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Formation of Protocorm-like Bodies (PLB) and Shoot Development through In Vitro Culture of Outer Tissue of *Cymbidium* PLB

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Summary

Outer tissue (OT) excised from protocorm-like bodies (PLB) of *Cymbidium* ×Thanksgiving, cultivar 'Nativity' produced PLB on hormone-free Murashige and Skoog (MS) medium and MS media supplemented with hormones. The PLB initiation from OT was earlier on hormone supplemented media compared to hormone-free MS medium. Explants showed the highest PLB formation abilitiy on the medium supplemented with 0.1 mg · liter^{-1} α-naphthaleneacetic acid (NAA) and 0.5 mg · liter^{-1} benzyl adenine (BA). On the medium of this combination, rate of cell division was high and the cell division occurred from surface to deeper tissue in the protuberance (an initial structure of PLB formed on OT explants) after 14 days of culture. OT explants turned light brown after 7 days of culture and a gray transparent protuberance was observed on the outer surface of the explant after 14 days of culture. The protuberance gradually increased in size and turned into a green globular PLB after 21 days of culture. This pattern of PLB formation was similar in both hormone supplemented MS media and hormone-free MS medium. Thus it suggests that exogenous hormones have no fundamental effects on PLB formation. OT-derived PLB formed 100% shoot on the medium supplemented with 0.1 mg · liter^{-1} NAA and 1.0 mg · liter^{-1} BA within 8 weeks, but shoot formation was markedly suppressed on the media supplemented with NAA alone.

Histological study showed that OT segments consisted of epidermal and sub-epidermal cells, which were parenchymatous, large and vacuolated. After one week culture, all the cells of epidermis were ruptured. A small group of cells with dense cytoplasm and deeply stained nuclei was observed just below the ruptured epidermis. These cells developed into a PLB. This result showed that OT of *Cymbidium* PLB has ability to produce PLB directly from explants through organogenesis.

Introduction

Cymbidium is one of the commercially important orchids throughout the world (Bilton, 1980). This genus is very popular in Japan as pot flowers (Kako and Ohno, 1980). It was the first orchid to be propagated by shoot-tip culture in vitro (Champagnat et al., 1966; Morel, 1960, 1964; Morel and Martin, 1955; Wimber, 1963). Afterwards several tissue culture procedures have been succeeded for different explants of *Cymbidium* such as flower stalks and roots (Kim and Kako, 1984a; Yasugi, 1989). Most of the explants grew in relatively simple media without hormones and/or natural substances. But in some cases, complex media supplemented with these additives were necessary to obtain plantlets from the explants. To obtain plantlets, the type and concentration of hormones varied with the species and cultivar as well as explants (Fonnesbech, 1972; Gu et al., 1987; Kim and Kako, 1984a; Matsui et al., 1970; Ueda and Torikata, 1968). It may be due to a wide range of adaptation of this genus from epiphytes to terrestrials (Lin, 1977; Wu and Chen 1980). In a preliminary experiment, we observed that the color, size, number and growth behaviors of PLB varied among cultivars of *Cymbidium*.

There is a report on the development of PLB from the peripheral area of PLB (Kanase et al., 1993). Previously we reported that cells of vascu-

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lar bundle in sections of inner tissue (IT) of *Cymbidium* PLB have abilities to divide and to form new PLB. It was observed that IT did response to NAA and 2, 4-dichlorophenoxyacetic acid (2, 4-D), but failed to show morphological potentiality unless it was transferred to hormone-free MS medium (Begum et al., 1993). It seem that IT requires exceptional growth condition compared to other explants. In the present study we have described about the effects of growth hormones on formation of PLB and shoot development from OT of PLB, using the same cultivar reported previously (Begum et al., 1993) and also compared the developmental properties of OT of PLB with those of IT of PLB.

Materials and Methods

1. Plant materials

PLB of *Cymbidium* × Thanksgiving 'Nativity' derived from shoot tip culture, were propagated in the MS liquid culture medium (Murashige and Skoog, 1962) with 3% sucrose. pH of the medium was adjusted to 5.7 to 5.8 before autoclaving at 120 °C for 15 minutes. Culture flasks were incubated at 25° ± 1 °C on a rotary shaker at 1 rpm and lighted continuously with intensity about 20 μ mol · S^{-1} · m^{-2} received by white fluorescence lamps (FL 20 S. W. Toshiba, Tokyo). Newly developed PLB were maintained by subculturing into the same medium at 1 month intervals and PLB were used for experiments.

