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https://doi.org/10.5109/23865

出版情報：九州大学大学院農学研究院紀要. 32 (1/2), pp.31-39, 1987-12. Kyushu University
バージョン：
権利関係：

J. Fac. Agr., Kyushu Univ., 32 (1•2), 31-39 (1987)

Comparative Organogenesis between Terrestrial and Epiphytic *Cymbidium* Species

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(Received May ***6, 1987)***

In the terrestrial ***cymbidium*** species (*C. kanran* Makino and C. ***georingii*** Reichenbach fil.), rhizome development was induced in axillary buds of pseudobulb by the treatment of auxins. The rate of rhizome induction in the pseudobulbs was enhanced by the application of higher concentrations of NAA and 2,4-D. The application of auxins as lanolin paste was efficient for the rhizome induction.

However, in the epiphytic type (*C.* ***dayanum*** Reichenbach fil. var. *austro-japonicum* Tuyama), auxin had no effect on rhizome induction from pseudobulb, but, shoot formation was directly induced in the form of axillary bud of pseudobulb by both auxin and cytokinin treatments.

INTRODUCTION

Significant diversity of ecological and morphological types are observed between tropical and temperate cymbidiums. Epiphytic types are typical in the tropical regions, and scarcely forms a rhizome system. On the other hand, the terrestrial types generally distribute in the temperate regions, and rhizome formation is frequently observed as a dormant organ.

In the tropical group, seed germinates readily and develops protocorms on the sterilized culture medium (Knudson, 1922, 1946), and their shoot formation can be seen soon after the protocorm formation. However, in the temperate group, both seed germination and shoot formation are rather difficult comparing with tropical group. After seed germination, rhizome development occurs in the apical meristem of protocorm. Shoot formation from the rhizome would be difficult without addition of auxin and cytokinin (Ueda and Torikata, 1968, 1969-a, 1969-b, Kokubu et. al, 1980). The method for rapid propagation *in vitro* in the tropical group was developed by G. M. Morel (1960). He noted that protocorm-like body (PLB), from which young seedlings were formed, were developed around the shoot tip cultures. Since this first study, morphological study in *vitro* (Wimber, 1963, Morel, 1964, Matsui et. al, 1964, Sagawa and Shoji, 1966, Vacherot, 1966, Wirfret, 1966, Bivins and Hacket, 1969, Steword and Mapes, 1971, Thompson, 1971, Wirckmeister, 1971, Kusumoto, 1980) has rapidly progressed.

However, in the terrestrial types, instead of PLB formation, rhizome development is observed in the meristem tissue culture in *vitro*. Difficulty in plantlet formation from the rhizome culture is similar to the seedling formation.

In this study, the effects of the chemicals on the morphological developments in *vivo* in terrestrial and epiphytic *Cymbidium* species are examined.

MATERIALS AND METHODS

1. Application of auxins and cytokinin as lanolin pastes

a) Mature plants of C. ***kanran*** Makino without rhizome were collected in Kochi Prefecture, Japan. After detaching of each pseudobulb having leaves and roots, auxins such as α-naphthylacetic acid (NAA), indole-3-butyric acid (IBA) and cytokinin, N^6-benzylaminopurine (BA) were applied separately at concentrations of 0.1, 1, 10, 50 or 100 ppm, respectively. These agents were dissolved at first in small volume of 95 % ethanol and then dissolved in distilled water, and added to the lanolin paste. Lanolin paste with ethanol and distilled water was used as the control. These treatments were conducted on April 23, 1983. All plants were grown in a green house at Kyushu Univ., and the results were estimated on September 22, 1983.

b) Mature plants of C. *georingii* Reichenbach fil. Without rhizome were collected in Fukuoka Prefecture, Japan. After detaching each pseudobulb having leaves and roots, NAA, IBA, 2,4-dichlorophenoxyacetic acid (2,4-D) or BA were applied in the same manner stated above.

