Table 4. Effect of auxins and activated charcoal on *in vitro* rooting characters of banana plantlets cv. Grand naine.

Treatment	Time taken (days)	Culture rooting (%)	Number of roots per culture	Length of longest root (cm)	Length of shoot (cm)	Number of leaves per shoot
MS (half strength) + IBA (0.50 mg/l)	10.66	63.33 (52.71)	3.66	4.70	4.20	3.66
MS (half strength) + IBA (1.00 mg/l)	7.00	82.00 (65.62)	6.00	7.00	6.70	5.33
MS (half strength) + NAA (0.50 mg/l)	13.66	52.66 (46.51)	1.66	2.20	2.30	1.66
MS (half strength) + NAA (1.0 mg/l)	12.00	60.66 (51.15)	2.66	3.50	4.10	3.00
MS (half strength) + IBA 0.50 mg/l + AC 200 mg/l	8.00	79.33 (62.95)	5.33	6.00	6.20	5.00
MS (half strength) + IBA 1.00 mg/l + AC 200 mg/l	6.33	98.66 (83.70)	6.66	7.80	7.40	6.33
MS (half strength) + NAA 0.50 mg/l + AC 200 mg/l	12.66	55.66 (48.23)	2.33	3.40	3.70	2.33
MS (half strength) + NAA 1.00 mg/l + AC 200 mg/l	13.33	54.66 (47.66)	2.00	3.10	3.20	2.00
MS (half strength) + IBA 0.50 mg/l + NAA 0.50 mg/l	10.00	64.33 (53.33)	4.00	5.20	4.80	3.66
MS (half strength) + IBA 0.50 mg/l + NAA 1.00 mg/l	12.33	60.33 (50.96)	2.33	3.40	3.80	2.66
MS (half strength) + IBA 1.00 mg/l + NAA 0.50 mg/l	6.66	95.33 (77.69)	6.33	7.20	7.20	6.00
MS (half strength) + IBA 1.00 mg/l + NAA 1.00 mg/l	7.00	82.33 (65.12)	6.00	7.00	6.80	5.66
MS (half strength) + IBA 0.50 mg/l + NAA 0.50 mg/l + AC 200 mg/l	9.00	71.66 (57.83)	4.33	5.20	5.40	4.33
MS (half strength) + IBA 0.50 mg/l + NAA 1.00 mg/l + AC 200 mg/l	9.66	68.33 (55.73)	4.00	5.40	5.00	4.00
MS (half strength) + IBA 1.00 mg/l + NAA 0.50 mg/l + AC 200 mg/l	7.66	82.66 (65.51)	5.66	6.30	6.30	5.33
MS (half strength) + IBA 1.00 mg/l + NAA 1.00 mg/l + AC 200 mg/l	8.33	75.66 (60.53)	4.66	5.70	5.80	4.66
MS basal medium (half strength)	14.33	50.66 (45.36)	1.00	2.10	2.50	1.33
SE (m) ±	0.25	1.61	0.17	0.14	0.13	0.12
CD (0.05)	0.51	4.64	0.48	0.40	0.38	0.49

Values in bracket indicate that they are transformed values. Data recorded for different parameters were subjected to completely randomized design (CRD). Statistical analysis by (ANOVA) technique of CRD software used for data analysis is O.P. State.

Maximum length of shoot (18.30 cm) was obtained on the medium containing BAP (4.00 mg/l) and IAA (2.00 mg/l). In case of number of leaves on longest shoot BAP (4.00 mg/l) and IAA (2.00 mg/l) obtained highest of 13.00 leaves.

