BIOLOGIA PLANTARUM 47 (1): 133-136, 2003/4

BRIEF COMMUNICATION

Effect of carbon source on the shoot proliferation potential of epicotyl explants of *Syzygium cuminii*

N. JAIN* and S.B. BABBAR

Department of Botany, University of Delhi, Delhi-110007, India

Abstract

Black plum (*Syzygium cuminii*) explants were grown *in vitro* on Murashige and Skoog medium. Among the various saccharides tested, the best caulogenic response was afforded by sucrose both in terms of explant response and shoot developing potential. Within monosaccharides, mannose was totally inhibitory as on the medium supplemented with this the shoot buds failed to develop, while, fructose and xylose completely inhibited the opening as well as the elongation of shoot buds. Glucose and galactose did not completely inhibit the caulogenic response. Among disaccharides, other than sucrose, maltose totally inhibited the elongation of the developed shoot buds while lactose supported it to a limited extent. Sugar alcohols, though not as good as sucrose, proved better sources of carbon and energy than the other tested sugars. Sucrose at concentration 4 % proved to be the best in developing 4.2 shoots per explant.

Additional key words: black plum, caulogenesis, fructose, galactose, glucose, lactose, maltose, mannitol, mannose, *Myrtaceae*, sorbitol, sucrose, xylose.

A sugar, generally sucrose, is an indispensable ingredient of all culture media, as the photosynthetic ability of the cultured tissues is limited because of low irradiance and limited gas exchange (Kozai 1991). Besides this, it is also required as an osmotic agent (Thorpe 1985). Being easily translocatable and resistant to enzymatic degradation due to its non-reducing nature, sucrose has been the sugar of choice for most of the studies (Pontis 1978). Sugars, mainly sucrose, facilitates the deposition of cellulose as well as lignin in the lattices of the cellulosic microfibrils (for review see Thorpe 1985). Besides, this sucrose plays a vital role in controlling various enzymatic processes as well as hormonal action (Pontis 1978). Despite the wide use of sucrose, other sugars have also been reported to be suitable carbon sources for tissue culture. In the present study an attempt has been made to assess the effect of quantity and quality of sucrose on the shoot proliferation capacity of the epicotyl explants of *S. cuminii*. Besides this, experiment was also conducted to see if sucrose could be satisfactorily replaced by any other sugar.

Seedlings of *Syzygium cuminii* (L.) Skeels, 7 - 8 cm long, raised aseptically on Knop medium, were used as the source of epicotyl explants. The responding cultures were obtained by planting the epicotyl explants on Murashige and Skoog medium (1962; MS) supplemented with 1 mg dm^{-3} 6-benzyladenine (BA; shoot multiplication medium; Jain and Babbar 2000). To study the effect of quantity and quality of sucrose, the shoot multiplication media gelled with 0.9 % bacteriological grade agar were supplemented with different concentrations of sucrose (2, 3, 4, 5 or 6 %) as well as different sucrose sources, viz. ExcelaR grade (*Qualigens, Glaxo Fine Chemicals*, Mumbai, India), AR grade (*SRL*, Mumbai, India), sugar cubes (*Daurala*, Meerut, India) and table sugar (*Mawana*, Meerut, India). An experiment was also conducted to see if sucrose could be satisfactorily replaced by any other sugar. A wide range of sugars were tried to assess their suitability, which includes monosaccharides (galactose, glucose, fructose, mannose and xylose), disaccharides (lactose, maltose and

Received 9 May 2002, *accepted* 24 June 2002.
Acknowledgements: The financial assistance provided by the University Grants Commission (UGC), New Delhi to SBB as a research scheme and to NJ as a Senior Research Fellowship is gratefully acknowledged.
* Corresponding author present address: Department of Biological Regulation, The Weizmann Institute of Sciences, P.O. Box 26, IL-76100, Rehovot, Israel; e-mail: neeru.jain@weizmann.ac.il

sucrose), sugar alcohols (sorbitol and mannitol) and trialcohol (glycerol). Each of these were supplemented in equimolar concentration (0.12 M, *i.e.* 4 % sucrose). pH of the media was adjusted to 5.8 before autoclaving. The media were dispensed in glass tubes (25 × 150 mm, *Borosil*, Mumbai, India) plugged with cotton plugs (non-adsorbent cotton wrapped in two layers of cheese cloth). The cultures were incubated at 25 ± 2°C and exposed to continuous light (60 μmol m^{-2} s^{-1}) provided by cool daylight fluorescent tubes (40 W, *Philips*, Calcutta, India).