2. Application of auxins and cytokinin as aqueous solution

Mature plants of C. ***goeringii*** Reichenbach fil. and C. ***dayanum*** Reichenbach fil. var. *austra-japonicum* Tuyama were grown in a green house at Kyushu Univ.. After shoots and roots were excised, pseudobulbs were detached each other. 2,4-D and BA were applied on pseudobulbs as aqueous solutions singly or in combinations at concentrations of 0, 0.1, 1, 10 and 100 ppm, respectively. These pseudobulbs were soaked in the solutions for 24 hours. The pH of the solutions were adjusted to 5.5 with 0.1-0.5 N NaOH or HCl. These treatments were conducted on April 19, 1986. All plants were grown at 25°C under dark condition, and examined on August 2, 1986.

RESULTS

l-a) Effects of auxins and a cytokinin on organogenesis in axillary buds of C. *kanran* Makino are summarized in Table 1. Rhizome development as an axillary bud in pseudobulb was observed in all cases except for the case of higher concentrations of BA or IBA. Application of NAA was effective for rhizome development in axillary buds. Additional organogenesis as a shoot was observed in an apex segment of the rhizome by the treatment of lower concentrations of NAA. Higher concentrations of NAA inhibited shoot formation in an apex segment of rhizome. Rhizome elongation was promoted by the application of 10 or 50 ppm NAA (Fig. 1). The number of rhizomes also increased by the application of NAA and the best result was obtained at 1 ppm. The shoot development as an axillary bud was enhanced at higher concentrations of BA. N^6-benzylaminopurine at concentrations ranging from 1 to 50 ppm are efficient for shoot formation. Application of IBA inhibited both shoot and rhizome development.

l-b) Effects of auxins and a cytokinin on organogenesis in axillary buds of C. ***georingii*** Reichenbach fil. are summarized in Table 2. Rhizome development as an axillary bud of C. ***georingii*** Reichenbach fil. was induced by the application of higher concentrations of NAA, 2,4-D or IBA. Lanolin paste without chemicals or only BA

Fig. 1. Rhizome development in pseudobulb axillary buds of C. *kanran* Makino induced by the application of 50 ppm of NAA.

Table 1. Effects of auxins and cytokinin on organogenesis in pseudobulbs of C. *kanran* Makino.

Treatment (ppm)		Number		Length (mm)		Formation rate (%)	
		Rhizome	Shoot	Rhizome	Shoot	Rhizome	Shoot
Control		0.2	0.6	4.2	57.0	20	60
BA	0.1	0.5	0.4	5.8	4.8	50	25
	1	0.5	0.5	5.4	41.9	20	80
	10	0.2	0.8	1.8	75.2	20	100
	50	0	1.0	–	75.2	0	100
	100	0	1.0	–	16.1	0	100
NAA	0.1	0.3	1.0	1.3	32.8	25	75
	1	1.3	0.8	8.1	24.0	100	75
	10	0.8	0.8	6.8	42.1	80	80
	50	1.0	1.0	11.2	46.2	100	100
	100	1.0	0.5	11.9	8.6	100	50
IBA	0.1	0.2	0.8	1.1	13.9	20	75
	1	0.5	0.8	3.6	18.6	60	60
	10	0.3	0.8	4.1	26.8	50	75
	50	0	0.8	–	58.1	0	60
	100	0.2	1.0	1.3	44.4	20	100

had no effect on the rhizome development. Higher concentrations of NAA or IBA slightly promoted rhizome elongation. 2,4-dichlorophenoxyacetic acid at concentrations ranging from 1 to 50 ppm were efficient for rhizome formation and the best result was obtained at 1 ppm. Shoot elongation was inhibited by the treatments of NAA, 2, 4-D and higher concentrations of BA. Lower concentrations of BA was effective for shoot elongation. The number of induced rhizome in axillary buds were increased when 2,4-D at concentrations ranging from 1 to 10 ppm IBA or 50 ppm NAA were applied and the best result was obtained at 1 ppm concentration of 2,4-D. Shoot formation was observed in all plants treated with NAA, IBA, BA, or lanolin paste without chemicals. 2,4-D at concentrations ranging from 1 to 50 ppm inhibited shoot formation.

