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MICROPROPAGATION OF BANANA CV. MALBHOG

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ABSTRACT
Malbhog, one of the most important and delicious local cultivar of banana in Bihar, is on verge of becoming extinct because of panama wilt and non-availability of disease free quality propagules. The culture of shoot tips taken from suckers on Murashige and Skoog (MS) medium supplemented with different concentrations and combinations of Indole acetic acid (IAA) and Benzyl amino purine (BAP) resulted in differentiation of adventitious shoots. The maximum differentiation of shoots (92.05 %) was observed on MS medium with 0.57 µM IAA and 17.74 µM BAP. The number of shoots per culture was 16.75. The subculture of differentiated shoots on the same medium resulted in further differentiation (91.97 %) of more than 15 shoots per culture. The *in vitro* developed shoots showed 100% rooting on MS medium supplemented with 4.92 µM Indole butyric acid (IBA). The plantlets were acclimatized and field transferred. A suitable and efficient protocol for micropropagation of Malbhog cultivar of banana was developed.

INTRODUCTION

Malbhog is the one of the most important cultivar grown in Vaishali region of Bihar, because of its unique and delicious taste. Its fruits are longer and have more weight and the bunch is also big. It is very sweet in taste and used as dessert. Malbhog is greatly affected by the Panama wilt caused by *Fusarium oxysporum* f. sp. *cubense*. Because of this disease, the choicest cultivar is on the verge of becoming extinct. Plantation of 'Malbhog' in new areas will reduce the problem of Panama wilt, but can only be done if large amount of quality propagules are available for planting, which is only possible through micropropagation. Further, micropropagated banana plants produce bunch with higher weight, more fingers and hands and more uniform fruit size and shape (Lalrinsanga *et al.*, 2013). Though, the protocol for micropropagation of some genotypes of banana are available (Suman *et al.*, 2013a, b) and one for cv. Malbhog of North East region (Roy *et al.*, 2010), there is none for cv. Malbhog of Bihar. Thus, micropropagation of banana cv. Malbhog will save the genotype from being extinct and help its expansion in large new areas.

MATERIALS AND METHODS

Suckers of banana cv. Malbhog were collected from the local farms and brought to the laboratory. The roots and the outer leaves with leaf sheaths were removed. A cube of tissue of about 2 cm^3 containing the apical meristem was excised from the base of the sucker. The rhizome shoot tips were prepared, washed, pretreated and surface sterilized following the method of Suman *et al.* (2013a). The outer two to three layers of the rhizome shoot tips were carefully removed using a scalpel and a cube of tissue of about 1 cm^3 containing the apical meristem was excised. The individual explants were inoculated and cultured on Murashige and Skoog (1962) medium supplemented with different concentrations and combinations of IAA and BAP in culture tubes and bottles. The cultured tubes and bottles were incubated in the thermal insulated tissue culture room with temperature around 25ºC and relative humidity 50 - 80 %. A continuous light of 2 kilo lux intensity was provided through fluorescent tubes.

The differentiated shoots developed from the cultured shoot tips were also subcultured on some of the selected media from the experiment to increase the number of shoots and on the two new media for development of roots. The regenerated plantlets having healthy roots were selected for pot transfer. The rooted plantlets along with the agar medium were carefully taken out. The agar medium was removed without damaging the root. Plantlets were now transferred to pots having sterilized sand and farm yard manure in 1:1 ratio and progressively acclimatized to reduced humidity for their hardening and acclimatization (Suman *et al.*, 2013b). The plants were finally transferred to the field. The data were subjected to one way analysis of variance to test the significance of the observed result and analyzed statistically according to completely randomized block design (CRD) and a comparison between mean values of treatments was made by the least significant difference (LSD) to identify the best treatments. The effects of treatments were tested by Analysis of Variance. Duncan's Multiple Range Test (DMRT) (Duncan, 1955) was used to test the difference among means.

RESULTS AND DISCUSSION

Shoot tip culture of banana cv. Malbhog resulted in elongation of existing shoot, callus formation and formation of multiple shoots and roots from the base of explant. The existing shoot

Table 1: The effect of the type and concentration of phytohormones on tissue culture responses from shoot tip cultures of cv. Malbhog