The response has been expressed in terms of percentage of responding explants, average number of shoots per explant and average length of shoots. The data were subjected to analysis of variance (*ANOVA*) by Fischer's least significant difference (P = 0.05). Each experiment was repeated at least once maintaining 48 explants each time.

Sucrose is mainly used at 2 - 5 % concentration, as it modifies the osmotic strength of the medium (Thompson and Thorpe 1987). Initial increase in concentration of sugar increases the availability of carbon source, thus stimulating the response, while at the higher concentrations the osmotic potential declines, thus reducing the response (Thompson and Thorpe 1987, Short *et al.* 1987). To assess the optimum concentration of sucrose for BA induced shoot multiplication and their subsequent development, the epicotyl explants were cultivated on MS medium containing 1 mg dm^{-3} BA along with different concentrations (0, 2, 3, 4, 5 or 6 %) of sucrose.

Table 1. Effect of different concentrations of sucrose on caulogenic response of the epicotyl explants. Means ± standard errors. Values followed by the same letter are not significantly different at 5 % level (F-test).

Sucrose [%]	Number of explants	Explant response [%]	Number of shoots [explant^{-1}]	Shoot length [cm]
0	48	0^b	0^d	0^c
2	40	60.0^a	0^d	0^c
3	42	83.3^a	2.26^c	0.36 ± 0.32^b
4	38	84.2^a	4.18^a	1.05 ± 0.50^a
5	38	73.7^a	3.18^b	0.44 ± 0.53^b
6	40	80.0^a	0^d	0^c

In the absence of sucrose, none of the explants developed shoot buds. The induction of shoot buds took place on all the media containing 2 % or higher concentrations of sucrose. However, at 2 and 6 % shoot buds failed to develop into shoots. Among the other concentrations, 4 % supported the best caulogenic response (Table 1). Similarly, in *Prunus* low concentrations (1 - 2 %) proved to be inhibitory (Morini *et al.* 1992), while high concentrations (6 - 10 %) were detrimental in *Albizzia* (Tomar and Gupta 1988).

After ascertaining the optimal oncentration of sucrose for shoot multiplication, various sources of sucrose differing in their quality were tried at the same concentrations, *i.e,* 4 %. In all the four cases, shoot buds were induced. However, on medium containing sugar cubes, these failed to elongate. The explant response was not significantly different in all the four cases. However, explant productivity (4.21 shoots explant^{-1}) and average shoot length (1.10 ± 0.27 cm) were best on the medium supplemented with ExcelaR grade sucrose (Table 2). As for the quality, the epicotyl explants of *S. cuminii* exhibited the high caulogenic response on the medium supplemented with ExcalaR grade sucrose unlike the explants of *Leucaena leucocephala*, which responded well on the medium containing sugar cubes (Dhawan and Bhojwani 1984).

Table 2. Effect of sucrose (4 %) quality on caulogenic response of the epicotyl explants. Means ± SE. Values followed by the same letter are not significantly different at 5 % level (F-test).

Sucrose quality	Number of explants	Explant response [%]	Number of shoots [explant^{-1}]	Shoot length [cm]
Excela R grade	40	95.0^a	4.21^a	1.10 ± 0.27^a
AR grade	40	80.0^a	2.72^b	0.96 ± 0.28^{ab}
Sugar cubes	38	81.6^a	0^c	0^c
Table sugar	40	67.5^a	2.59^b	0.71 ± 0.34^b

The best caulogenic response was afforded by sucrose among the various sugars tested (Table 3). Among monosaccharides, mannose was totally inhibitory as on this medium not even shoot buds were developed, while, fructose and xylose completely inhibited further development of shoot buds. Earlier reports demonstrate a variable effect of fructose as it was promotory for cultures of *Morus* (Oka and Ohyama 1986), while was inhibitory for *Syringa* (Welander *et al.* 1989).

