BIOLOGIA PLANTARUM 50 (4): 782-784, 2006

BRIEF COMMUNICATION

Effects of carbon sources and auxins on *in vitro* propagation of banana

P. MADHULATHA, S.I. KIRUBAKARAN and N. SAKTHIVEL*

Department of Biotechnology, Pondicherry University, Pondicherry 605014, India

Abstract

The effects of carbon sources (sucrose, glucose, fructose and mannitol) and auxins [indolebutyric acid (IBA) and α-naphthaleneacetic acid (NAA)] on *in vitro* propagation of banana (*Musa* spp. AAA) were studied. Over all carbon sources tested, sucrose induced highest frequency of shoot proliferation. Optimal shoot proliferation rates were achieved on the Murashige and Skoog (MS) medium supplemented with sucrose and glucose combination (1:1) at the concentration of 30 g dm^{-3}. Similarly, higher frequency of root induction was obtained at IBA and NAA combination (1:1; concentration of 2 mg dm^{-3}) than at other concentrations of IBA or NAA alone or their combinations.

Additional key words: carbon sources, auxins, banana (*Musa* spp. AAA), *in vitro* propagation.

Banana is clonally propagated because all cultivated bananas are triploid, seedless or seed sterile. Conventional propagation through corms, suckers and sward suckers (Cronauer-Mitra and Krikorian 1984) are not ideal because they carry pathogens, nematodes and viruses and also suffer from slow multiplication, bulkiness, and poor phytosanitory quality (Sagi *et al.* 1998). In recent time, tissue culture propagation of banana has gained attention due to its potential to provide genetically uniform, pest and disease free planting material. Micropropagation of banana through shoot tips have been successfully demonstrated (Vuylsteke and De Langhe 1985, Ganapathi *et al.* 1995) and the influence of liquid pulse treatment with growth regulators on *in vitro* propagation of banana also has been reported (Madhulatha *et al.* 2004). Effects of carbon sources, glucose, sorbitol, fructose, and sucrose, and auxins, α-naphthaleneacetic acid (NAA) and indolebutyric acid (IBA) have been examined in micropropagation of other plants (Harada and Murai 1996, Jain and Babbar 2003/4, Custodio *et al.* 2004). However, no previous report has appeared on the efficacy of media supplemented with different carbon sources as well growth regulators on *in vitro* propagation of banana. This paper examines the effects of sucrose, glucose, fructose and mannitol as well as NAA and IBA on *in vitro* propagation of banana.

To study the influence of carbon sources on multiple shoot proliferation, meristem-tip cultures of banana (*Musa* spp. AAA) cv. Nendran were excised from shoot apexes following standard method described earlier (Ma and Shii 1972). Explants (*ca*. 10 × 10 × 6 mm) were excised from decapitated shoot apexes of suckers. Shoot meristems were isolated following the method of Banerjee and De Langhe (1985). Isolated shoot tips were surface sterilized in ethanol and in 1.5 % calcium hypochlorite solution for 30 s and 20 min, respectively, followed by repeated washing with sterile distilled water. Explants were cultured in tubes (2.5 cm in diameter and 10 cm in height) containing Murashige and Skoog medium (1962; MS) supplemented with sucrose or glucose or fructose or mannitol alone or their combinations (1:1). Explants cultured on media without carbon source served as controls. Cultures were incubated at temperature of 25 ± 2°C, 16-h photoperiod with

Received 1 March 2005, *accepted* 18 April 2005.

Abbreviations: IBA - indolebutyric acid; NAA - α-naphthaleneacetic acid; MS medium - Murashige and Skoog medium.

Acknowledgements: The authors are thankful to the Principal, PKVK, Pondicherry for providing facilities and Mr. Ramamurthy for his technical assistance during the course of this investigation. We also thank Dr. S.R. Prabagaran, CCMB, Hyderabad for his statistical analyses. The financial assistance provided by the Department of Science and Technology (DST), New Delhi to NS is gratefully acknowledged.

* Author for correspondence; fax: (+91) 413 2655211, e-mail: puns2005@gmail.com

irradiance of 50 µmol m^{-2} s^{-1} provided by cool-white fluorescent tubes, and 60 - 65 % relative humidity. After 30 d, number and length of the shoots were recorded.

