In Vitro Shoot Formation from Rhizome Apices of Cymbidium ensifolium and C. kanran

Ogura, Yuki
Laboratory of Horticultural Science, Division of Agricultural Botany, Department of Plant Resources, Graduate School of Bioresource and Bioenvironmental Sciences, Kyushu University

Okubo, Hiroshi
Laboratory of Horticultural Science, Division of Agricultural Botany, Department of Plant Resources,Faculty of Agriculture, Kyushu University

https://doi.org/10.5109/4499

出版情報：九州大学大学院農学研究院紀要. 47 (2), pp.301-306, 2003-02-01. Faculty of Agriculture, Kyushu University
バージョン：
権利関係：

J. Fac. Agr., Kyushu Univ., **47** (2), 301–306 (2003)

In Vitro Shoot Formation from Rhizome Apices of *Cymbidium ensifolium* and *C. kanran*

Yuki OGURA*† and Hiroshi OKUBO

Laboratory of Horticultural Science, Division of Agricultural Botany, Department of Plant Resources, Faculty of Agriculture, Kyushu University, Fukuoka 812–8581, Japan

(Received October 31, 2002 and accept November 7, 2002)

Shoot formation from rhizome apices of *Cymbidium ensifolium* was promoted on Murashige and Skoog (MS) medium supplemented with 1 or 10 mgl^{-1} with or without NAA, and on MS medium with the reduction of ammonium and potassium nitrates to 25 and 50%, respectively from their original concentrations (modified MS medium, MMS). The optimal time and length of culture of *C. ensifolium* and *C. kanran* on MMS or MS medium with 1 mgl^{-1} BA and 0.1 mgl^{-1} NAA (BN medium) for shoot formation were when the explants were cultured for at least one month and at about two months after the initial culture on MS medium, respectively.

INTRODUCTION

Temperate *Cymbidium* species are generally terrestrial and they form rhizomes before shoot regeneration. Their seed germination is difficult and shoot formation takes longer time from the rhizomes than from epiphytic *Cymbidium* protocorms, resulting the *in vitro* micropropagation of terrestrial *Cymbidium* species being difficult to achieve (Kako, 1968; Sawa and Torikata, 1968). Shimasaki and Uemoto (1990) showed that shoot formation from rhizomes of *C. kanran* was promoted by the application of N^6– benzyladenine (BA) and α–naphthaleneacetic acid (NAA) in Murashige and Skoog (MS) medium (Murashige and Skoog, 1962). They found that the promotion of shoot formation was achieved also on MS medium with the reduction of ammonium nitrate and potassium nitrate (modified MS medium, MMS).

In this study, effects of BA and NAA application in MS medium and the reduction of nitrogen salts in MS medium on shoot formation from the rhizomes of *C. ensifolium* were investigated to certify the response of these shoot inductive treatments being effective in another terrestrial *Cymbidium* species. Effects of time and length of culture on these media on shoot formation in *C. ensifolium* and *C. kanran.* were also examined.

MATERIALS AND METHODS

Plant materials

Plants of *C. ensifolium* and *C. kanran* were used. They have been maintained *in vitro* in our laboratory by subcultures for long years.

* Laboratory of Horticultural Science, Division of Agricultural Botany, Department of Plant Resources, Graduate School of Bioresource and Bioenvironmental Sciences, Kyushu University

† Corresponding author (E–mail: oguy@agr.kyushu–u.ac.jp)

Culture conditions

Five and 10 mm–long apical segments of the rhizomes of *C. ensifolium* and *C. kanran*, respectively, were aseptically prepared and cultured on basal MS medium (pH 5.6) with 8 gl^{-1} agar and 30 gl^{-1} sucrose for three to four months before the start of the experiments in order to obtain the explant sources of the similar age (pre–culture). After the pre–culture, 5 and 10 mm–long apical segments of the rhizomes of *C. ensifolium* and *C. kanran*, respectively, were prepared and used for the experiments. Five explants were cultured in one 100 ml Erlenmeyer flask containing 30 ml medium. Each treatment consisted of 15 to 60 replicates. All cultures were maintained at 25±2 °C in continuous light condition (44.73 $\mu molsec^{-1}m^{-2}$).