Glucose and galactose did not completely inhibit the caulogenic response of *S. cuminii*. However, on medium containing either of these sugars, the response was considerably lower than that obtained on sucrose-containing medium. Contrary to the present observations, earlier reports indicate a promotory effect of glucose in *Alnus* (Tremblay and Lalonde 1984) and *Quercus* (Romano *et al.* 1995). Galactose supported the development of shoots of *S. cuminii*, to a limited extent. However, in some cases, as in embryogenic response of *Citrus* cell culture, galactose proved to be the best carbon source (Cabasson *et al.* 1995). Among disaccharides, other than sucrose, maltose totally inhibited further development of the shoot buds while lactose supported it to a limited extent. Sucrose also increased the size of

leaves of *in vitro* regenerated shoots of *Ceratonia siliqua*, in comparison to shoots regenerated on medium containing fructose or glucose (Vinterhalter *et al.* 2001). However, maltose which proved inhibitory for shoot development in this case, proved beneficial for shoot regeneration from calli in *Humulus lupulus* (Smýkalová *et al.* 2001). Sugar alcohols, though not as good as sucrose, were better sources of carbon and energy than the other tested sugars. Earlier, sugar alcohols have proved to the most effective carbon sources for shoot

Table 3. Caulogenic response of the epicotyl explants cultivated on medium containing different carbon sources. Means ± SE. Values followed by the same letter are not significantly different at 5 % level (*F*-test).

Carbon source		Number of explants	Explant response [%]	Number of shoots [explant^{-1}]	Shoot length [cm]
Monosaccharides	galactose	40	70.0^{a}	1.21^{bc}	0.66 ± 0.34^{a}
	glucose	38	76.3^{a}	1.17^{c}	0.81 ± 0.45^{a}
	fructose	40	72.5^{a}	0^{e}	0^{b}
	mannose	34	0^{b}	0^{e}	0^{b}
	xylose	38	71.1^{a}	0.26^{d}	0.31 ± 0.08^{a}
Disaccharides	lactose	40	65.0^{a}	0.81^{c}	0.52 ± 0.23^{a}
	maltose	40	62.5^{a}	0^{e}	0^{b}
	sucrose	42	80.9^{a}	4.12^{a}	1.02 ± 0.28^{a}
Sugar alcohols	mannitol	40	75.0^{a}	0.93^{bc}	0.62 ± 0.35^{a}
	sorbitol	38	63.1^{a}	1.75^{b}	0.65 ± 0.39^{a}
Trialcohol	glycerol	34	61.7^{a}	1.62^{b}	0.66 ± 0.30^{a}

proliferation by nodal segments of *Prunus armeniaca* (Marino *et al.* 1989, 1993), *Malus robustus* (Pua and Chong 1989) and *Malus pumila* (Welander *et al.* 1989).

The differential morphogenic response by the plants to various sugars could be probably due to *1*) their differential role in vascular differentiation compared to sucrose, *2*) differences in the endogenous content of reducing sugar in cultured tissue (Romano *et al.* 1995), and *3*) differential sensitivity of the tissues to the breakdown products such as furfural and hydroxy-furfurol, formed during autoclaving (Hsiao and Bornman 1989).

References

Cabasson, C., Ollitrault, P., Côte, F.X., Nicole, M.F., Dominique, D., Dalnic, R., Tesson, C.: Characteristics of *Citrus* cell cultures during undifferentiated growth on sucrose and somatic embryogenesis on galactose. - Physiol. Plant. **93**: 464-470, 1995.

Dhawan, V., Bhojwani, S.S.: Reduction of cost of tissue culture of *Leucaena leucocephala* (Lam.) De Wit. by replacing AR sucrose by sugar cubes. - Curr. Sci. **51**: 1159-1161, 1984.

Hsiao, K.C., Bornman, C.H.: Cyanide initiated oxygen consumption in autoclaved culture media containing sugars. - Plant Cell Rep. **8**: 90-92, 1989.