2. Preparation of OT explants

The PLB of 3～4 mm in diameter were horizontally sectioned into two or three pieces at a right angle with the main axis aseptically under a zoom stereomicroscope. Then these pieces were peeled by taking off a sheet of epidermal cells. This sheet was designated as OT and it was composed of 10 to 12 layers of cells both with epidermal and subepidermal tissue as described by Morel (1974). Several slices of OT were obtained from one PLB. The OT slices which have neither buds nor any outgrowth were used as explants (Fig. 1-A).

3. Effects of growth hormones on growth and development of PLB and shoot from OT of PLB

The OTs were inoculated on MS solid media (pH 5.7 to 5.8) supplemented with NAA and BA alone or in combinations (from 0.1 mg · $liter^{-1}$ to 1 mg · $liter^{-1}$). Hormone-free MS medium was used as control. Ten slices of OT were used for each treatment (each flask contained 5 slices of OT). This experiment was repeated three times. The flasks were incubated at a temperature of 25° ± 1 °C under light intensity obout 30 μmol · S^{-1} · m^{-2} for 16 hours per day, received from white fluorescent lamps (FL 20 S. W. Toshiba, Tokyo). Growth and development of new tissue or organs were observed weekly. A protuberance developed into a green globular structure was considered as PLB (Fig. 1-C) and PLB with two leaf primordia was considered as shoot (Fig. 1-D).

4. Histological observations.

To observe formation and development of PLB, the OT samples of 0, 7, 10, 14, 21 and 28 days of culture were fixed in FAA, which was prepared by formalin, acetic acid and ethanol (50% v/v) at the ratio of 5 : 5 : 90 respectively, dehydrated, embedded in paraffin, cut longitudinally into 10～12 μm section, stained with Delafield's hematoxyline and observed under a light microscope.

To clarify the initial stage of PLB formation, OT segments cultured for 7 days and 14 days were chosen to observe the cell division activity (from metaphase to anaphase stage), direction of anticlinal and periclinal cell division and to measure the cell sizes.

In case of OT segments cultured for 7 days (Fig. 2-B), all the meristematic cells, which were composed in a group and distinct from surrounding cells (about 15 to 20 cells in width and 3 to 8 cells in depth) were observed under a light microscope (magnification, ×400).

In case of OT segments cultured for 14 days (Fig. 2-D), the cells of apical meristematic area of globular protuberance (left, central top and right sites of cut section) were chosen. For each of three areas of a protuberance, the cells from surface (about 25 cells in width) to inside (about 10 cells in depth) were observed. Cell division frequency (%) was calculated by the formula, (number of dividing cells/total number of cell in a layer) × 100. For the above observation, three consecutive sections at the middle of selected samples were used.