Table 2. Effects of auxins and cytokinin on organogenesis in pseudobulbs of C. *goeringii* Reichenbach. fil..

Treatment (ppm)		Number		Length (mm)		Formation rate (%)	
		Rhizome	Shoot	Rhizome	Shoot	Rhizome	Shoot
Control		0	1.0	_	169.3	0	100
BA	0.1	0	1.4		182.5	0	100
	1	0	1.5	_	153.3	0	100
	10	0	1.2	_	33.4	0	100
	50	0	1.3	_	58.8	0	100
	100	0	1.1	_	179.5	0	100
NAA	0.1	0	1.0		86.4	0	100
	1	0	1.0	_	72.7	0	100
	10	0	1.0	_	79.8	0	100
	50	0.3	1.1	2.8	51.8	28	100
	100	0.2	1.4	1.0	67.2	14	100
IBA	0.1	0.4	1.0	4.3	67.1	14	100
	1	0.3	1.0	2.1	72.1	14	100
	10	0.2	1.1	1.6	96.8	20	100
	50	0.2	1.5	1.8	99.7	22	100
	100	0.3	1.0	1.7	56.2	25	100
2,4-D	0.1	0	1.4	–	86.3	0	100
	1	0.8	1.0	9.8	18.1	50	80
	10	1.1	0.8	6.8	27.1	100	80
	50	0.7	0.6	6.3	42.8	71	57
	100	0.2	1.2	1.0	58.2	17	100

2-a) Effects of BA and 2,4-D as aqueous solution on organogenesis in axillary buds of C. ***georingii*** Reichenbach fil. are summarized in Table 3. Rhizome development in axillary buds was occurred by the treatment at higher concentrations of 2,4-D with BA and the best result was obtained at 10 ppm 2,4-D with 0.1 ppm BA. Shoot elongation was promoted when BA/2,4-D ratios were ranged from 1 to 10. One hundred ppm of BA with 0.1 or 1 ppm 2,4-D gave the best result on shoot formations.

2-b) Effects of BA and 2,4-D on the organogenesis in axillary buds of C. ***dayanum*** Reichenbach fil. var. *austro-japonicum* Tuyama are summarized in Table 4. BA applied singly promoted shoot elongation and gave the best result at 10 ppm of BA.

Table 3. Effects of BA and 2,4-D on organogenesis in pseudobulbs of C. *goeringii* Reichenbach. fil..

Treatment (ppm)		Number		Length (mm)		Formation rate (%)	
BA	2,4-D	Rhizome	Shoot	Rhizome	Shoot	Rhizome	Shoot
0	0	0	1.0	_	63.2	0	30
0	0.1	0	1.4	_	57.7	0	70
0	1	0.6	1.0	5.4	33.4	22	44
0	10	0	1.0	_	30.7	0	71
0	100	0	0.3	_	15.2	0	33
0.1	0	0	1.2	_	28.5	0	45
0.1	0.1	0	1.2	_	76.9	0	60
0.1	1	0	1.0	_	61.2	0	60
0.1	10	0.7	0.8	13.8	44.3	25	36
0.1	100	0.4	1.0	3.8	8.6	22	44
1	0	0	1.3	_	19.0	0	67
1	0.1	0	1.0	_	67.7	0	60
1	1	0	1.0	_	58.2	0	40
1	10	0	1.2	_	26.3	0	71
1	100	0.5	1.0	2.5	5.1	33	33
10	0	0	1.2	_	29.5	0	69
10	0.1	0	1.4	_	45.2	0	50
10	1	0	1.0	_	46.5	0	40
10	10	0	0.3	_	50.0	0	10
10	100	0	1.0	_	9.0	0	43
100	0	0	1.7	_	35.0	0	38
100	0.1	0	2.6	_	30.2	0	70
100	1	0	2.7	_	27.1	0	33
100	10	0	1.0	_	53.5	0	10
100	100	3.0	1.0	3.0	5.7	13	50

However, addition of 2,4-D resulted in an inhibition of shoot elongation. Rhizome development in the axillary buds in each plant did not occur. Both BA and 2,4-D had no effect on the number of shoot developed in pseudobulbs. The application of 10 or 100 ppm of 2,4-D resulted in the death of all plants.