Jain, N., Babbar, S.B.: Recurrent production of plants of black plum, *Syzygium cuminii* (L.) Skeels, a myrtaceous fruit tree, from *in vitro* cultured seedling explants. - Plant Cell Rep. **19**: 519-524, 2000.

Kozai, T.: Micropropagation under photoautotrophic conditions. - In: Debergh, P.C., Zimmerman, R.H. (ed.): Micropropagation – Technology and Application. Pp. 447-469. Kluwer Academic Publishers, Dordrecht 1991.

Marino, G., Bertazza, G., Margnanini, E., Altan, A.D.: Comparative effects of sorbitol and sucrose as main carbon energy sources in micropropagation of apricot. - Plant Cell Tissue Organ Cult. **34**: 235-244, 1993.

Marino, G., Margnanini, E., Battistini, S., Rishetti, D.: Effect of hormones and main carbon energy sources on *in vitro* propagation of apricot (*Prunus armeniaca* L.) cvs. 'SanCastrese' and 'Portici'. - Acta Hort. **293**: 355-362, 1989.

Morini, S., Sciutti, R., Fortuna, P.: *In vitro* growth responses of *Prunus cressifera* shoots as influenced by different light-dark cycles and sucrose concentrations. - Plant Cell Tissue Organ Cult. **28**: 245-248, 1992.

Murashige, T., Skoog, F.: A revised medium for rapid growth and bioassay with tobacco tissue cultures. - Physiol. Plant. **15**: 473-479, 1962.

Oka, S., Ohayama, K.: Mulberry (*Morus alba* L.). - In: Bajaj, Y.P.S. (ed.): Biotechnology in Agriculture and Forestry. Vol. 1. Trees I. Pp. 384-392. Springer-Verlag, Berlin 1986.

Pontis, H.G.: On the scent of riddle of sucrose. - TIBS **6**: 137-139, 1978.

Pua, E.C., Chong, E.B.: Regulation of *in vitro* shoot and root regeneration in 'Macspur' apple by sorbitol (D-glucitol) and related carbon sources. - J. amer. Soc. hort. Sci. **110**: 705-709, 1989.

Romano, A., Nornha, C., Martinus-Loção, M.A.: Role of carbohydrates in micropropagation of cork oak. - Plant Cell Tissue Organ Cult. **40**: 159-167, 1995.

Short, K., Warburton, J., Robert, A.: *In vitro* hardening of cultured cauliflower and chrysanthemum plantlets to humidity. - Acta Hort. **212**: 329-334, 1987.

Smýkalová, I., Ortová, M., Lipavská, H., Patzak, J.: Efficient

in vitro micropropagation and regeneration of *Humulus lupulus* on low sugar, starch-*Gelrite* media. - Biol. Plant. **44**: 7-12, 2001.

Thompson, M., Thorpe, T.: Metabolic and non-metabolic roles of carbohydrates. - In: Bonga, R.M., Durzan, D.J. (ed.): Cell and Tissue Culture in Forestry. Vol. 1: General Principles and Biotechnology. Pp. 89-112. Martinus-Nijhoff Publishers, Dordrecht 1987.

Thorpe, T.A.: Carbohydrate utilization and metabolism. - In: Bonga, J.M., Durzan, D.J. (ed.): Tissue Culture in Forestry. Pp. 325-368. Martinus Nijhoff Publishers, Dordrecht 1985.

Tomar, U.K., Gupta, S.C.: *In vitro* plant regeneration of leguminous tree (*Albizzia* spp.). - Plant Cell Rep. **7**: 385-388, 1988.

Tremblay, F.M., Lalonde, M.: Requirements for *in vitro* propagation of seven nitrogen fixing *Alnus* species. - Plant Cell Tissue Organ Cult. **3**: 189-199. 1984.

Vinterhalter, B., Vinterhalter, D., Nešković, M.: Effect of irradiance, sugars and nitrogen on leaf size of *in vitro* grown *Ceratonia siliqua* L. - Biol. Plant. **44**: 185-188, 2001

Welander, M., Welander, N.T., Brackman, A.S.: Regulation of *in vitro* shoot multiplication in *Syringa, Alnus* and *Malus* by different carbon sources. - J. hort. Sci. **64**: 361-366, 1989.