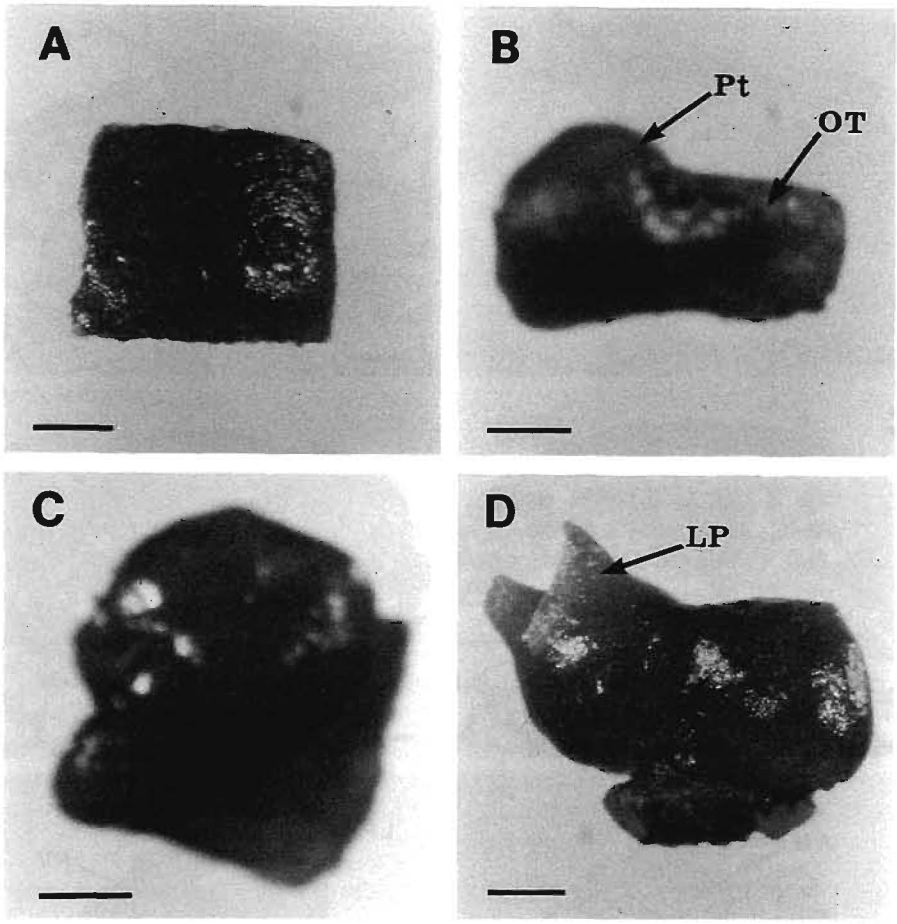

Fig. 1. *Different stages of PLB formation from the outer tissue (OT) segment of PLB in MS medium supplemented with NAA 0.1 mg · liter^{-1} and BA 0.5 mg · liter^{-1}. A : OT at zero day. B : protuberance on the surface of the OT after 14 days culture. C : globular PLB after 21 days culture. D : PLB with leaf primordia after 35 days culture. OT, outer tissue; Pt, protuberance; LP, leaf primordia. Scale bar=1 mm.*

Results

1. Effects of NAA and BA on PLB formation from OT of PLB

OT of PLB produced PLB on hormone-supplemented media as well as hormone-free MS medium (Table 1). The initiation of PLB formation was observed earlier in hormone-supplemented media, on 14 days after culture. Whereas, in hormone-free MS medium, initiation of PLB formation was delayed and observed on 21 days after culture.

The pattern of PLB formation was similar in both hormone-supplemented and hormone-free MS medium. Explants turned light brown after 7 days of incubation. A gray transparent protuberance was observed on the outer surface of the explant after 14 days of culture (Fig. 1-B). This protuberance was gradually increased in size and turned into a green globular PLB after 21 days (Fig. 1-C). A leaf primordium was also observed on PLB within 35 days (Fig. 1-D).

Media supplemented singly with NAA or BA increased the number of explants which produced PLB at higher concentrations of both hormones