To determine the influence of auxins on root induction of banana, 60-d old explants of uniform size were transferred to the culture tubes (2.5 cm in diameter and 10 cm in height) containing MS medium supplemented with IBA or NAA alone or IBA and NAA combination (1:1) at various concentrations such as 0.5, 1.0, 1.5, 2.0, 2.5, and 3.0 mg dm^{-3}. Explants cultured on media without growth regulator served as controls. Cultures were incubated at environmental conditions mentioned above.

Experiments were set up in a completely randomized design with 14 replications for each treatment. Data on number and length of shoots per proliferating bud and number, length of roots per explant were recorded after 30 d. Mean and standard error (SE) were calculated and differences between means were tested using Duncan's multiple range test at the level of $P = 0.05$.

Table 1. Effects of carbon sources (sucrose or glucose or fructose or mannose) or their combination (1:1) at a concentration of 30 g dm^{-3} for shoot proliferation of banana cv. Nendran. Means ± SE. Means within the column followed by different letters are significantly different according to Duncan's multiple range test ($P = 0.05$). Data represents the mean of 14 replications with 10 explants per replication.

Carbon source	Number of shoots [explant^{-1}]	Shoot length [cm]
Control	2.10 ± 0.57^{e}	3.10 ± 0.49^{d}
Sucrose	10.90 ± 0.81^{bc}	3.90 ± 0.71^{bc}
Glucose	9.70 ± 0.45^{c}	3.80 ± 0.24^{bc}
Fructose	5.30 ± 0.21^{d}	3.40 ± 0.62^{cd}
Mannitol	5.50 ± 0.78^{d}	3.00 ± 0.91^{d}
Sucrose + glucose	14.20 ± 0.62^{a}	5.20 ± 0.69^{a}
Sucrose + fructose	11.10 ± 0.58^{b}	4.00 ± 0.18^{bc}
Sucrose + mannitol	10.40 ± 0.29^{bc}	4.40 ± 0.82^{b}
Glucose + fructose	8.60 ± 0.72^{c}	3.50 ± 0.87^{cd}
Glucose + mannitol	8.90 ± 0.68^{c}	3.70 ± 0.12^{c}
Fructose + mannitol	4.60 ± 0.39^{de}	3.80 ± 0.76^{bc}

Present study revealed the efficacy of combinations of carbon sources and auxins on *in vitro* propagation of banana cv. Nendran, a leading commercial cultivar in India. Carbon sources are components of the medium as a source of energy and for maintaining the osmotic potential (Sul and Korban 1998, Cuenca and Vieitez 2000). In general, sucrose is commonly used in tissue culture media to induce adventitious shoots or buds. However, there are few reports whereby glucose has been found to be better source of carbon than sucrose (Hsia and Korban 1996, Sul and Korban 1998, Cuenca and Vietiez 2000). Among carbon sources tested, sucrose was found best for multiple shoot proliferation of banana. However, optimal shoot proliferation rates were achieved due on MS media supplemented with sucrose and glucose combination (1:1) at the concentration of 30 g dm^{-3} (Table 1).

Table 2. Effects of auxins, IBA or NAA or their combination (1:1) at the concentration of 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 mg dm^{-3} for root induction of banana cv. Nendran. Means ± SE. Means within the column followed by different letters are significantly different according to Duncan's multiple range test ($P = 0.05$). Data represents the mean of 14 replications with 10 explants per replication.

Auxin	Concentration [mg dm^{-3}]	Number of shoots [explant^{-1}]	Shoot length [cm]
Control	0.0	1.29 ± 0.36^{i}	4.60 ± 0.65^{f}
IBA	0.5	2.40 ± 0.94^{h}	4.70 ± 0.78^{ef}
	1.0	3.20 ± 0.16^{g}	4.80 ± 0.66^{e}
	1.5	4.80 ± 0.34^{e}	4.20 ± 0.49^{g}
	2.0	7.20 ± 0.52^{bc}	4.70 ± 0.22^{ef}
	2.5	6.50 ± 0.12^{c}	4.20 ± 0.17^{g}
	3.0	5.00 ± 0.77^{de}	4.20 ± 0.13^{g}
NAA	0.5	3.10 ± 0.22^{g}	4.10 ± 0.92^{g}
	1.0	4.00 ± 0.71^{f}	4.40 ± 0.33^{eg}
	1.5	4.10 ± 0.42^{f}	5.50 ± 0.19^{cd}
	2.0	6.70 ± 0.69^{bc}	6.10 ± 0.81^{b}
	2.5	6.50 ± 0.23^{c}	5.80 ± 0.76^{bc}
	3.0	5.80 ± 0.27^{d}	5.70 ± 0.21^{c}
NAA+IBA	0.5	4.60 ± 0.13^{ef}	5.40 ± 0.27^{d}
	1.0	4.90 ± 0.51^{de}	6.00 ± 0.59^{bc}
	1.5	6.20 ± 0.29^{cd}	6.20 ± 0.74^{ab}
	2.0	10.80 ± 0.78^{a}	6.50 ± 0.76^{a}
	2.5	7.60 ± 0.34^{b}	6.20 ± 0.21^{ab}
	3.0	6.90 ± 0.21^{bc}	5.90 ± 0.49^{bc}

