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Effect of Carbon Sources in *In Vitro* Morphogenesis from Rhizomes of *Cymbidium sinense*

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Response of the rhizomes of *Cymbidium sinense* to four carbohydrates, sucrose, glucose, fructose and lactose, was compared to determine the most suitable carbon source for the efficient *in vitro* multiplication. Lactose had negative effect on morphogenesis. Sucrose was less effective on plantlet development than glucose and fructose. Glucose at the concentration of 5% was considered to be the best carbon source that may bring the maximum number of plantlets.

INTRODUCTION

Explants in *in vitro* culture require exogenous carbon as energy source for their growth and differentiation because they do not have complete autotrophism. Although sucrose is the most generally used carbohydrate with the concentration of 2–3%, other carbohydrates are also used. There are some reports available that compared the response to carbohydrates in growth and differentiation in some plant species. For example, Fukai (1986) showed that the callus weight was most increased with maltose, but shoot differentiation occurred only with sucrose, glucose or fructose among 10 carbon sources examined in callus and organ formation from leaf segments of chrysanthemum (*Dendranthema grandiflorum* Kitamura).

Many attempts were given in micropropagation of terrestrial *Cymbidium* species. Shoot formation from rhizomes by using various plant hormones has been studied in *C. goeringii* (Ueda and Torikata, 1969; Hasegawa, 1987), *C. faberi* (Hasegawa *et al.*, 1985), *C. kanran* (Shimasaki and Uemoto, 1990) and *C. forrestii* (Paek and Yeung, 1991). Lowering the ammonium and potassium nitrate concentrations in Murashige and Skoog (MS) medium (Murashige and Skoog, 1962) was also shown to be effective in promotion of shoot formation in *C. kanran* (Shimasaki and Uemoto, 1990; Ogura and Okubo, 2003), *C. ensifolium* (Ogura and Okubo, 2003) and *C. sinense* (Huang and Okubo, 2005). Promotion of shoot formation by ethylene inhibitors, aminoethoxyvinyl glycine (AVG) or silver thiosulfate (STS), in MS medium was reported in rhizome cultures of *C. kanran* (Shimasaki, 1992, Ogura 2003), *C. ensifolium* (Ogura, 2003) and *C. sinense* (Ogura, 2003).

Hirai *et al.* (1991) studied the effects of sugars on nutrient absorption and growth of epithytic *Cymbidium* protoccorm-like bodies (PLBs) through which new shoots were

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obtained. However, no carbon sources in the culture medium have been yet studied in micropropagation of terrestrial *Cymbidium* species. In this study we compared the response of the rhizomes of *C. sinense* to four carbohydrates to determine the most suitable carbon source for the efficient *in vitro* multiplication.

MATERIALS AND METHODS

Plant materials and culture

Apices of the rhizomes, 0.5 cm in length, were prepared aseptically from one or two year old and seed-derived plants of *C. sinense* cv. Shue Pai Tsoa, and have been cultured *in vitro* in Horticultural Science, National Chiayi University, Chiayi, Taiwan. Well-grown and branched rhizomes of the cultures were cut into 1.5 cm long segments and used for the experiments.

All cultures were incubated on MS medium (pH 5.5–5.7, before autoclaving at 121 °C for 15 minutes) solidified with 2.7 gl^{-1} Gelrite and supplemented with 1 mgl^{-1} naphthyl acetic acid (NAA) + 5 mgl^{-1} N^6-benzyladenine (BA) or with 5 mgl^{-1} NAA + 1 mgl^{-1} BA. Seven explants were cultured in one 500 ml-bottle containing 50 ml medium. Each treatment consisted of six explants with five replicates. The cultures were incubated at 25±2 °C in 16 h daylength (28–36 $\mu molsec^{-1}m^{-2}$).

Experiments

One, 3 or 5% sucrose, glucose, fructose or lactose was added to the MS medium before autoclaving. Measurement took place after 90 days of culture.

RESULTS AND DISCUSSION

Culture with 1 mgl^{-1} NAA + 5 mgl^{-1} BA

There were no significant differences in number of rhizomes (0 to 0.5) by sugars and their concentrations (Table 1). Higher number of buds (>3) was obtained with 1 and 3% glucose and 1% fructose. Similarly, shoots developed well in these concentrations of the sugars as well as in 3% fructose and 3% sucrose. The highest frequency of shoot development with 3% fructose did not lead to the highest number of plantlet. There is, however, still the possibility that the shoots may develop to plantlets later. No shoot was obtained with lactose. Glucose at 5% brought the highest numbers of roots and plantlets. Explant browning during culture least occurred with glucose. Figure 1 shows the shoot development with 3% sucrose (A), 5% glucose (C) and 5% fructose (E).