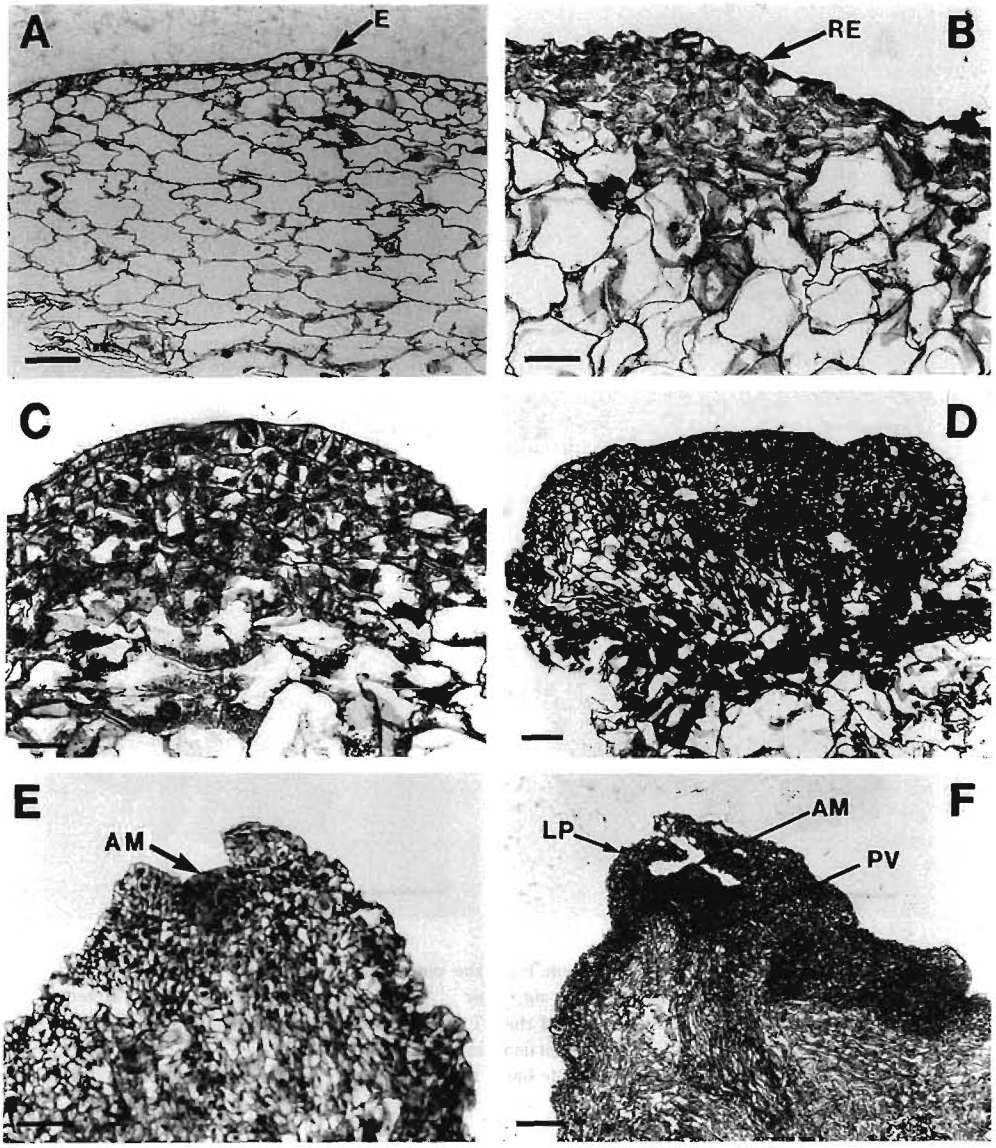

Fig. 2. Histological observation of PLB formation from the outer tissue (OT) segment of PLB in MS medium supplemented with NAA 0.1 mg · liter^{-1} and BA 0.5 mg · liter^{-1}.
A : section of OT segment of PLB at zero day. B : section of OT segment after 7 days culture. C : longitudinal section of protuberance after 10 days culture. D : longitudinal section of globular protuberance after 14 days culture. E : longitudinal section of globular structure showing shoot apical meristematic tissue after 21 days culture. F : longitudinal section of PLB showing leaf primordia and provascular strand after 28 days culture. E, epidermis; RE, ruptured epidermis; AM, apical meristem; LP, leaf primordia; PV, provascular strand. Scale bar : A, B, D, E=100 μm; C=50 μm; F=200 μm.

(Table 1). Fifty three percent, 53% and 70% explants produced PLB on the media supplemented with 0.1, 0.5 and 1.0 mg · liter^{-1} NAA, respectively. Similarly, 63%, 73% and 83% explants formed PLB on the media supplemented 0.1, 0.5 and 1.0 mg · liter^{-1} BA, respectively. The media supplemented with 0.1 mg · liter^{-1} NAA and 0.5 mg · liter^{-1} BA showed the highest PLB forma-

Table 1. Effects of NAA and BA on PLB formation from outer tissue (OT) of PLB[z].