Effects of BA, NAA and nitrogen salts in MS medium on shoot formation of rhizome apices of *C. ensifolium*

The explants were cultured on MS medium with various concentrations of BA and NAA for 50 days or on MS medium with the reduction of NH_4NO_3 and KNO_3 to 25 and 50%, respectively from their original concentrations (modified MS, MMS) for four months. The combination and the reduction rates of the nitrogen salts were reported to be optimal for shoot formation in *C. kanran* (Shimasaki and Uemoto, 1990).

Effects of time and length of culture on MMS or BN medium on shoot formation of rhizome apices of *C. ensifolium* and *C. kanran*

The rhizome apices of *C. ensifolium* and *C. kanran* were cultured on MS (control), MMS or MS with 1 mgl^{-1} BA and 0.1 mgl^{-1} NAA (BN medium). Medium changes during culture were given as follows: Control explants were cultured on MS medium for four months (Treatment 0, Fig. 1). The other explants were cultured on MMS or BN medium for three (Treatment 0–3), two (Treatment 0–2) or one (Treatment 0–1) month(s) prior to the transfer to MS medium, on MS medium for one month, transferred to MMS or BN medium and cultured for two (Treatment 1–2) or one (Treatment 1–1) month(s), and then on MS medium again, or on MS medium for two months followed by the culture on MMS or BN medium for one month (Treatment 2–1), then on MS medium again for the rest of the culture period.

RESULTS AND DISCUSSION

Effects of BA, NAA and nitrogen salts in MS medium on shoot formation of rhizome apices of *C. ensifolium*

Shoot formation from the rhizomes of *C. ensifolium* was promoted by the addition of 1 or 10 mgl^{-1} BA irrespective of the addition of NAA (Table 1). Among the treatments 1 mgl^{-1} BA with 1 or 10 mgl^{-1} NAA and 10 mgl^{-1} BA with 0.1, 1 or 10 mgl^{-1} NAA gave >20% shoot formation. The highest value (50%) was obtained with 10 mgl^{-1} BA and 10 mgl^{-1} NAA. The presence of BA in MS medium reduced the number of branches, and the addition of NAA increased the fresh weight of the rhizomes. Number of shoots and fresh weight of the explants were maximum with 10 mgl^{-1} BA and 10 mg l^{-1} NAA. Presence of BA in culture medium promoted shoot formation from the rhizomes of other terrestrial *Cymbidium* species such as *C. goeringii* and *C. faberi* (Hasegawa, 1987), *C. forrestii*

Table 1. Effects of BA and NAA in MS medium on development of rhizome apices of *C. ensifolium*

Treatment (mgl^{-1})		% of shoot formation[a]	Number of branches of rhizomes	Number of shoots[b]	Fresh weight of explants (g)
BA	NAA				
0	0 (control)	0	3.9±0.16[c]	0	88.4± 3.97
0	0.1	0	3.6±0.16	0	103.2± 4.66
0	1	0	2.8±0.21**[d]	0	101.7± 6.05
0	10	0	1.3±0.16**	0	46.4± 3.70**
0.1	0	0	1.1±0.12**	0	39.5± 1.93**
0.1	0.1	0	1.2±0.16**	0	51.1± 2.69**
0.1	1	5.4	1.6±0.15**	1.00	87.2± 6.06**
0.1	10	0	1.1±0.12**	0	63.1± 4.85**
1	0	9.1	1.4±0.19**	1.00	37.0± 2.96**
1	0.1	20.4	1.2±0.12**	1.08	35.7± 2.26**
1	1	37.7	1.1±0.13**	1.03	55.2± 5.12**
1	10	29.1	1.0±0.12**	1.38	76.7±12.06**
10	0	13.3	0.4±0.10**	1.13	29.1± 2.42**
10	0.1	20	0.4±0.09**	1.08	36.3± 3.72**
10	1	30.1	0.7±0.15**	1.35	50.7± 5.65**
10	10	50.0	1.3±0.15**	1.90	112.4±13.78**

[a] (number of explants that formed shoots/number of explants survived)×100
[b] Total number of shoots/number of explants that formed shoots.
[c] Values are means±S.E.
[d] The values followed by** are significantly different from those of control treatment at 1% level.
Shoot formation was recorded after 50 days of culture. Survival rate of the explants was 86.7–100%.

