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Research Article

NATURAL AND LOW-COST SUBSTITUTES OF SYNTHETIC PGR FOR MICROPROPAGATION OF BANANA

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ABSTRACT
Micropropagation is the process where synthetic PGRs are utilized to obtain maximum desired growth. The most widely used synthetic cytokinin BA (3-5 mg/l) is used for *in vitro* shoot-multiplication. In present study, impact of various fruit juices were evaluated on *in vitro* shoot-multiplication of banana var. Grand naine for the purpose of cost-reduction. Sweet-lime juice was found useful as the substitute of such costlier synthetic cytokinin.

Key Words: *Plant Growth Regulator, Cytokinin, Banana, Micropropagation, Sweet- lime*

INTRODUCTION
Banana (*Musa paradisiaca* Linn.) is nutritionally significant, one of the most important food crops, which is widely grown and consumed throughout the world. Conventionally, banana is cultivated through suckers (5-10 in number per plant) produced from underground rhizome of the mother plant. Banana plantlets raised through micropropagation remain always high in demand (Anonymous, 2005). This technique provides a large number of uniform, high quality and disease-free planting material to meet demand in a short span of time on a year-round basis anywhere, irrespective of the season and weather (Anonymous, 2004).
The most widely used MS medium (Murashige and Skoog, 1962) is used for commercial production of plantlets through shoot-apical meristem culture of banana. High cost of plantlet production through micropropagation technique is a major concern limiting its wide application, despite its obvious advantages. Due to the high cost of production, 32 out of 90 commercial micropropagation units were closed down in India after the tremendous growth in 1990s (Prakash, 2001 and Savangikar, 2004). In developed countries also this industry has undergone a pause, as it is difficult to remain cost-effective (Govil and Gupta, 1997).
The expenditure for all the components incorporated in the protocol for media preparation, is chiefly constituted 49.61 % by gelling agent (Agar-agar), 38.49 % of sucrose (tissue-culture grade), 7.78 % of BA and 4.12 % of rest of the components (Vora, 2011). The present communication deals with the study of impact of various natural substitutes of PGR on *in vitro* shoot-multiplication of banana.

MATERIALS AND METHODS
Suckers of Grand naine variety of banana (*Musa paradisiaca* Linn.) were collected from Gujarat Green Revolution Company, Umareth and Gujarat. They were washed under running tap-water (30 min) and trimmed to remove outer scales. The washed suckers were pretreated (20 min) with a mixture of 0.05 % of Carbendazim (Bavistin) and 0.1 % of activated charcoal on gyratory shaker (100 rpm). After thorough washing with distilled water (3 times) the apical shoots were isolated for inoculation (Vora and Jasrai, 2011). The multiplication was carried out in MS medium (Murashige and Skoog, 1962) containing 3 mg/l BA, 3 % sucrose and 0.8 % Agar-Agar (Cronauer and Krikorian, 1984) in culture bottles (10X5 cm). Shoot-clumps with 5 shoots each were transferred to MS medium containing 4 mg/l BA during shoot-multiplication cycles. The multiple-shoots were sub-cultured at every four week intervals.
The extracts of fresh fruits were used at different concentrations (3-10 %) in place of BA. These included Sweet-lime, Tomato extract and Sweet corn juice. MS medium containing 4 mg/l BA was used as the control. pH of all media was adjusted to 5.8 with either 0.1 N KOH or 0.1 N HCl prior to its sterilization

in an autoclave each time. Regenerated shoots were rooted on half-strength MS medium with 3 mg/l IBA. Hardening of the rooted shoots was carried out after 4 weeks (Jasrai *et al.*, 1999).
All the cultures were kept in the culture room with 25 ºC temperature and 16 h photoperiod. For each treatment, 15 replicates were used during 6th subculture cycle. Data for each treatment was analyzed after 4 weeks in terms of increase in number of shoots, shoot-length and number of leaves.

RESULTS AND DISCUSSION

The fresh juice of Sweet-lime (5 %) was found comparatively better for shoot-multiplication of banana among rest of the treatments (Table 1, Figure 1A). This was higher by about 12 % than that of the control. Increase in length of shoot was found to be maximum with 3 % of Sweet-lime juice, followed by 3 % and 5 % of Tomato extract. Increase in number of leaves was also found to be maximum in 3 % of Sweet-lime juice (Table 1).