Treatment (mg · liter^{-1})		No. of PLB produced (days of culture)			
NAA	BA	7	14	21	28
0.0	0.0	0.0	0.0a	4.0a	6.3bc
0.1	0.0	0.0	2.7c	4.3ab	5.3a
0.5	0.0	0.0	2.0b	4.7bc	5.3a
1.0	0.0	0.0	2.7c	4.7bc	7.0cd
0.0	0.1	0.0	3.0cd	4.7bc	6.3bc
0.0	0.5	0.0	3.3d	5.3cd	7.3de
0.0	1.0	0.0	4.0de	6.0e	8.3fg
0.1	0.1	0.0	5.7fg	7.3f	8.0f
0.1	0.5	0.0	6.7g	9.3g	9.3g
0.1	1.0	0.0	4.0de	5.7de	8.7g
0.5	0.1	0.0	5.0ef	6.7f	7.3de
0.5	0.5	0.0	5.3f	7.3f	7.7ef
0.5	1.0	0.0	5.0ef	8.0fg	8.3fg
1.0	0.1	0.0	3.0cd	5.7de	6.0ab
1.0	0.5	0.0	5.3f	6.3ef	6.3bc
1.0	1.0	0.0	2.3bc	4.0a	6.0ab

[z] Values with different letters in the same column are significantly different at 5% level of probability according to Duncan's multiple range test.

Table 2. Effects of NAA and BA on shoot formation from protocorms derived from OT[z].

Treatment (mg · liter^{-1})		No. of protocorm produced shoots (days of culture)				
NAA	BA	35	42	56	70	90
0.0	0.0	0.0	0.0a	3.7cd	4.3cd	5.0ef
0.1	0.0	0.0	0.0	0.0	0.0	0.7a
0.5	0.0	0.0	0.0	0.0	0.0	1.0ab
1.0	0.0	0.0	0.0	0.0	0.0	2.3bc
0.0	0.1	0.0	2.7cd	5.3ef	5.3de	5.7g
0.0	0.5	0.0	3.3de	5.7fg	6.0ef	6.3g
0.0	1.0	0.0	3.0d	6.3gh	6.3fg	7.7hi
0.1	0.1	0.0	1.7bc	3.7cd	4.7d	6.7gh
0.1	0.5	0.7a	4.7f	7.0hi	7.3h	9.3j
0.1	1.0	1.0b	4.3ef	8.7j	8.7i	8.7ij
0.5	0.1	0.0	1.0ab	1.7a	3.7bc	5.0ef
0.5	0.5	0.0	1.3b	3.3bc	3.7bc	4.3de
0.5	1.0	0.0	3.0d	4.7de	5.3de	6.7gh
1.0	0.1	0.0	0.0a	1.7a	2.7a	3.3cd
1.0	0.5	0.0	0.7ab	2.7ab	3.0ab	3.3cd
1.0	1.0	0.0	3.0d	5.3ef	5.0d	5.3fg

[z] Values with different letters in the same column are significantly different at 5% level of probability according to Duncan's multiple range test.

tion. In this combination 93% explants produced PLB.

2. Effects of NAA and BA on shoot formation from OT-derived PLB.

The incubation of PLB was continued in the same flasks to observe shoot formation. Media supplemented with BA alone or with both NAA and BA showed earlier shoot initiation followed by hormone-free MS medium (Table 2). In the medium supplemented with NAA 0.1 mg · liter^{-1} and BA 1.0 mg · liter^{-1}, 100% of OT-derived PLB gave shoots within 56 days. In a few explants, more than one new PLB were also appeared along with shoot development.

Shoot initiation was markedly suppressed by NAA alone, compared to other hormone-supplemented and hormone-free MS media (Table 2). Shoot initiation in MS media supplemented with both NAA and BA, or with BA singly was observed on 35 days and 42 days, respectively. On the other hand the media supplemented with NAA alone produced shoot after 84 days of culture.

3. Histological study on the development of PLB from OT

OT having neither buds nor any outgrowth was used as explant. Histological study showed that OT segments consisted of epidermal and subepidermal cells, which were parenchymatous, large and vacuolated (Fig. 2-A). After one week culture, all the cells of epidermis were ruptured. A small group of dividing cells (about 15 to 20), with dense cytoplasm, less vacuolated and containing deeply stained nuclei was observed just below the ruptured cells of epidermis (Fig. 2-B). These cells increased in number and formed a protuberance after 10 days of culture (Fig. 2-C). It became globular in shape after 2 weeks of culture (Fig. 2-D). In the media supplemented with NAA and BA, the cells of protuberance divided both anticlinally and periclinally (Fig. 4-H) and protruded toward the epidermal layer. All cells of this globular

mass were small in size with dense cytoplasm and dark nucleus. Thus they were easily distinguished from the parent cells of the explants. The remaining parent cells of the explants started to degenerate third weeks after culture. Apical meristematic tissue of shoot with protoderm was observed after 21 days (Fig. 2-E). Provascular strand and leaf primordia were observed in samples 28 days after incubation (Fig. 2-F). Two or more meristematic areas were observed in some samples after 28 days of incubation (Fig. 3-D).