DISCUSSION

Cymbidium kanran Makino and C. *georingii* Reichenbach fil. are the representative terrestrial species in the temperate regions, Japan. *Cymbidium dayanum* fil. is an epiphytic type distributing both in the tropical and temperate regions. Significant differentiations in the morphological growth responses by chemicals were observed between these two types. These differenciations supposed to attributed in the differences in growth cycles, i. e., the epiphytic *Cymbidium* species (Table 5 and 6) develop shoots immediately after the protocorm formation. However, the terrestrial types (Table 6) develop rhizome as a dormant organ soon after the protocorm formation. The rhizome continues to grow and branch out under the ground for comparatively long durations. Therefore, shoot formation cannot be observed. In temperate zone plant, rhizome is formed as a sort of dormant organ. Therefore, in the terrestrial type species of *Cymbidium*, shoot formations observed after their dormancy would be

Table 4. Effects of BA and 2,4-D on organogenesis on shoot formation of C. *dayanum* Reichenbach fil. var. *austro-iaponicum* Tuyama.

Treatment (ppm)		Number	Length (mm)	Formation rate (%)
BA	2,4-D			
0	0	0.3	33.0	28
0	0.1	0.7	15.1	50
0	1	0.2	22.4	20
0	10	0.2	3.9	20
0	100	0.2	2.6	20
0.1	0	1.0	50.0	71
0.1	0.1	0.4	14.7	43
0.1	1	0.3	25.6	30
0.1	10	0.2	9.7	20
0.1	100	0	–	0
1	0	0.5	70.0	50
1	0.1	0.3	28.7	33
1	1	0.7	5.6	43
1	10	0.3	13.8	15
1	100	0	–	0
10	0	0.6	73.6	57
10	0.1	0.1	45.0	14
10	1	0.3	13.7	33
10	10	0.1	2.5	14
10	100	0	–	0
100	0	0.5	50.4	50
100	0.1	0.2	20.3	16
100	1	0.7	17.6	67
100	10	0	–	0
100	100	0		0

finished. Dormant growth stage in the form of rhizome is supposed to be obtained during expansion or movement of geographical distribution. By a terrestrial growth habit, they could show an expansion of distribution under various environments. ***Cymbidium*** *kanran* Makino have achieved movement and expansion of distribution from southern area of the peoples' Republic of China through Shizuoka Prefecture in Japan. As for C. ***georingii*** Reichenbach fil. have achieved movement and expansion of geographical distribution from southern area in the peoples' Republic of China to Hokkaido in Japan. Similar ecological and morphological differences can be observed in the case of bamboo (Uchimura, 1978), i.e., bamboo species like *Bambusa,* ***Dendrocalamus*** and *Schizostachyum* genera which grow in the tropical region, clump of culms is observed, i. e., shoot formation directly occures at the lower portion of culms. On the other hand, non clump-forming type represented by the genera *Phyllostachys,* ***Semiarundinaria*** and *Melocanna,* are distributed in the temperate and higher latitude region, extend rhizome under the ground after seed germination.

In the terrestrial ***Cymbidium*** species, the protocorm stage is supposed to play a role as predormant growth stage. This stage is observed both tropical and temperate cymbidiums on sterized culture media. This fact suggested that protocorm is a originate growth stage in these species. Protocorm as a predormant growth stage is supposed to be induced by instability in a premature embryo of seed. Similar aspects

were seen in the meristem culture of the shoots. This response was also supposedly caused by instability in the meristem tissue. The results in these experiments suggested that auxin play an important role to the induction of development of the dormant organ in the form of rhizome in mature plants. By the application of higher concentrations of auxin, rhizome continue to elongate and branch out because of the dormancy in the plant. By the application of lower concentrations of auxin, shoot formation was observed in the apical segment of rhizome. This fact suggested that dormancy in the plant was broken immediately after rhizome formation. On the other hand, higher concentrations of cytokinin inhibited the induction of dormancy. In the tropical types, 2,4-D inhibited shoot elongation. These results suggested the dormant growth stage in the form of rhizome have not been obtained for these species. For these reasons, the tropical epiphytic types are supposed to have difficulty in the movement and expantion of distribution to the temperate regions. The tropical epiphytic types are supposed to have difficulty in the movement and expansion of distribution to the temperate region.