Auxins have been found to influence the cell enlargement, root initiation and suppress lateral buds (Cronauer-Mitra and Kirikorian 1984, Jarret *et al.* 1985). The most efficient root induction was produced on the media supplemented with IBA and NAA combination (1:1) at the concentration of 2.0 mg dm^{-3} compared to the media supplemented with other concentrations (0.5, 1.0, 1.5, 2.5 and 3.0 mg dm^{-3}) of IBA or NAA alone or their combinations (Table 2). Data generated in this study demonstrate the usefulness of combination of carbon sources and auxins for efficient *in vitro* propagation of banana.

References

Banerjee, N., De Langhe, E.: A tissue culture technique for rapid clonal propagation and storage under minimal growth conditions of *Musa* (banana and plantain). - Plant Cell Rep. **4**: 351-354, 1985.

Cronauer-Mitra, S.S., Krikorian, A.D.: Multiplication of *Musa* from excised stem tips. - Ann. Bot. **53**: 321-328, 1984.

Cuenca, B., Vieitez, A.M.: Influence of carbon source on shoot multiplication and adventitious bud regeneration in *in vitro* beech culture. - Plant Growth Regul. **32**: 1-12, 2000.

Custodio, L., Martins-Laucao, M.A., Romano, A.: Influence of sugars on *in vitro* rooting and acclimatization of carob tree. - Biol. Plant. **48**: 469-472, 2004.

Ganapathi, T.R., Mohan, J.S.S., Suprasanna, P., Bapat, V.A., Rao, P.S.: A low-cost strategy for *in vitro* propagation of banana. - Curr. Sci. **68**: 646-649, 1995.

Harada, H., Murai, Y.: Micropropagation of *Prunus mume*. - Plant Cell Tissue Organ Cult. **46**: 265-267, 1996.

Hsia, C.N., Korban, S.S.: Organogenesis and somatic embryogenesis in callus cultures of *Rosa hybrida* and *Rosa chinensis minima*. - Plant Cell Tissue Organ Cult. **44**: 1-6, 1996.

Jain, N., Babbar, S.B.: Effect of carbon source on the shoot proliferation potential of epicotyl explants of *Syzygium cuminii*. - Biol. Plant. **47**: 133-136, 2003/4.

Jarret, R.L., Fisher, J.B., Litz, R.E.: Organ formation in *Musa* tissue cultures. - Plant Physiol. **121**:123-130, 1985.

Ma, S.S., Shii, C.T.: *In vitro* formation of adventitious buds in banana shoot apex following decapitation. - J. chin. hort. Sci. **18**: 35-142, 1972.

Madhulatha, P., Anbalagan, M., Jayachandran, S., Sakthivel, N.: Influence of liquid pulse treatment with growth regulators on *in vitro* propagation of banana (*Musa* spp. AAA). - Plant Cell Tissue Organ Cult. **76**:189-192, 2004.

Murashige, T., Skoog, F.: A revised method for rapid growth and bioassays with tissue cultures. - Physiol. Plant **15**: 473-497, 1962.

Sagi, L., Gregory, D.M., Remy, S., Swennen, R.: Recent developments in biotechnological research on bananas (*Musa* spp.). - Biotechnol. genet. Rev. **15**: 313-317, 1998.

Sul, I.W., Korban, S.S.: Effects of media, carbon sources, and cytokinins on shoot organogenesis in the christmas tree *Pinus sylvestris* L. - J. hort. Sci. Biotechnol. **73**: 822-827, 1998.

Vuylsteke, D., De Langhe, E.: Feasibility of *in vitro* propagation of bananas and plantains. - Trop. Agr. **62**: 323-328, 1985.