It is considered that 5% glucose is the best carbon sources among four sugars in the rhizome culture on MS medium with 1 mgl^{-1} NAA + 5 mgl^{-1} BA.

Culture with 5 mgl^{-1} NAA + 1 mgl^{-1} BA

Quite similar results were obtained with different concentrations of NAA and BA in the MS medium (Table 2). The highest frequency of plantlet formation was obtained also with 5% glucose. Lactose was ineffective. Shoot development with 3% sucrose (Fig. 1B), 5% glucose (Fig. 1D) and 5% fructose (Fig. 1F) is shown.

From the results of the two experiments by changing the concentration of NAA and

Table 1. Effects of carbon sources in MS medium[z] on morphogenesis from rhizomes of *Cymbidium sinense*.

Carbon source	Conc. (%)	No. of rhizomes	No. of buds	No. of shoots	No. of roots	No. of plantlets	Browning (%)
None		0.3 a[y]	2.4 bcd	0.1 b	0	0	7
Sucrose	1	0.3 a	1.7 cde	0	0	0.1 d	21
	3	0.1 a	2.6 bc	0.6 a	0.5 c	0.4 cd	0
	5	0	1.8 cde	0.1 b	2.2 b	1.5 b	21
Glucose	1	0	4.8 a	0.7 a	0.3 c	0.2 cd	0
	3	0.1 a	3.6 ab	0.5 ab	2.1 b	1.2 b	0
	5	0	2.4 bcd	0.1 b	4.1 a	2.9 a	0
Fructose	1	0	3.7 ab	0.5 ab	0.5 c	0.3 cd	0
	3	0.3 a	2.5 bc	0.8 a	2.1 b	1.5 b	0
	5	0.5 a	1.7 cde	0	2.5 b	1.0 bc	29
Lactose	1	0	3.0 bc	0	0	0	0
	3	0.2 a	0.7 de	0	0	0	7
	5	0.1 a	0.4 e	0	0	0	7

[z] MS medium was supplemented with 1 mgl^{-1} NAA and 5 mgl^{-1} BA.
[y] Values followed by different letters are significantly different at 5% level.

Table 2. Effects of carbon sources in MS medium[z] on morphogenesis from rhizomes of *Cymbidium sinense*.

Carbon source	Conc. (%)	No. of rhizomes	No. of buds	No. of shoots	No. of roots	No. of plantlets	Browning (%)
None		0	1.3 def	0.2 ab	0	0	14
Sucrose	1	0.1 a[y]	2.2 cde	0	0.7 c	0.7 cd	0
	3	0.1 a	3.9 b	0.1 b	2.1 bc	1.3 bc	0
	5	0.1 a	2.4 bc	0	1.4 c	1.0 bc	14
Glucose	1	0	6.7 a	0.2 ab	0.9 c	0.7 cd	0
	3	0.4 a	1.5 cdef	0	4.1 ab	1.7 ab	0
	5	0.4 a	1.7 cdef	0.1 b	5.4 a	2.3 a	14
Fructose	1	0.2 a	2.9 bc	0.2 ab	1.4 c	1.3 bc	0
	3	0.4 a	1.9 cdef	0.5 a	4.8 a	1.3 bc	21
	5	0.4 a	0.9 def	0.1 b	0	0	56
Lactose	1	0	1.9 cdef	0	0	0	29
	3	0.2 a	0.7 ef	0	0	0	7
	5	0	0.5 f	0	0	0	36

[z] MS medium was supplemented with 5 mgl^{-1} NAA and 1 mgl^{-1} BA.
[y] Values followed by different letters are significantly different at 5% level.

