AJH
eISSN- 0976-724X

THE ASIAN JOURNAL OF HORTICULTURE
Volume **11** | Issue 1 | June, 2016 | 141-145
Visit us -*www.researchjournal.co.in*

RESEARCH PAPER

DOI : 10.15740/HAS/TAJH/11.1/141-145

Article history :
Received : 19.01.2016
Revised : 27.04.2016
Accepted : 06.05.2016

Enhancing propagation efficiency of banana cv. MONTHAN (ABB) through micropropagation

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ABSTRACT : An investigation was carried out at Plant Tissue Culture Laboratory of Horticultural College and Research Institute, Coimbatore during 2012-2013. Shoot tip of banana cv. MONTHAN were cultured in MS medium fortified with different growth regulators (BAP @ 4, 5 and 6 mg l^{-1}) either alone or in combination with NAA (0.5 mg l^{-1}) and kinetin (2 mg l^{-1}) to study the influence of growth regulators on multiplication rate. The data on number of multiple shoots, days for multiple shoot induction and micro shoot length were recorded. MS+BAP 6 mg l^{-1} + kinetin 2 mg l^{-1} recorded maximum number of multiple shoots (5.07). MS + BAP 6 mg l^{-1} recorded least days for multiple shoot induction (27.06 days) and highest micro shoot length (6.73cm). Further to enhance multiplication best growth regulator combination (MS + BAP 6 mg l^{-1} + Kinetin 2 mg l^{-1}) along with different concentration of coconut water (5, 10 and 15%) and casein hydrolysate (100, 200 and 300 mg l^{-1}) were supplemented. MS + BAP 6 mg l^{-1} + kinetin 2 mg l^{-1}+coconut water 5 per cent recorded the highest number of multiple shoots at all the intervals of subculture (5.13). The best growth regulator combination (MS+BAP 6 mg l^{-1} + kinetin 2 mg l^{-1}) along with B5 vitamins in specific to different thiamine concentration (5, 10 and 15 mg l^{-1}) for further multiplication were supplemented and B_5 vitamins with thiamine combinations did not increase the multiplication rate. The degree of efficiency of shooting was found to be dependent on type of hormone.

KEY WORDS : Banana, Multiplication, BAP, Kinetin, Coconut water, Thiamine

HOW TO CITE THIS ARTICLE : Udaya, C.Y. Shalini, Porika, H.K., Sindhupriya, V. and Kumar, P. Prasanna (2016). Enhancing propagation efficiency of banana cv. MONTHAN (ABB) through micropropagation. *Asian J. Hort.*, **11**(1) : 141-145, **DOI : 10.15740/HAS/TAJH/11.1/141-145.**

Banana is popularly known as 'apple of paradise'. It is a rich source of energy (137 K.cal/100g). Banana ranks fourth in terms of production in the world and second most significant fruit crop in India next to mango (Subramanyam *et al*., 2011). India is the largest producer of banana with an area of 8.02 lakh ha, production of 297.24 lakh MT and with productivity 37.0 MT/ha (NHB, 2015). Tamil Nadu has the largest area (1.18 lakh ha) with highest share (19.0%) and which ranks first in production (56.50 lakh MT) followed by Maharashtra and Gujarat.

Production of banana in India is entirely dependent on unimproved clones that were often selected from nature, domesticated and maintained in culture. Conventionally, the suckers arising from the corms are separated and used as planting material (Sathiamoorthy, 1973). Inevitably, this leads to transmission of pathogen inoculum during subsequent crop cycles leading to poor productivity. Biotic stresses like Panama wilt disease *(F. oxysporum* f. sp *cubense*), leaf spot disease (*Mycosphaerella musicola* and *Mycosphaerella fijiensis*) and many viral diseases (Banana bunchy top virus, Banana Bract Mosaic virus) are continuing to be the major threats to banana cultivation (Arias, 1992 and

Sagi *et al*., 1998). Hence, the use of disease free planting materials primarily becomes the way to keep the crop productivity at reasonably high levels. In addition, the suckers / corms suffer from slow multiplication, bulkiness and poor phytosanitary quality (Vuylsteke, 1989). Micropropagation has been reported to be the best tool for eliminating virus from the planting materials apart from other advantages like high multiplication rate and faster growth in early stages compared to conventional materials (Vuylsteke, 1989; Daniells and Smith, 1991 and Arias, 1992).