4. Effects of NAA and BA on cell division activity, direction of cell division and cell size of OT-derived protuberance

After 7 days of culture active cell division at the area of explants where PLB would form was observed in all the treatments (Figs. 4-A, B, C and D). The frequency of cell division ranged between 1.8% and 4.4%. At this stage, only in the combination of NAA and BA, cell division at the outer most cell layer (the first layer) was observed at a rate of 4.0%. This cell layer was located just inside of the epidermis. The direction of cell division in this case was equal in the rate between anticlinal-wise and periclinal-wise. And the cell size became significantly shorter in length (Table 3), compared to the hormone-free treatment (control).

In the hormone-free medium, cell division occurred only at second and third layers at the rate of 2.6% and 2.6%, respectively (Fig. 4-A) and the cell size was significantly longer and wider among all treatments (Table 3). In the other hormone treatments, cell division occurred in the tissue cells inside from the second layer (Figs. 4-B, C).

In the sample of 14-days-culture cell division at the three areas, i.e., left, central top and right areas of the apical region of protuberance was observed. In all treatments, the cell division at the central

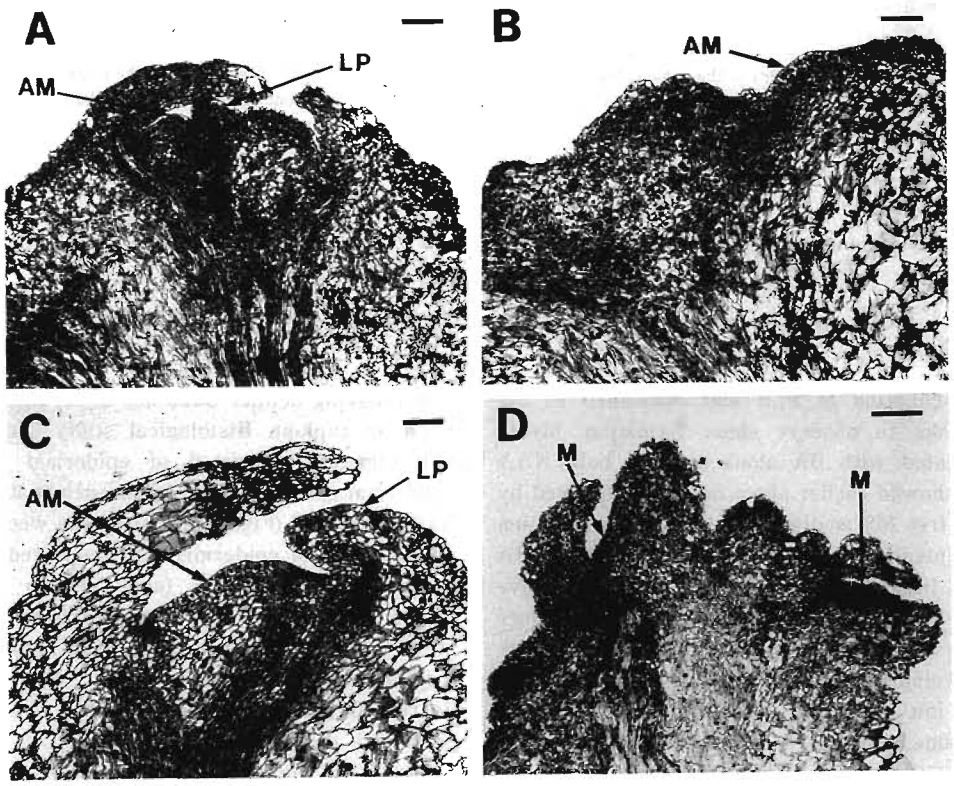

Fig. 3. Longitudinal sections of PLB after 28 days culture. A : hormone-free MS medium. B : MS media supplemented with : 1 mg · liter^{-1} NAA. C : 1 mg · liter^{-1} BA. D : 0.1 mg · liter^{-1} NAA and 0.5 mg · liter^{-1} BA. AM, apical meristem; LP, leaf primordia; M, meristematic area. Scale bar : A, B, C =100 μm; D=200 μm.

