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Rhizome induction and plantlet regeneration of *Cymbidium goeringii* from flower bud cultures in vitro

Kazuhiko Shimasaki[1] & Shunpei Uemoto
Laboratory of Horticulture, Faculty of Agriculture, Kyushu University, Fukuoka 812, Japan ([1]present address: Faculty of Agriculture, Saga University, Saga 840, Japan)

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Abstract

Apical flower buds of *Cymbidium goeringii* Reichenbach fil. (ca 2 mm long) excised from inflorescences (ca 5 cm long) were explanted on modified Murashige & Skoog medium (=MS medium) supplemented with N^6-benzyladenine (BA) and α-naphthaleneacetic acid (NAA). Within 107 days of culture, swelling growth, chlorophyll synthesis, and subsequent rhizome differentiation were observed. MS medium containing 0.1 mg l^{-1} BA and 10 mg l^{-1} NAA was found to be optimal for initiating rhizome development and subsequent plantlet regeneration.

Explants cultured on MS medium supplemented with 1 mg l^{-1} NAA alone formed a mass of rhizome branches. Multiple shoots of rhizome branches were induced from apical segments when rhizomes were transferred to MS medium containing 0.1 mg l^{-1} BA and 10 mg l^{-1} NAA.

Abbreviations: NAA – α-naphthaleneacetic acid, BA – N^6-benzyladenine

Introduction

The spring orchid (*Cymbidium goeringii* Reichenbach fil.) is one of the most popular terrestrial species indigenous to temperate Eastern Asia, that has been cultivated as an ornamental, and the flowers are sometimes used as ingredients of a soup, an alcoholic drink, tea, etc. Conventionally, spring orchids are propagated through the division of pseudobulbs. Propagating material is always in short supply, because of its slow rate of vegetative propagation. Since the first experiments of Rotor (1949), numerous authors have reported plantlet regeneration from tissue cultures of orchid using in vitro culture techniques. However, plantlet regeneration of the temperate *Cymbidium* species such as *C. goeringii* and *C. kanran*, using seed, tissue and organ culture techniques in vitro has been considered rather difficult (Kako 1976; Kokubu et al. 1980; Sawa 1969; Ueda & Torikata 1968, 1969a).

Several investigators have reported successful plant regeneration from floral organs and inflorescences in orchid species (Intuwong & Sagawa 1973; Kim & Kako 1984). In the terrestrial *Cymbidium* species, few reports deal with morphogenesis from floral tissue and organ cultures (Sawa & Hara 1973).

In the present paper we describe an efficient

method for plantlet regeneration from young buds of *C. goeringii*.

Materials and methods

Plant material

Inflorescences ca 5 cm long were taken from *C. goeringii* plants that were collected in Fukuoka Prefecture, Japan, on October 8, 1986. Apical flower buds ca 2 mm long served as explant sources.

Sterilization and preparation of explants

Flower stalk sections were washed under running water. Apical segments of the washed stalks were excised, each containing an apical flower bud. They were immersed for 10 min in 1% aqueous sodium hypochlorite solution containing 0.01% Tween 20 and subsequently rinsed 3 times with sterile distilled water. Excess stalk tissues were removed from apical flower buds after the rinsing.

Culture conditions

Flower buds were placed horizontally on the agar surface. Cultures were maintained under continuous illumination of 75 $\mu mol\ m^{-2}\ s^{-1}$ provided by fluorescent lamps (Toshiba Bio-light), and a temperature 25 ± 1°C. These cultures were maintained from October 8, 1986 to April 8, 1987 (6 months).

Culture media

The culture medium consisting of Murashige & Skoog solution (1962) with 20 $g\ l^{-1}$ sucrose and 7.5 $g\ l^{-1}$ agar(GIBCO) was used as the basal medium. Several concentrations of BA and NAA were tested (Table 1). Explants were placed in the test tubes (25 × 100 mm) containing 10 ml of medium. Culture bottles, 200 ml capacity containing 40 ml of medium, were employed for the subculture of the rhizome mass. These were autoclaved at 120°C for 15 min. Replications consisted of 12 test tubes, each containing one explant.