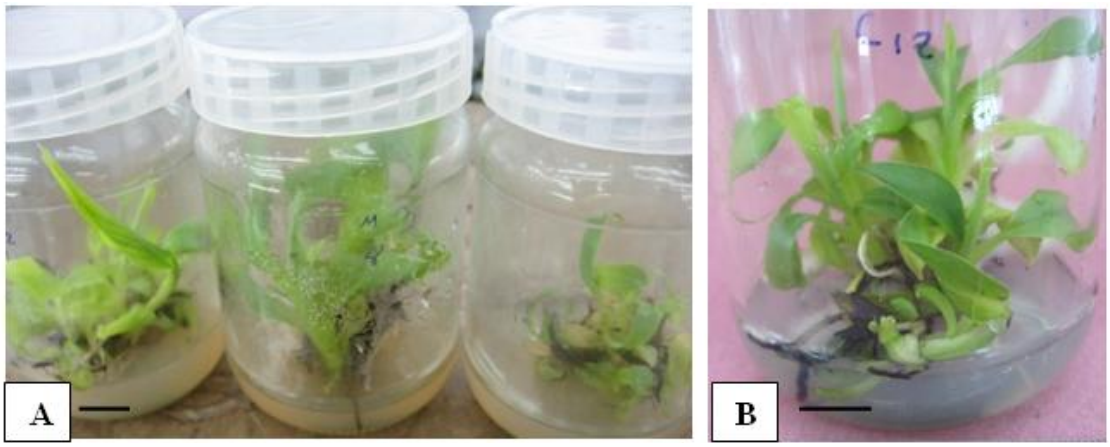

Figure 1: *In vitro* shoot-multiplication of banana A- Sweet-lime juice (5%) **B-** Control (Horizontal Bar = 1 cm)

Sweet corn extract is known to contain cytokinin, namely zeatin, zeatin riboside and C-3 (Letham, 1966) with cell-division activity in various plant species (Miller, 1967). However, shoot-multiplication of banana was found comparatively lower in medium with different levels of Sweet-corn juice (Table 1). A low-cost substitute of hormone, called Pectimorf, derived from citrus fruit rinds, showed high rate of *in vitro* shoot-multiplication and 90 % of survival during acclimatization of *Anthurium cubense* (Montes *et al.*, 2000). Similarly, Pectimorf (10 mg/l) as compared to BA (0.5 mg/l) demonstrated higher multiplication of *Spathiphyllum* (Hernandez *et al.*, 2009).
Similarly, induction of fruit explant cultures of citron (*Citrus medica*), lemon (*C. limon*), grapefruit (*C. paradisi*), sweet orange (*C. sinensis*), and mandarin (*C. reticulata*) was reported with orange juice (10% v/v) in MS basal medium (Einset, 1978; Duran-vila, 1989). Earlier, coconut milk was incorporated in tissue culture media for embryo culture of various plant species (Overbeek *et al.*, 1941 and Caplin and Steward, 1948). Coconut milk contains rebosyl-zeatin, which is very similar to zeatin isolated from young maize endosperm (Letham, 1974). According to modern definition, any substance that promotes growth and cell-division is known as cytokinin (Salisbury and Ross, 1992). In other words, cytokinins have biological activities similar to those of trans-zeatin, including induction of cell division and promotion of bud formation (Tez and Zeiger, 2010).
In addition to nutritional constituents (Table 2), Sweet lime (*Citrus limetta* Risso) contains volatile compounds in its essential oil, namely d-limonene, β-pinene, bergamol, linalool, sabinene, β-myrcene, α-pinene, β-bisabolol, β-bisabolene, α-terpineol, neryl acetate, geranyl acetate, neral, geranial, cis-geraniol, isopinocarveol, citronellal, nonane, Aromadendrene, Epi-β-santalene, α-Terpineol acetate, terpinen-4-ol,

Trans-sabinene hydrate, farnesol, camphene, undecanal, nonanal, α-bisabolol, myrcenil acetate, (Z) sabinene hydrate, octil ester, 1-cyclohexen-1-methanol, 4-1 methylenil acetate, trans-nerolidol, octal cyclopropane, cis-myrtanol, aldehyde peril, β-farnesene, trans-β-santalol, isopropyl palmitate, β-santalene, camphor and α-farnesene (Maria *et al.*, 2012).