Shoot tip culture Media	Growth regulatorsIAA (μm) + BAP(μm)	Explants showing elongation of existing shoot (% ± SE)	Explants showing callogenesis (% ± SE)	Explants showing caulogenesis (% ± SE)	No.of shoots/ explants (% ± SE)	Explants showing rhizogenesis (% ± SE)
BM_1	5.71 + 0	81.25 ± 1.09 [b]	36.93 ± 1.09 [f]	-	-	46.59 ± 1.47 [b]
BM_2	11.42 + 0	-	82.96 ± 1.47 [a]	-	-	64.77 ± 1.47[a]
BM_3	0 + 17.74	-	-	68.18 ± 1.89 [d]	11.08 ± 0.11 [c]	-
BM_4	0 + 26.61	-	-	73.86 ± 1.47 [c]	9.64 ± 0.10 [d]	-
BM_5	0.57 + 8.87	87.50 ± 1.79 [a]	-	23.61 ± 1.80 [g]	4.76 ± 0.32 [f]	-
BM_6	5.71 + 8.87	43.06 ± 1.79 [f]	76.39 ± 1.79 [b]	-	-	31.95 ± 1.79 [e]
BM_7	11.42 + 8.87	-	64.77 ± 1.47 [c]	-	-	37.50 ± 1.47 [f]
BM_8	0.57 + 13.30	76.39 ± 1.79 [c]	-	87.50 ± 1.80 [b]	12.19 ± 0.08 [b]	-
BM_9	5.71 + 13.30	78.47 ± 1.33 [b c]	56.94 ± 1.80 [d]	45.83 ± 1.80 [f]	7.13 ± 0.16 [e]	-
BM_{10}	11.42 + 13.30	-	45.83 ± 1.80 [e]	-	-	43.06 ± 1.80 [c]
BM_{11}	0.57 + 17.74	62.50 ± 1.46 [e]	-	92.05 ± 1.47 [a]	16.75 ± 0.07 [a]	-
BM_{12}	5.71 + 17.74	67.71 ± 1.35 [d]	-	64.74 ± 1.47 [d e]	9.64 ± 0.10 [d]	-
BM_{13}	11.42 + 17.74	-	31.95 ± 1.80 [g]	-	-	42.21 ± 1.76 [cd]
SEm ±		1.13	1.19	1.23	0.11	1.11
CD at 5%		3.24	3.41	3.53	0.33	3.18
Differentiated shoot subculture						
BM_3	0 + 17.74	-	-	72.32 ± 1.15 [d]	12.55 ± 0.07 [c]	-
BM_4	0 + 26.61	-	-	77.89 ± 1.24 [c]	10.35 ± 0.07 [d]	-
BM_8	0.57 + 13.30	-	-	93.27 ± 1.24 [a]	13.34 ± 0.05 [b]	-
BM_{11}	0.57 + 17.74	-	-	91.97 ± 1.15 [a b]	15.65 ± 0.05 [a]	-
SEm ±				1.20	0.06	
CD at 5%				3.73	0.18	
	IBA(μm)					
BM_{14}	4.92	-	-	-	-	100
NAA(μm)						
BM_{15}	½ MS + 5.37	-	-	-	-	100

Values followed by the same letter in columns are not significantly different using Duncan's multiple range test at 5% level

present in the explant elongated after 10 - 15 days of culture (Fig. 1a) and the frequency of such elongation was the maximum (87.50 %) on medium BM_5 (MS + 0.57 μM IAA + 8.87 μM BAP, Table 1). Elongation of existing shoot was also observed on media BM_1 (MS + 5.71 μM IAA) and BM_9 (MS + 5.71 μM IAA + 13.30 μM BAP). All these media have lower concentrations of phytohormones which resulted in less other tissue culture responses and thus showed enhanced existing shoot development. The phytohormones particularly cytokinin promotes calcium uptake from the medium; calcium regulates exocytosis through cytoskeleton and thus promotes existing shoot development. Shoot elongation was found to be the maximum in a medium having lower concentrations of cytokinins (Akbar and Roy, 2006; Ali *et al.*, 2011).

The callus formation was observed after 20-25 days of culture. It generally developed from the swelled basal region of the explants that was in contact of the medium. The callus initiation was observed from the outermost cells of the explant and may have developed as a result of onset of cell division. The nature of callus was compact and the colour was light yellow, white yellow and light grey in all the media. Callogenesis was the maximum (82.96 %) on medium BM_2 (MS + 11.42 μM IAA). Callogenesis in good frequency was also observed on other media having either higher concentrations of auxin compared to cytokinins or more or less equal concentrations of both (Table 1). Callus is an important source of variation and can be exploited through somaclonal variation for the improvement of vegetatively propagated crop like banana. (Sahu and Khalkho, 2012).