Propagation of banana through *in vitro* techniques has been reported by several workers using different explant sources, as well as regeneration pathways (Krikorian and Cronauer, 1982; Banerjee *et al*., 1986 and Navarro *et al*., 1997). Micropropagation of banana through shoot tips and floral apices have been successfully demonstrated (Vuylsteke and De Langhe, 1985; Balakrishnamurthy and Sreerangaswamy, 1988); Ganapathi, 1995; Mukunthakumar *et al*., 2011 and Karthika, 2012).

A number of protocols for *in vitro* propagation are available for all banana cultivars. The important factor affecting the efficiency of micropropagation system is the rate of multiplication. Rate of multiplication in banana is genotypic dependent (Israeli *et al*., 1995 and Mendes *et al*., 1996). Hirimburegama and Gamage (1996) reported that the rate of shoot proliferation is less in ABB genomes compared to other groups of banana genome. They also reported that in Monthan, Alukehel and Seenikehel belong to ABB group; the production of multiple shoots is a problem.

Among the commercial cultivars, the cultivar Grand Naine is extensively grown in Tamil Nadu. The new prolific 'Monthan' type clones of economic value are recently becoming popular in Tamil Nadu. Monthan is a widely cultivated culinary cultivar for processing and production of leaves. It is widely cultivated for production of leaves in Trichy and Tanjore districts of Tamil Nadu. Apart from its culinary use of fruits, pseudostem core is a highly relished vegetable with many medicinal properties. It has many desirable qualities like immune to Banana Bunchy Top Virus (BBTV) disease and salt tolerance and normal bunch mass even under marginal condition, but it is highly susceptible to Fusarium wilt disease. Balakrishnamurthy and Sreerangaswamy (1988) made attempts to standardize the protocols for multiplication of cv. MONTHAN from shoot tip and floral apices and also reported that Monthan has slow rate of multiplication under *in vitro* shoot tip culture. Monthan contains high levels of phenolic substances which contribute to browning and death of tissue due to oxidation of polyphenols (Hirimburegama and Gamage, 1996). This substance inhibits the proliferation under *in vitro* culture. So the present work was planned to enhance the rate of multiplication of cv. MONTHAN from shoot tips under *in vitro* condition.

RESEARCH METHODS

An investigation was carried out at Plant Tissue Culture Laboratory of Horticultural College and Research Institute, Coimbatore during 2012-2013 using Completely Randomized Design (CRD). The Murashige and Skoog medium (1962) was used as the basal medium. The nutrient media included sucrose as carbon source (30 g l^{-1}). Difco agar was used at 0.8 per cent as gelling agent. The washed glasswares were allowed to dry and sterilized in an autoclave at a pressure of 15 pounds per square inch and a temperature of 121°C for 30 minutes and stored away free of contamination until use. The shoot tips were collected from two to three months old sword suckers. The suckers were separated from the mother rhizome, detopped and reduced to the size of nearly 7 to 8 cm in length having the terminal meristematic bud, along with a small portion (3-4 cm) of the rhizome. The explants were then taken to the laboratory immediately, where they were first washed thoroughly in running tap water for 30 minutes to remove all the dirt and soil particles adhering to them, followed by soaking of suckers in pretreatment solution (Carbendazim 0.5% WP + 5% NaOCl + Teepol (1-2 drops/ 100 ml) for 15 minutes) to reduce the microbial load. Again the explants were washed in running tap water and trimmed. Then, the explants were washed with antioxidant solution containing 300 mg/lit. of citric acid and 150 mg/lit. ascorbic acid in order to reduce browning of explant due to excessive polyphenol exudation. In order to obtain the aseptic cultures, efficient sterilization technique was followed. After antioxidant treatment the explants were surface sterilized under Laminar Air Flow Chamber. The explants were surface sterilized with 70 per cent ethanol for 30 seconds. Then the explants were rinsed with sterile distilled water four times followed by washing with mercuric chloride 1 per cent for 5 minutes. After surface sterilization, the explants were rinsed with sterile distilled water four times. To remove moisture from the surface,

the explants were transferred to the sterile filter paper placed over sterile Petri dishes. Then, the shoot tip was isolated by removing the surrounding leaf sheaths and a portion of the rhizomatous base remained intact with the shoot tip (length: 2.5 to 3.0 cm; diameter 1.5 to 2.0 cm). After sterilization, the explants were inoculated by inserting one-fourth the length of shoot tip explant into the medium.