Table 1: Effect of natural substitutes and BA on shoot-multiplication of banana in MS medium*

Substitutes (%)		No. of Shoots	Length of Shoot (cm)	No. of Leaves
	3	2.0 + 1.73 b	3.6 ± 0.64 ab	2.8 ± 0.58 b
Sweet-lime juice	5	2.5 ± 0.49 b	1.5 ± 1.13 b	1.0 ± 0.81 a
	10	1.1 ± 1.21 a	0.5 ± 1.25 a	1.0 ± 0.92 a
	3	1.8 ± 0.97 a	3.2 ± 0.24 ab	1.0 ± 1.09 a
Tomato extract	5	1.3 ± 1.31 a	2.6 ± 0.85 b	1.6 ± 1.08 a
	10	1.5 ± 1.34 a	1.5 ± 1.23 b	1.3 ± 0.71 a
	3	1.36 ± 0.47 a	1.90 ± 0.33 b	1.36 ± 0.33 a
Sweet corn juice	5	1.6 ± 0.38 a	2.50 ± 0.23 b	1.60 ± 0.32 a
	10	1.54 ± 0.45 a	1.81 ± 0.33 b	2.09 ± 0.39 b
Control (BA)	4	2.23 ± 0.50 b	0.97 ± 0.19 a	1.76 ± 0.30 a

** Data represent Mean ± SE of 15 replicates recorded after four weeks; Values followed by different letter(s) in a column are significantly different at p≤ 0.05 by Tukey's test*

Table 2: Nutritional value of Sweet lime, Tomato and Sweet corn*

Components (%)	Sweet lime	Tomato	Sweet corn
Carbohydrate	11	Traces	69.3
Sugars	1.7	2.6	3.22
Dietary fibers	3	1.2	2.9
Fat	0.2	0.2	1.18
Protein	0.7	0.9	12.9
Water	83.5	94.5	75.96
Vitamin A	Traces	42 µg	9 µg
Vitamin C	56.60 mg	14 mg	6.8 mg

**Value in percentage or else as mentioned (Anonymous 2013a, b)*

Similarly, tomato extract contains lycopene, phytoene, phytofluene, β-carotene, tocopherols, sterols, various fatty acids and acylglycerols (myristic acid, palmitic acid, stearic acid, oleic acid, linoleic acid, linolenic acid, rachidic acid, behenic acid, free fatty acids), lactic acid, phosphorus, phospholipids, nitrogen with ash (Rath, 2013). However, the stimulatory effect of these fruit juice cannot be attributed to sucrose or organic growth factors already present in the basal medium (Einset, 1978). Morphogenesis is a complex interplay of water potential, pH, nitrate and ammonium ions, autoclaving, osmotic potential, in addition sugars, organic acids, nutritional uptake, enzymatic activities and starch metabolism (George *et al.*, 2008).

Table 3: Cost of natural substitutes of BA

Component	Concentration (%)	Cost/l media (`)
BA	0.004	3.64
Sweet-lime	5	1.68
Sweet corn	5	0.75
Tomato	5	0.50

Culture media are often supplemented with a variety of organic extracts like coconut milk, protein hydrolysates, yeast and malt extract, ground banana, potato extract, orange juice and tomato juice (Razdan, 2003; Molnar *et al.*, 2011). In the present study, Sweet-lime juice (5 %), used as substitute for highly priced synthetic BA was found effective for *in vitro* shoot-multiplication of banana. While both Tomato extract and Sweet corn juice showed comparatively lower shoot-multiplication, when used at different concentrations (Table 1). In conclusion, fresh juice of Sweet-lime (5 %) provided higher rate of multiplication as well as being cost-effective (Table 3) as compared to BA.

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