Table 2. Effect of MMS medium on development of rhizome apices of *C. ensifolium*

Medium	% of shoot formation[a]	Number of branches of rhizomes	Number of shoots[b]	Fresh weight of explants (g)
MS (control)	5.0	37.35±2.13[c]	1.00	855.63±41.63
MMS	65.0	15.10±1.40	1.08	423.68±37.33

[a,b] See Table 1.
[c] Values are means±S.E.
Shoot formation was recorded after four months of culture. All explants survived.

(Paek and Yeung, 1991) and *C. niveo-marginatum* (Niimi *et al.*, 1993).

Percentage of shoot formation and number of shoots increased, whereas number of branches and fresh weight of the rhizomes decreased on MMS medium (Table 2). These results were in agreement with the previous report in *C. kanran* (Shimasaki and Uemoto, 1990). It was clarified that the shoot formation from the rhizome apices of *C. ensifolium*

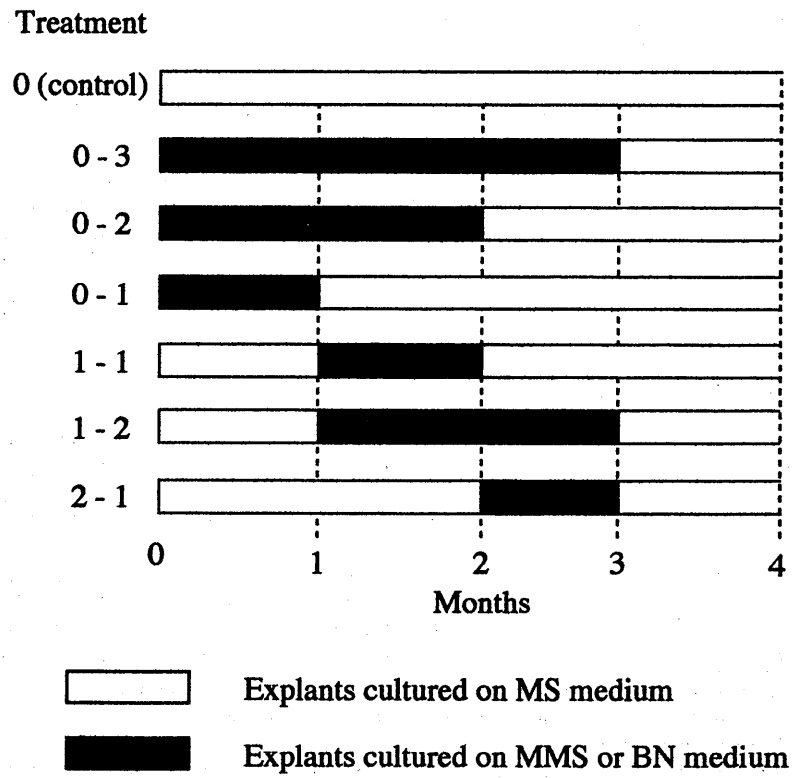

Fig. 1. Experimental design for the effect of time and length of culture on MMS or BN medium

can be induced on MMS medium without adding any plant hormones. It was also recognized that the response of *C. ensifolium* to these media is similar to that of *C. kanran*.

Effects of time and length of culture on MMS or BN medium on shoot formation of rhizome apices of *C. ensifolium* and *C. kanran*

High percentage (50–60%) of shoot formation was achieved when the explants of *C. ensifolium* were cultured on MMS medium for three months (Treatments 0–3), two months after one month culture on MS medium (Treatment 1–2) and one month after two months on MS medium (Treatment 2–1) (Table 3). Number of shoots ranged from 1.0 to 1.17. The shoot formation rate was low (15%) even when the explants were cultured on MMS medium for two months from the beginning of the culture (Treatment 0–2). Growth of the explants to some extent seems to be necessary before responding to the MMS medium treatment.

Treatment 2–1 gave the highest (55%) shoot formation rate and the highest number of shoots (1.64) (Table 4). The explants of two to three months of ages seem to be more sensitive to the plant hormones in MS medium than the initial explants.