Table 5. Representative epiphytic ***Cymbidium*** species and their distributions

Species	Distributions
C. *aloifolium* Sw.	Philippines, Burma, Srilanka
C. ***dayanum*** Reichb. fil.	Japan, China, Taiwan, Indonesia, Malaysia
C. ***devonianum*** Paxt.	India, Himalaya,
C. *erythraecum* Lindl.	China, India, Bhutan, Nepal, Burma
C. *finlaysonianum* Lindl.	Philippines, Indonesia, Burma, Vietnam
C. *floribundum* Lindl.	China, Taiwan
C. ***giganteum*** Wall	India, Himalaya
C. *grandiflorum* Griff.	India, Bhutan
C. *hookerianum* Reichb. fil.	China, India, Bhutan, Nepal
C. ***i'ansonii*** Lindl.	Burma
C. ***iridioides*** D. Don	China, India, Bhutan
C. ***javanicum*** Bl.	China, Taiwan,
C. *lancifolium* Hook.	Tropical region of Asia
C. *longifolium* D. Don	China, Nepal, India, Burma
C. *parishii* Reichb. fil.	Burma
C. ***pendulum Sw.***	China, India, Burma, Vietnam,
C. *pumilum* Rolfe	China
C. ***simulans*** Rolfe	China, India, Burma, Vietnam, Thailand

Table 6. Representative semi-epiphytic ***Cymbidium*** species and their distributions.

Species	Distributions
C. ***canaliculatum*** R. Br.	Australia
C. *eburneum* Lindl.	Himalaya, Burma
C. *erythrostylum* Rolfe	Vietnam
C. ***insigne*** Rolfe	China, Bietnum
C. ***lowianum*** Reichb. fil.	Burma
C. *masterii* Par.	India, Himalaya
C. *tigrinum* Par.	Burma
C. ***tracyanum*** Rolfe	Burma

Table 7. Representative terrestrial ***Cymbidium*** species and their distributions.

Species	Distributions
C. *aberrans* Schltr.	Japan
C. *allo-marginatum* Makino	China
C. *ensifolium* Sw.	China
C. faberi Rolfe	China, Taiwan
C. formosanum Hay. et Fukuyama	Taiwan
C. *forrestii* Rolfe	China
C. *goeringii* Reichb. fil.	Japan
C. *gracillimum* Fukuyama	Taiwan
C. ***gyokuchin*** Makino	China, Taiwan
C. ***javanicum*** Blume	Japan, Taiwan, Malaysia, Himalaya
C. *kanran* Makino	Japan, China, Taiwan
C. *koran* Makino	Japan
C. ***lancifolium*** Hook.	Japan, Taiwan, India
C. *linearisepalum* Yamamoto	Taiwan
C. *macrorhizum* Lindl.	China, India
C. *misericors* Hay.	Taiwan
C. *nipponicum* Makino	Japan
C. ***niveo-marginatum*** Makino	China
C. ***oiwakensis*** Hay	Taiwan
C. *purpureo-hiemale* Hay.	Taiwan
C. *rubrigemmum* Hay.	Taiwan
C. ***sinense*** Willd	China, Taiwan
C. ***yakibaran*** Makino	China, Japan
C. *yakusimense* Masamune	Japan

ACKNOWLEDGEMENT

The authers wish to dedicate this paper to the Associate Professor T. Kawarabata, Institute of Biological Control, Faculty of Agriculture, Kyushu Univ. for his providing samples.

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