In vitro rooting

The data pertaining to the response of different auxins (IBA and NAA) on *in vitro* rooting of banana buds are presented in Table 4 and Figure 4. Out of 17 treatments

Figure 4. *In vitro* rooting.

tried, only 3 treatments (MS half strength) with IBA (1.00 mg/l) and activated charcoal showed maximum rooting of cultures (98.66%) which was at par with MS (half strength) with IBA (1.00 mg/l) and NAA (0.50 mg/l) registering 95.33% rooting. However, the shootlet took least time (6.33 days) for root initiation on half strength MS medium supplemented with 1.00 mg/l IBA and 200 mg/l activated charcoal. The maximum number of roots per shootlet (6.66) and the maximum length of root (7.80 cm) was produced on the medium containing 1.00 mg/l IBA and 200 mg/l activated charcoal. Treatments MS (half strength) augmented with 1.00 mg/l IBA and activated charcoal supported the maximum length of shoot (7.40 cm), which was significantly superior to the rest of the treatments tested. Considering the leaves per shoot, the maximum number of leaves (6.33) were produced on MS (half strength) supplemented with 1.00 mg/l IBA and activated charcoal. Addition of activated charcoal avoids callus formation at the base of the shoot before rooting.

DISCUSSION

Surface sterilization

Titov et al. (2006) studied that for *in vitro* propagation of banana cv. Kanthali, huge number of explants die due to microbial contamination; it studies that contamination free culture were established by $HgCl_2$ for 6 min followed by several washes in sterile water and obviated the need to develop extensive and complicated surface sterilization protocol, also Suneeta and Das (2008) studied that the investigation on the effect of surface sterilization agents showed variation in respect to their sterilizing property; out of sterilizing chemicals, calcium hypochlorate, hydrogen peroxide and bromine water did not show any good response and Jaisy and Ghai (2011) who worked on *in vitro* propagation of banana also found treatment of explants with $HgCl_2$ (0.1%) for 6 min most effective surface sterilization procedure registering maximum culture establishment with minimum contamination.

Culture establishment

The results obtained in the present investigation regarding culture establishment are supported by the findings of Muhammad et al. (2007) who worked on micropropagation of banana. Rahman et al. (2005) also found that in case of shoot multiplication of dessert banana, MS medium supplemented with 4.0 mg/l BAP, 2 mg/l NAA and 2.0 mg/l IAA to be the best. However, in the present investigation MS medium augmented with 4.00 mg/l BAP and 2.00 mg/l IAA gave best results registering per cent establishment and took least time for shoot initiation. The differences obtained in the requirement of phytoharmones as reported by different researchers and also in the present investigation may be attributed to the differences in the levels of endogenous phytoharmones, nutrients, metabolites and interaction between various factors.

According to Skoog and Miller (1957), quantitative interaction between diverse growth factor may have decisive role in organogenesis. Ammirato (1986) 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.

Culture proliferation

Likewise, Muhammad et al. (2007) found highest response of shoot multiplication in 4 mg/l BAP and 2 mg/l IAA while Rahman et al. (2005) obtained maximum multiplication on treatment involving 4 mg/l BAP, 2 mg/l IAA and 2.0 mg/l NAA. However, in the present study, a higher level of BAP (4.00 mg/l) was found to be more suitable. The basic phenomenon involved in the explants establishment, induction of multiple shoots and subsequent plantlet production *in vitro* are reported to be due to action of plant hormones. Little is known about how hormones evoke the particular pattern of morpho-genesis (Thorpe, 1978). One hypothesis is that hormone treatment starts the cell on a specific development pathway; the alternative view is that hormone responsive cells are already determined and hormone evokes the expression of the combined state. Hence, the observed differences in the requirement of growth hormones for shoot proliferation of explant as reported by different researchers as well as in the present investigation could be attributed to the differential requirement of the growth hormones.

In vitro rooting

In vitro multiplication of banana is normally carried in the presence of high cytokinins levels, which inhibit root formation and elongation. Addition of 200 mg/l charcoal enhanced rooting and stopped callus formation. It was also obvious from the result that incorporation of activated charcoal reduced the time taken for root initiation and further increased the root and shoot length. Reports of Sharma et al. (1997); Gubbuk and Pekmezci (2006) and Roy et al. (2010) support the results as they obtained rooting only with 1.00 mg/l IBA and 200 mg/l activated charcoal.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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