Multiple shoot differentiation *i.e.*, caulogenesis is essential for propagules multiplication during micropropagation of banana. Caulogenesis from the cultured shoot tips was observed after 30 - 35 days of culture. Caulogenesis mostly occurred directly from the base of the explants (Fig. 1b and 1c) as found by other workers (Jafari *et al.*, 2011). Callus mediated caulogenesis was also found but only on medium BM_9 (MS + 5.71 μM IAA + 13.30 μM BAP, Table 1). Direct caulogenesis was the maximum (92.05 %) on medium BM_{11} (MS + 0.57 μM IAA + 17.74 μM BAP, Table 1). High frequency of shoot differentiation was also observed on media BM_8 (MS + 0.57 μM IAA + 13.30 μM BAP) and BM_4 (MS + 26.61 μM BAP). Medium BM_{11} even gave the highest number of differentiated shoots (16.75) per explant (Table 1). Differentiated shoots subculture on medium BM_{11} resulted in further differentiation of shoots (91.97 %), with 15.65 shoots per culture. All these media supporting good caulogenesis have high BAP alone or with IAA in lower concentrations. The frequency of caulogenesis increased with increase in concentration of BAP but its higher concentration showed mutagenic effects and resulted in more appearance of off type plants (Jafari *et al.*, 2011). The shoot differentiation in such media was in conformation with the classical hypothesis about phytohormonal regulation of organogenesis in plant tissue culture.

The high performance of BAP over the other cytokinins in inducing caulogenesis in shoot tip cultures has been found in

