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BRIEF COMMUNICATION

Promotion of direct somatic embryogenesis of *Oncidium* by adjusting carbon sources

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Abstract

To further optimize a culture medium for induction of direct embryo formation of *Oncidium* cvs. Gower Ramsey and Sweet Sugar, five kinds of carbon sources, cellibiose, fructose, glucose, maltose and sucrose at 10, 20, 30 and 60 g dm^{-3} were tested in this study. Cellibiose supply had an inhibitory effect and resulted in high percentage of explant browning in both cultivars. By contrast, fructose, glucose and sucrose were all effective for direct embryo induction. In cv. Gower Ramsey, the suitable ranges of concentration were found at 30 - 60 g dm^{-3} of sucrose, 10 - 20 g dm^{-3} of glucose and 20 - 30 g dm^{-3} of fructose, respectively. The suitable ranges for cv. Sweet Sugar were at 20 - 60 g dm^{-3} of sucrose, 10 - 30 g dm^{-3} of glucose, 10 - 20 g dm^{-3} of fructose and 30 - 60 g dm^{-3} of maltose, respectively. The highest amount of embryos was obtained at 30 g dm^{-3} of sucrose for cv. Gower Ramsey and at 20 g dm^{-3} of glucose for cv. Sweet Sugar.

Additional key words: embryogenic response, leaf explant, orchid.

Oncidium is a popular orchid genus with high economical value in flower market worldwide. For the purposes of propagation and breeding, many of *in vitro* culture methods had been developed (Arditti and Ernst 1993). Plant regeneration through direct somatic embryogenesis of *Oncidium* was established using young leaves as explants (Chen *et al*. 1999). The requirement of this leaf culture system is simple and the regeneration process is efficient and easily to be recognized. Thus, this is a suitable model system to study the physiology of direct somatic embryogenesis. Indeed, in previous reports, we had studied the effects of growth regulators (auxins, cytokinins, gibberelins, growth retardants, polar auxin transport inhibitors, and ethylene inhibitors), explant length, explant orientation and medium additives (NaH_2PO_4, nitrogen sources) on direct somatic embryogenesis (Chen *et al*. 1999, Chen and Chang 2001, 2002, 2003a, 2003b, 2004). Jheng *et al*. (2006) reported that regeneration efficiency from callus cultures of *Oncidium* Gower Ramsey was affected by carbon sources. Therefore, this paper attempts to further optimize the amount of embryo production of *Oncidium*, by testing the effects of five sugars on direct somatic embryogenesis.

In vitro grown plantlets derived from leaf somatic embryos of *Oncidium* cv. Gower Ramsey and cv. Sweet Sugar were maintained on hormone-free 1/2 Murashige and Skoog (1962; MS) medium with a 2-month-interval of subculture as described by Chen *et al.* (1999). These plantlets were used as donor plants. Tips (about 1 cm long) taken from young leaves of donor plants were used as explants to induce direct somatic embryogenesis (Chen *et al.* 1999). Plant growth regulators were sterilized by filtration using 0.2 μm porosity filters (*Gelman Sciences*, USA), and added to 1/2 MS medium. Explants were placed adaxial-side-up on the surface of culture media supplemented with 1 mg dm^{-3} of 1-phenyl-3-(1,2,3-thiadiazol-5-yl)-urea (TDZ) and incubated in Petri dishes under a 16-h photoperiod with irradiance of 28 - 36 μmol m^{-2} s^{-1} (daylight fluorescent tubes *FL-30D/29*, 40 W, *China Electric Co*., Taipei, Taiwan) and temperature of 26 ± 1 °C. Two independent experiments were performed to evaluate the effects of carbon sources of media including cellibiose, fructose, glucose, maltose and sucrose at concentrations 10, 20, 30 and 60 g dm^{-3} on direct embryo formation from leaf explants of cv. Gower Ramsey and cv. Sweet Sugar. The frequency of embryo-forming explants and the mean number of embryos per explant were determined for each trial. Four explants were placed in each dish as one replicate. Five replicates were established for each treatment. The percentage of

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Abbreviations: GA_3 - gibberellic acid; TDZ - 1-phenyl-3-(1,2,3-thiadiazol-5-yl)-urea
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explants forming somatic embryos was recorded as formed from entire explants or different locations of the explants. The number of embryos formed from each responding explant was counted under a stereomicroscope (*SZH*, *Olympus*, Tokyo, Japan) at the protocorm stage. Data expressed as percentage were transformed using arc sine prior to *ANOVA* and converted back to the original scale (Compton 1994). Treatment means were compared by following Duncan's Multiple Range Test (Duncan 1955).