Table 3. Effects of time and length of culture on MMS medium on shoot formation of rhizome apices of *C. ensifolium*

Treatment[a]	% of shoot formation[b]	Number of shoots[c]
0(control)	5	1.00
0–3	60	1.00
0–2	15	1.00
0–1	28	1.07
1–1	40	1.00
1–2	60	1.17
2–1	50	1.04

[a] See Fig. 1.
[b,c] See Table 1.
Shoot formation was recorded after four months of culture.
All explants survived.

Table 4. Effects of time and length of culture on BN medium on shoot formation of rhizome apices of *C. ensifolium*

Treatment[a]	% of shoot formation[b]	Number of shoots[c]
0(control)	5	1.00
0–3	0	0
0–2	20	1.00
0–1	0	0
1–1	0	0
1–2	5	1.00
2–1	55	1.64

[a] See Fig. 1.
[b,c] See Table 1.
Shoot formation was investigated after four months of culture.
All explants survived.

Table 5. Effects of time and length of culture on BN medium on shoot formation of rhizome apices of *C. kanran*

Treatment[a]	% of shoot formation[b]		Number of shoots[c]	
	3 months	4 months	3 months	4 months
0(control)	3.3	6.7	1.00	1.00
0–3	86.6	86.6	1.58	1.69
0–2	53.3	37.9	1.44	1.00
0–1	3.4	3.4	1.00	1.00
1–1	89.3	39.3	1.84	1.27
1–2	96.4	78.6	2.52	1.86
2–1	92.9	100	3.62	2.64

[a] See Fig. 1.
[b,c] See Table 1.
Shoot formation was recorded after three and four months of culture.
All explants survived.

The treatments on BN medium for two months or longer period (Treatments 0–3, 0–2 and 1–2) or for one month started after one or two month(s) initial culture on MS medium (Treatments 1–1 and 2–1) gave high percentages of shoot formation (53.3– 96.4%) and high number of shoots (1.44–2.62) in *C. kanran* after three months of culture (Table 5). When the explants were cultured on BN medium for the first one month only, the shoot formation rate and number of shoots were low. Shoot formation rates in the treatments except Treatments 0 and 0–1 decreased from 53.3–96.4% to 37.9– 78.6% after four months of culture. It was caused by the reformation of rhizomes from the regenerated shoots.

It is concluded that the explants of *C. ensifolium* and *C. kanran* require at least one month treatment of MMS or BN medium to initiates shoots, and that the optimal time of the treatments is about two months after the initial culture on MS medium.

REFERENCES

Hasegawa, A. 1987 Studies on the propagation of oriental *Cymbidium*. *Mem. Fac. Agric. Kagawa Univ.*, **50**: 1–108

Kako, S. 1968 Studies on the seed germination of *Cymbidium goeringii* Reichenbach fil. *In* "Seed formation and sterile culture of the orchids", ed. by H. Torikata, Seibundo Shinkosha, Tokyo, pp. 174–237 (translated into English from the original Japanese by Y. O.)

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

Niimi, Y., C. Tanaka and Y. Hayata 1993 Proliferation and organogenesis of *Cymbidium* using rhizome. *Proc. Nagoya Inter. Orchid Congress*, pp. 72–79

Paek, K. Y. and E. C. Yeung 1991 The effects of 1–naphthaleneacetic acid and N^6–benzyladenine on the growth of *Cymbidium forrestii* rhizomes *in vitro*. *Plant Cell, Tiss. Org. Cult.*, **24**: 65–71

Sawa, Y. and H. Torikata 1968 Studies on aseptic germination of *Cymbidium* seed and ecological factors for germination. *In* "Seed formation and sterile culture of the orchids", ed. by H. Torikata, Seibundo Shinkosha, Tokyo, pp. 153–173 (translated into English from the original Japanese by Y. O.)

Shimasaki, K. and S. Uemoto 1990 Micropropagation of a terrestrial *Cymbidium* species using rhizomes developed from seeds and pseudobulbs. *Plant Cell, Tiss. Org. Cult.*, **22**: 237–244