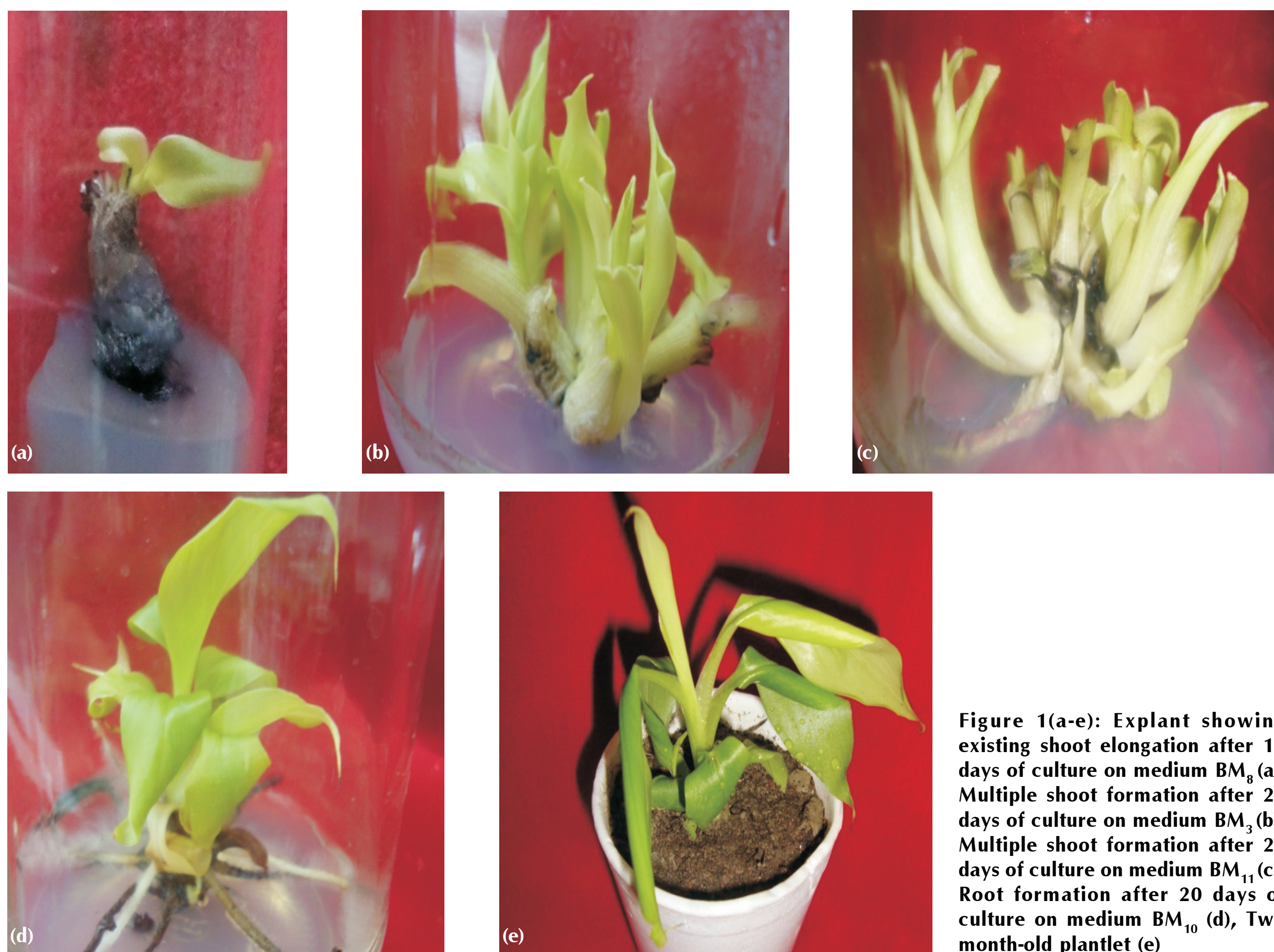

Figure 1(a-e): Explant showing existing shoot elongation after 15 days of culture on medium BM_8 (a), Multiple shoot formation after 20 days of culture on medium BM_3 (b), Multiple shoot formation after 25 days of culture on medium BM_{11} (c), Root formation after 20 days of culture on medium BM_{10} (d), Two month-old plantlet (e)

different cultivars of banana (Kulkarni *et al.*, 2004; Jafari *et al.*, 2011; Kanchanapoom and Promsorn, 2012; Lalrinsanga *et al.*, 2013 ; Ahmed *et al.*, 2014). The most favorable concentration of BAP for caulogenesis was 17.74 μm and this was also supported by Vani *et al.* (1999) and Ahmed *et al.* (2014). Caulogenesis from shoot tip culture at 20 μm BAP has been suggested by Vuylsteke (1985) and Lalrinsanga *et al.* (2013). The addition of an auxin IAA at lower concentration of 0.57 μm enhanced the caulogenesis. Kumar *et al.* (2002) got multiple shoots on modified MS medium supplemented with 22.18 μm BAP and 1.14 μm IAA. Good caulogenesis was also observed on medium with only BAP at concentrations 17.74 μm and 26.61 μm. Many workers reported that BAP at 22.18 μm was the optimum cytokinin concentration for multiple shoot formation in banana (Venkatachalam *et al.*, 2006; Bairu *et al.*, 2008; Shirani *et al.*, 2009; Lalrinsanga *et al.*, 2013). The *in vitro* developed shoot explants showed better response than *in vivo* explants because of lesser problems of phenols and surface sterilization.

The development of roots from the base of shoots was essential for the development of plantlets. Rhizogenesis was observed directly from the base of cultured shoot tips mostly along with callus formation. Direct rhizogenesis was also observed (Fig. 1d). Rhizogenesis was the maximum (64.77 %) on medium BM_2 (MS + 11.42 μM IAA, Table 1). All those media, which supported rhizogenesis has auxin only or higher concentration of auxin compared to the cytokinin. The differentiation of roots in such media was as per the classical hypothesis of hormonal regulation of organogenesis.

Rhizogenesis in the *in vitro* propagated cultivars was observed on MS media supplemented with 1.07 μm NAA (Acharjee *et al.*, 2004). Strosses *et al.* (2004) observed root induction on MS medium with 0.54 – 10.74 μm NAA also. Vani *et al.* (1999) suggested MS medium supplemented with 8.87 μm BAP + 11.42 μm IAA + 0.1% activated charcoal to be good for rhizogenesis from shoot tip culture, while Strosses *et al.* (2004) favored MS medium supplemented with 1 μm BAP + 1 μm IAA for root formation. The frequency of rhizogenesis was low on all these media and on most occasions callus formation also occurred simultaneously and thus inhibiting proper plantlet formation. Thus, the differentiated shoots were subcultured on two new media BM_{14} (MS + 4.92 μM IBA) and BM_{15} (1/2 MS + 5.37 μM NAA) which resulted in cent percent rhizogenesis without any aberrant callus formation resulting into proper plantlet formation. The medium BM_{14} showed better rhizogenesis compared to medium BM_{15} with respect to better root differentiation and root growth (Table 1). Most of the workers have found IBA, the most suitable hormone in banana

for rooting of *in vitro* developed shoots. Some of them have used the same concentration 4.92 μm IBA (Strosses *et al.*, 2004). However, Kumar *et al.* (2012) got better rooting when the medium with 4.92 μm IBA was further added to 2.85 μm IAA.

The well rooted regenerated plantlets of cv. 'Malbhog' showed 95 % survival during acclimatization (Fig. 1e) and field transfer. The plantlets did not show any morphological variation and were true to the parent type. Thus, a suitable and efficient protocol for a highly desired but endangered cultivar of banana (Malbhog) was developed.

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