The paper is one part of a series of studies on direct somatic embryogenesis of *Oncidium* orchids. This present communication mainly deals with the effects of carbon sources on direct somatic embryogenesis on leaf explants taken from *in vitro* grown plantlets derived from leaf embryos followed the method described by Chen *et al*. (1999). When leaf explants of two *Oncidium* cultivars were cultured on medium without any sugar as the control treatment, 100 % of explants became browning and eventually necrotic after 2 months of culture (Table 1).

In cv. Gower Ramsey, cellibiose at 30 and 60 g dm^{-3} resulted in 5 and 30 % of explants to form embryos, respectively (Table 1). However, at lower concentrations (10 and 20 g dm^{-3}) of cellibiose, no embryos were obtained after 2 months of culture (Table 1). High browning rates were found at 10, 20 and 30 g dm^{-3} of cellibiose (Table 1). In cv. Sweet Sugar, except for 60 g dm^{-3} of cellibiose, all concentrations tested resulted in high browning rates and no embryos were found (Table 2). As cellibiose is rare to be used in plant culture medium, it showed obviously inhibitory on direct embryo induction of *Oncidium*.

In cv. Gower Ramsey, fructose at 10, 20 and 30 g dm^{-3} resulted in high embryogenic responses, and 20 g dm^{-3} was the best among fructose treatments with 95 % of explants forming a mean number of 20.8 embryos per responding explant (Table 1). However, embryogenic response was dramatically decreased at 60 g dm^{-3} of fructose, only 10 % of explants formed embryos (Table 1). Almost the same result was obtained in cv. Sweet Sugar, as 20 g dm^{-3} of fructose was the best but 60 g dm^{-3} showed highly inhibitory (Table 2). In both cultivars, the embryogenic competence of adaxial sides was higher than leaf tips and other leaf regions at 10 – 30 g dm^{-3} of fructose (Table 1, 2).

In cv. Gower Ramsey, glucose at 10 and 20 g dm^{-3} resulted in high embryogenic responses as 80 - 90 % explants formed an average of 22.8 - 27.6 embryos per explant (Table 1). By contrast, higher concentrations (30 and 60 g dm^{-3}) of glucose were not suitable for direct embryo induction (Table 1). The suitable range of glucose concentration in cv. Sweet Sugar (10 - 30 g dm^{-3}) induced 60 - 75 % of explants formed embryos (Table 2). By contrast, glucose at 60 g dm^{-3} resulted in an obviously low embryogenic response (Table 2).

In cv. Gower Ramsey, maltose at 20, 30 and 60 g dm^{-3} had better embryogenic frequencies (45 - 50 %) when compared with 10 g dm^{-3} (35 %) (Table 1). In cv. Sweet Sugar, maltose at 30 and 60 g dm^{-3} was better in embryo induction with 65 - 70 % of embryogenic frequency

Table 1. Effects of carbon sources on direct somatic embryogenesis from leaf explants of *Oncidium* cv. Gower Ramsey. Data were scored after 60 d of culture. The amounts of direct embryo formation were counted at entire explants or at different explant locations (LT - leaf tips, Ad - adaxial surface, Ab - abaxial surface, CE - cut ends). Five replicates (dishes) each with four leaf explants were performed for each treatment. Means with the same letters within columns are not significantly different at $P < 0.05$ (Duncan 1955).

Treatments [g dm^{-3}]		Browning [%]	Embryogenesis on different positions [%]				Total embryo-genesis [%]	Number of embryos [explant^{-1}]
			LT	Ad	Ab	CE		
Control		100 a	0 g	0 f	0 a	0 c	0 g	0
Cellibiose	10	75 ab	0 g	0 f	0 a	0 c	0 g	0
	20	75 ab	0 g	0 f	0 a	0 c	0 g	0
	30	80 ab	0 g	5 ef	0 a	0 c	5 g	2.0
	60	15 cde	15 efg	15 cdef	0 a	0 c	30 efg	3.5
Fructose	10	10 de	60 abcd	80 ab	0 a	5 bc	85 abc	5.0
	20	5 e	85 a	95 a	0 a	0 c	95 a	20.8
	30	5 e	45 bcdef	80 ab	0 a	0 c	80 abcd	14.5
	60	25 cde	0 g	10 cdef	0 a	0 c	10 fg	1.0
Glucose	10	10 de	75 abc	95 a	0 a	20 a	90 ab	22.8
	20	10 de	70 abc	75 ab	5 a	0 c	80 abcd	27.6
	30	0 e	40 bcde	45 bcd	0 a	0 c	55 bcde	25.3
	60	0 e	20 efg	10 def	0 a	0 c	25 dfg	4.8
Maltose	10	35 cde	10 fg	15 cdef	0 a	15 ab	35 dfg	4.1
	20	45 bcd	50 bcde	45 bcd	0 a	5 bc	50 cde	10.5
	30	10 de	30 def	45 bcd	0 a	5 bc	45 def	15.1
	60	0 e	35 cdef	45 bcd	0 a	0 c	50 cde	15.8
Sucrose	10	50 bc	15 dfg	45 bcd	5 a	0 c	45 def	12.0
	20	20 cde	30 defg	50 bc	0 a	0 c	50 cde	22.1
	30	5 e	45 bcde	80 ab	5 a	0 c	80 abcd	33.7
	60	0 e	75 ab	80 ab	5 a	0 c	80 abcd	30.2