Physical environment for *in vitro* culture: Temperature - 25 ± 2°C; Humidity - 70-80 per cent; light source - white fluorescent lamps; light intensity - 2500 to 3000 Lux photoperiod.

Trial 1: BAP alone at (4.0 mg.l^{-1}, 5.0 mg.l^{-1} and 6.0 mg.l^{-1}) and in combination with NAA (0.5 mg.l^{-1}) and kinetin (2.0 mg.l^{-1}) were used along with MS medium. **Trial 2:** BAP 6.0 mg.l^{-1}+ kinetin 2.0 mg.l^{-1} in combination with coconut water (5, 10 and 15%) and casein hydrolysate (100, 200 and 300 mg/lit.). **Trial 3:** BAP 6.0 mg l^{-1}+ kinetin 2.0 mg l^{-1} in combination with B_5 vitamin in specific with different concentration of thiamine (5, 10 and 15 mg/lit.). The data generated from the various trials were subjected to statistical analysis in completely randomized design as per the methods of Panse and Sukhatme (1985). The critical values were worked out for five per cent (0.05) probability and the results were interpreted. Analysis was carried out with AGRES software package and MS Excel® spreadsheet.

RESEARCH FINDINGS AND DISCUSSION

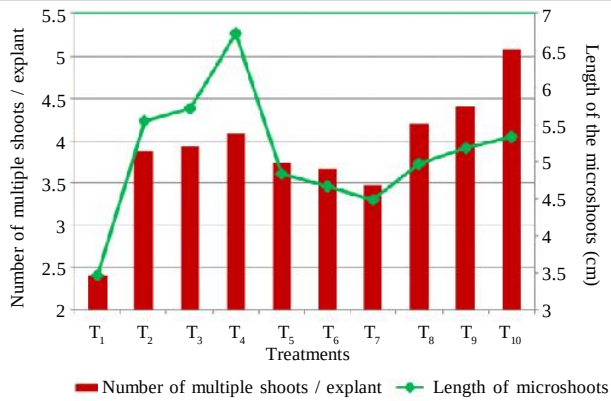
Rate of shoot multiplication is dependent on type of cytokinin, their concentration and cultivars (Hamide and Pekmezci, 2004). Variation in the activity of different cytokinins can be explained by their different uptake rate reported in different genomes (Blakesley, 1991), varied translocation rates to meristematic regions and metabolic processes, in which the cytokinin may be degraded or conjugated with sugars or amino acids to form biologically inert compounds as reported by Van and Trinh (1990) and Kaminek (1992). Pierik (1987) reported that the inherent endogenous auxins and cytokinins levels must

Table 1 : Influence of growth regulators on days for multiple shoot induction average number of multiple shoots / explant and length of the micro shoots (cm)

Treatments details	Mean days taken for multiple shoot induction	Average no. of multiple shoots	Mean length of micro shoots (cm)
T_1: (MS basal medium - control)	40.67	2.40	3.46
T_2: (MS + BAP 6 mg.l^{-1} + KIN 2 mg.l^{-1} + CW 5 %)	31.47	5.13	5.52
T_3: (MS + BAP 6 mg.l^{-1} + KIN 2 mg.l^{-1} + CW 10 %)	32.53	4.47	4.97
T_4: (MS + BAP 6 mg.l^{-1} + KIN 2 mg.l^{-1} + CW 15 %)	34.80	4.33	4.74
T_5: (MS + BAP 6 mg.l^{-1} + KIN 2 mg.l^{-1} + CH 100 mg.l^{-1})	35.93	3.13	4.08
T_6: (MS + BAP 6 mg.l^{-1} + KIN 2 mg.l^{-1} + CH 200 mg.l^{-1})	37.07	3.07	4.28
T_7: (MS + BAP 6 mg.l^{-1} + KIN 2 mg.l^{-1} + CH 300 mg.l^{-1})	38.13	2.93	4.53
Mean	34.99	3.84	4.51
S.E. ±	0.41	0.25	0.22
C.D. (P=0.05)	0.89	0.53	0.48