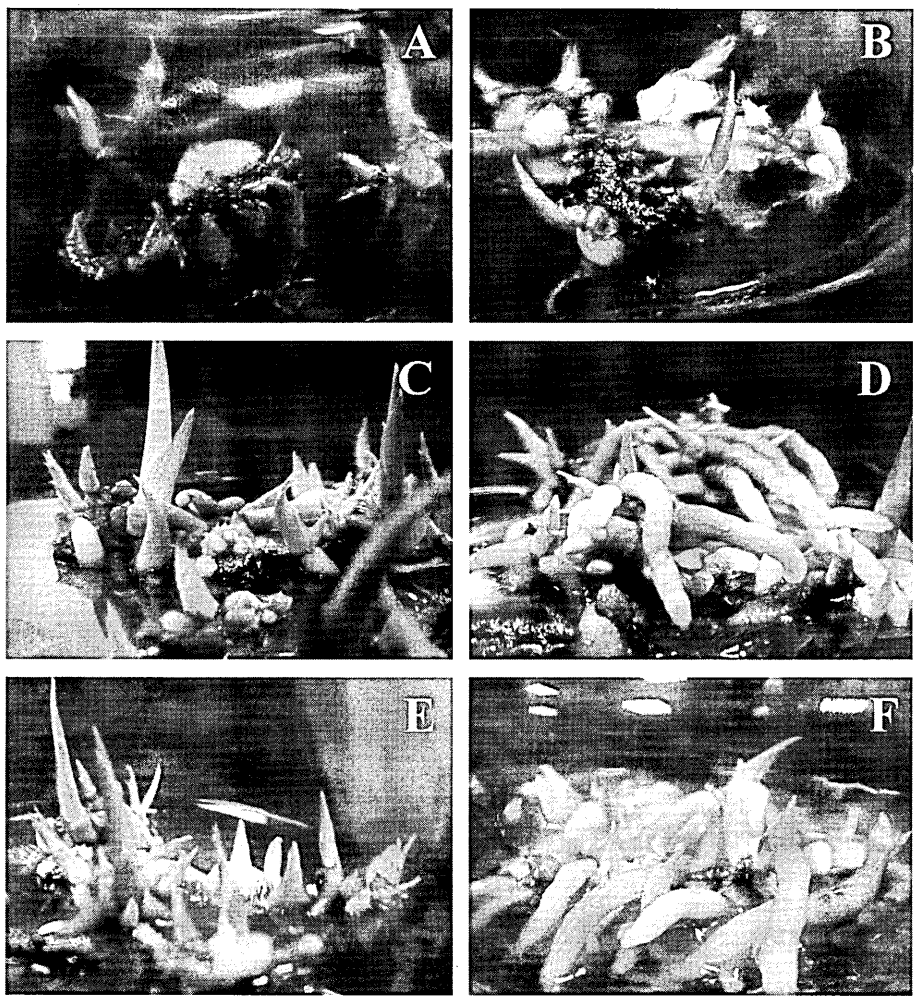

Fig. 1. Morphogenesis from the rhizomes of *Cymbidium sinense* cultured with various carbohydrates. A, B; 3% sucrose, C, D; 5% glucose, E, F; 5% fructose. A, C, E; MS medium supplemented with 1 mgl^{-1} NAA and 5 mgl^{-1} BA. B, D, F; MS medium supplemented with 5 mgl^{-1} NAA and 1 mgl^{-1} BA.

BA, it is clear that in the rhizome culture of *C. sinensis* glucose is the best carbon source among other sugars examined.

Sucrose was dehydrolyzed into its component sugars, glucose and fructose, and these mono–saccharides are absorbed during the multiplication process of *Cymbidium* PLBs (Hirai *et al.*, 1990). About 10% of the sucrose in the agar medium was hydrolyzed during autoclaving (Bach *et al.*, 1992). In the culture of hyacinth explants at 23/20 °C (day/night) initial amount of sucrose, glucose and fructose in the medium decreased to 26.9, 15.9 and 45.2%, respectively 12 weeks of culture (Bach *et al.*, 1992), indicating that

the glucose was most absorbed and utilized during the culture. These reports suggest the advantage of the use of monosaccharide. However, it is hard to explain the difference between glucose and fructose in their effectiveness although they are both monosaccharides.

Lactose is a disaccharide of fructose and galactose, and galactose is generally toxic to cultured plant tissues, although, for example, the adaptation of Japanese morning glory (*Pharbitis nil* L.) cells to lactose was reported (Hisajima *et al.*, 1985).

There are some other reports on carbon sources. Sucrose was more effective carbon source on the growth of Japanese morning-glory callus than glucose and fructose (Hisajima and Thorpe, 1985). Glucose- and sucrose-containing medium was superior to fructose-containing medium in induction of shoots and bulblets in *Hyacinthus orientalis* (Bach *et al.*, 1992). Sucrose, glucose and fructose were more suitable for adventitious bud formation in the culture of immature mulberry leaves than other sugars including maltose, lactose, galactose, xylose, mannose, sorbitol and mannitol (Yamanouchi *et al.*, 1999), where there were not significant differences among the three sugars. Multiple shoots obtained from the shoot apices of three-year-old *Shorea* died with sucrose in subcultures, whereas they were alive for two years with the mixture of glucose and maltose in the medium (Nakamura *et al.*, 1999). It is possible that the effects of carbon sources are plant species dependent.

In conclusion, glucose at the concentration of 5% may bring the maximum number of plantlets of *C. sinense* in the rhizome culture.

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