Table 2. Effects of carbon sources on direct somatic embryogenesis from leaf explants of *Oncidium* cv. Sweet Sugar. Data were scored after 60 d of culture. The amounts of direct embryo formation were counted at entire explants or at different explant locations (LT - leaf tips, Ad - adaxial surface, Ab - abaxial surface, CE - cut ends). Five replicates (dishes) each with four leaf explants were performed for each treatment. Means with the same letters within columns are not significantly different at $P < 0.05$ (Duncan 1955).

Treatments [g dm^{-3}]		Browning [%]	Embryogenesis on different positions [%]				Total embryo-genesis [%]	Number of embryos [explant^{-1}]
			LT	Ad	Ab	CE		
Control		100 a	0 d	0 e	0 a	0 c	0 e	0
Cellibiose	10	100 a	0 d	0 e	0 a	0 c	0 e	0
	20	100 a	0 d	0 e	0 a	0 c	0 e	0
	30	90 a	0 d	0 e	0 a	0 c	0 e	0
	60	45 b	0 d	10 de	0 a	0 c	10 de	1.5
Fructose	10	10 c	55 abc	90 a	0 a	5 bc	90 a	12.6
	20	0 c	50 bc	95 a	0 a	0 c	95 a	17.4
	30	0 c	35 c	70 abc	0 a	0 c	80 ab	4.7
	60	25 bc	5 d	5 e	0 a	0 c	5 de	0
Glucose	10	15 bc	45 bc	50 bc	0 a	15 abc	60 abc	22.6
	20	15 bc	85 a	75 abc	10 a	10 abc	85 a	30.1
	30	5 c	50 bc	75 abc	0 a	0 c	75 abc	23.6
	60	25 bc	35 c	10 de	0 a	0 c	35 cde	3.4
Maltose	10	15 bc	5 d	0 e	0 a	35 a	40 bcd	7.8
	20	30 bc	40 c	40 cd	0 a	20 abc	60 abc	9.6
	30	20 bc	55 bc	60 abc	5 a	30 a	70 abc	18.7
	60	10 c	50 bc	60 abc	0 a	0 c	65 abc	29.7
Sucrose	10	10 c	50 bc	80 ab	0 a	25 ab	80 ab	12.1
	20	20 bc	75 ab	75 abc	0 a	20 abc	75 abc	31.1
	30	5 c	75 ab	90 a	5 a	0 c	90 a	28.1
	60	30 bc	70 abc	65 abc	0 a	0 c	70 abc	23.5

and 18.7 - 29.7 embryos per responding explant than at 10 and 20 g dm^{-3} with 40 - 60 % of frequency and 7.8 - 9.6 embryos per responding explant (Table 2).

According to embryogenic frequency plus embryo numbers per responding explant, in cv. Gower Ramsey, sucrose at 30 and 60 g dm^{-3} were the most suitable for embryo induction than other sucrose treatments (80 % of embryogenic frequency and 30.2 - 33.7 embryos per responding explant; Table 1). In cv. Sweet Sugar, sucrose at 30 dm^{-3} was the most suitable with 90 % of embryogenic frequency and 28.1 embryos per responding explant (Table 2).

Overall, in cv. Gower Ramsey, the most suitable treatments were at 30 g dm^{-3} of sucrose, 10 - 20 g dm^{-3} of glucose and then 20 g dm^{-3} of fructose. Besides, in cv. Sweet Sugar, the most suitable treatments were at 30 g dm^{-3} of sucrose and then 20 g dm^{-3} of glucose. Higher concentrations of sucrose performed an enhancement on somatic embryo formation, and could be replaced by relatively lower concentrations of fructose and glucose (Arditti and Ernst 1993). It was suggested that the increase of osmolarity enhanced somatic embryogenesis (Arditti and Ernst 1993). However, in this communication, higher concentrations (30 or 60 g dm^{-3}) of sucrose had better response in direct embryo induction than lower concentrations (10 g dm^{-3}) of sucrose. In this study, the performance of the most suitable sucrose concentration (20 - 30 g dm^{-3}) was almost the same with 20 - 30 g dm^{-3} of glucose or fructose. Generally, in orchid tissue culture, the most common sucrose concentration was at 20 g dm^{-3} (Arditti and Ernst 1993). Our results showed that 30 - 60 g dm^{-3} of sucrose was the most suitable for direct embryo induction in *Oncidium* cv. Gower Ramsey, and 30 g dm^{-3} for cv. Sweet Sugar.

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