Table 2 : Influence of thiamine on days for multiple shoot induction average number of multiple shoots / explant and length of the microshoots (cm)

Treatments details	Mean days taken for multiple shoot induction	Average no. of multiple shoots	Mean length of micro shoots (cm)
T_1: MS (Control)	40.70	2.35	3.48
T_2: (MS+ BAP 6 mg.l^{-1} + KIN 2 mg.l^{-1} + Thiamine 5 mg.l^{-1} + Nicotinic acid 1 mg.l^{-1} + Pyridoxine 1 mg.l^{-1})	35.85	4.10	4.87
T_3: (MS+ BAP 6 mg.l^{-1} + KIN 2 mg.l^{-1} + Thiamine 10 mg.l^{-1} + Nicotinic acid 1mg.l^{-1} + Pyridoxine 1mg.l^{-1})	37.05	3.55	4.43
T_4: (MS+ BAP 6 mg.l^{-1} + KIN 2 mg.l^{-1} + Thiamine 15 mg.l^{-1} + Nicotinic acid 1 mg.l^{-1} + Pyridoxine 1 mg.l^{-1})	39.50	3.20	4.17
Mean	38.28	3.30	4.24
S.E.±	0.21	0.14	0.16
C.D. (P=0.05)	0.45	0.31	0.35



T_1 (MS basal medium – control)	T_6 (MS + BAP 5.0 mgl^{-1} + NAA 0.5 mgl^{-1})
T_2 (MS + BAP 4.0 mgl^{-1})	T_7 (MS + BAP 6.0 mgl^{-1} + NAA 0.5 mgl^{-1})
T_3 (MS + BAP 5.0 mgl^{-1})	T_8 (MS + BAP 4.0 mgl^{-1} + Kinetin 2.0 mgl^{-1})
T_4 (MS + BAP 6.0 mgl^{-1})	T_9 (MS + BAP 5.0 mgl^{-1} + Kinetin 2.0 mgl^{-1})
T_5 (MS + BAP 4.0 mgl^{-1}+ NAA 0.5 mgl^{-1})	T_{10} (MS + BAP 6.0 mgl^{-1} + Kinetin 2.0 mgl^{-1})

Fig. 1 : Influence of growth regulators on production of number of multiple shoot and length of the micro shoot at end of fourth subculture

have also played part in shoot proliferation of higher plants. BAP 6.0 $mg.l^{-1}$ + Kinetin 2.0 $mg.l^{-1}$ was found to be the best with respect to average number of multiple shoots / explants. Azam *et al*. (2010) found that rate of shoot proliferation increased considerably with the synergistic effect of BAP and Kinetin. BAP 6.0 $mg.l^{-1}$ was found to be the best with respect to days for multiple shoot induction and length of the micro shoots. Influence of growth regulators on production of average number of multiple shoots / explant in subsequent subcultureis been summarized in (Fig.1). Josekutty *et al*. (2003) reported that in variety Kufwafwa (ABB) the shoot multiplication started only after 92 days of inoculation. BAP 6.0 $mg.l^{-1}$ + kinetin 2.0 $mg.l^{-1}$ + coconut water 5 per cent was found to be best with average number of multiple shoots/ explant, days for multiple shoot induction and length of the micro shoots. Influence of growth regulators on days for multiple shoot induction average number of multiple shoots / explant and length of the micro shoots is been summarized in (Table 1). The combination of BAP with kinetin and coconut water produced higher number of shoots and highest length of the shoot (Rahman *et al*., 2005). BAP 6.0 $mg.l^{-1}$ + kinetin 2.0 $mg.l^{-1}$ + thiamine 5.0 $mg.l^{-1}$ + nicotinic acid 1.0 $mg.l^{-1}$ + pyridoxine 1.0 $mg.l^{-1}$ was found to be the best treatment with respect to average number of multiple shoots/ explant, days for multiple shoot induction and length of the micro shoots. Influence of thiamine on days for multiple shoot induction average number of multiple shoots / explant and length of the microshootsis been summarized in (Table 2). Balakrishnamurthy and Sreerangaswamy (1988) also reported highest regeneration of banana plantlets *in vitro* from floral apices using MS salts with B_5 vitamin.

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