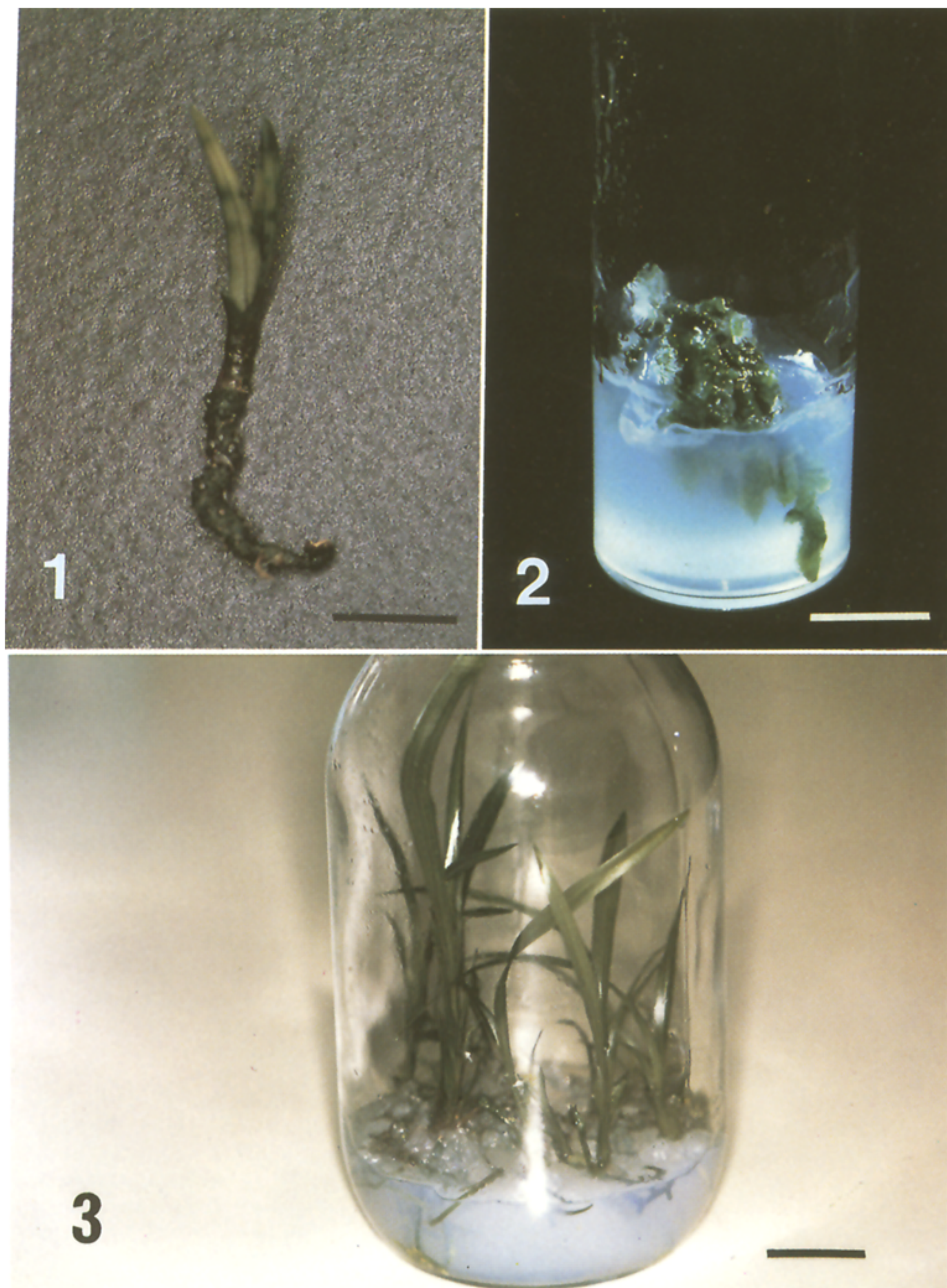
Results and discussion

More than half of the flower buds become swollen by the first week. Some swollen explants on MS_3, MS_6, MS_7, MS_8, MS_{10}, MS_{13}, MS_{14}, MS_{15}, and MS_{16} initiated chlorophyll synthesis within 2 weeks of culture. Following greening, explants on MS_3, MS_7, MS_8, and MS_{16} began to produce elongated rhizomes by the eighth week. Shoots differentiated from rhizome apex segments on MS_7, MS_8, and MS_{16} within 107 days of the start of culture (Table 1). MS_8 was most effective as a shoot induction medium, but failed to induce root formation (Fig. 1). The optimal medium for plantlet regeneration from rhizomes consisted of the basal solution plus 0.1 $mg\ l^{-1}$ BA and 1 $mg\ l^{-1}$ NAA (MS_7). Explants on MS_3 formed masses of rhizome branches, but failed to develop shoots after 6 months of culture (Fig. 2). Plantlets were produced when each mass was transferred to MS_7 (Fig. 3). All necrotic tissues died without signs of regeneration.

It has been pointed out that rhizome induction from shoot-tips was promoted by the addition of NAA to the medium. The addition of cytokinin had little effect on rhizome induction (Kokubu et al. 1980, Lee 1988a,b, Lee & So 1985, Sawa 1969, Ueda & Torikata 1969a). A medium containing cytokinin was efficient for shoot regeneration from rhizome apices (Ueda & Torikata 1969).

Attempts to culture *C. goeringii* inflorescence tissue and first floret explants were successful with swelling growth followed by callus formation. However, both cytokinin and auxin had a limited effect on induction of rhizome development and subsequent plantlet regeneration [Sawa & Hara 1973]. In experiments with apical flower buds (flower buds of second floret), rhizome formation and subsequent plantlet regeneration appears to require cytokinin and auxin. This result indicates that the sensitivity of flower buds to cytokinin and auxin varies significantly between first and second florets.

The present study shows that excised flower buds from *C. goeringii* can be induced to pro-



Figs. 1–3. Flower bud culture and plantlet regeneration of *Cymbidium goeringii*. *1.* Rhizome and shoot development from flower bud explant on MS_8 after 107 days. Bar = 2 mm. *2.* Rhizome branches on MS_3 after 107 days. Bar = 10 mm. *3.* Multiple shoots on rhizome branches following 6 months of subculture on MS_7. Bar = 10 mm.

Table 1. Development of *C. goeringii* explants in vitro.

Medium	Growth regulators ($mg\,l^{-1}$)[a]		Growth and development (%)			
	BA	NAA	Rhizome formation	Swelling and chlorophyll synthesis	Swelling	Dead
MS_1	0	0	0	0	50.0	50.0
MS_2	0	0.1	0	0	87.5	12.5
MS_3	0	1	11.1	0	77.8	11.1
MS_4	0	10	0	0	85.7	14.3
MS_5	0.1	0	0	0	50.0	50.0
MS_6	0.1	0.1	0	33.3	33.3	33.3
MS_7	0.1	1	12.5[b]	37.5	0	50.0
MS_8	0.1	10	37.5[b]	12.5	37.5	12.5
MS_9	1	0	0	0	87.5	12.5
MS_{10}	1	0.1	0	12.5	50.0	37.5
MS_{11}	1	10	0	45.5	54.5	0
MS_{12}	1	10	0	0	83.3	16.7
MS_{13}	10	0	0	25.0	50.0	25.0
MS_{14}	10	0.1	0	28.6	28.6	42.8
MS_{15}	10	1	0	42.8	28.6	28.6
MS_{16}	10	10	20.0	40.0	20.0	20.0

[a] BA, N^6-benzyladenine; NAA, α-naphthaleneacetic acid.
[b] Rhizome differentiated shoot(s) within 107 days.

duce rhizomes and plantlets in Murashige & Skoog media with $20\,g\,l^{-1}$ sucrose, BA and NAA.

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