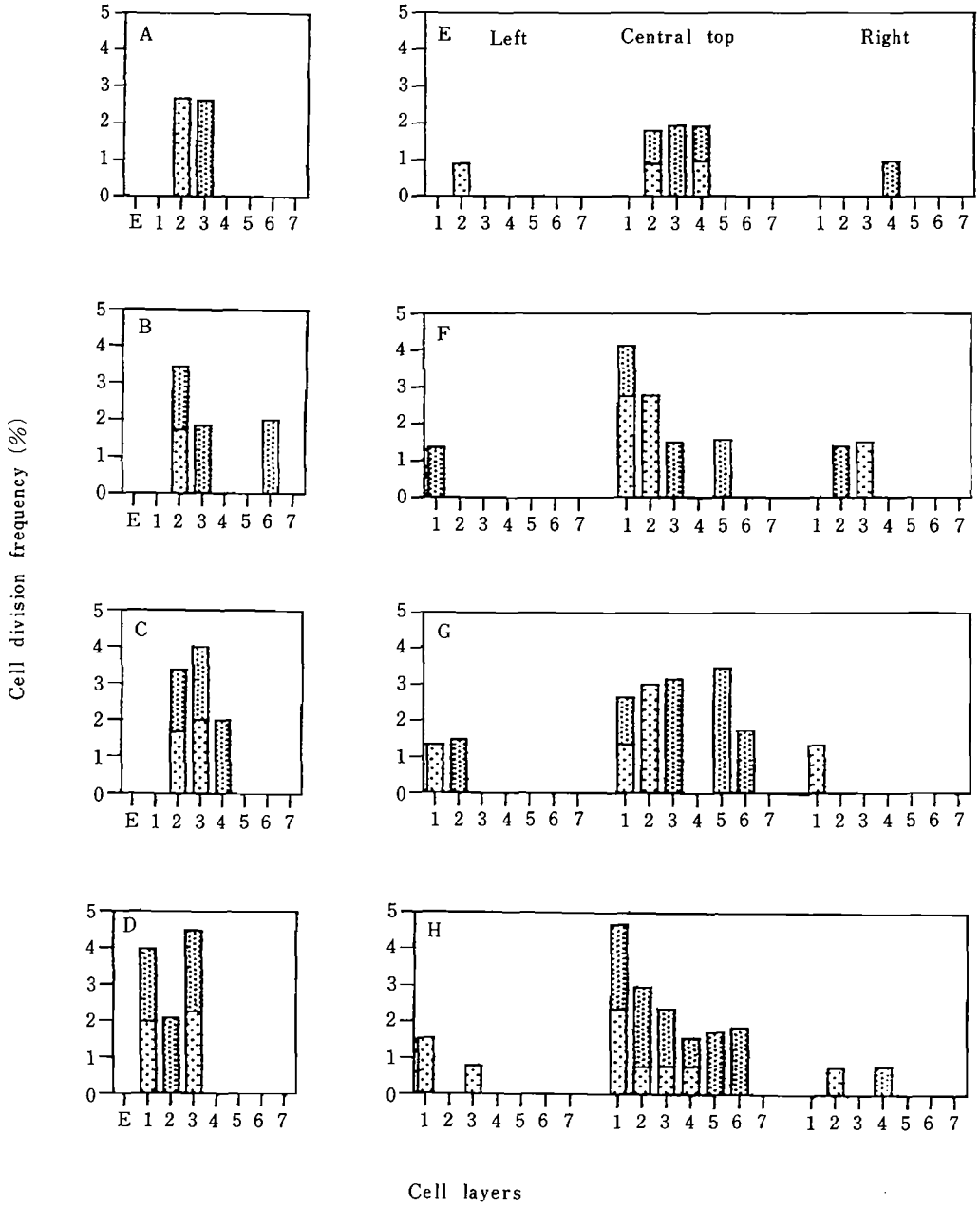

Fig. 4. Effects of growth hormones on cell division activities at the apical tissue of protuberances.

Figures from A to D are after 7 days culture and those from E to H are after 14 days culture. A, E; hormone-free. B, F; 1 mg · liter^{-1} NAA. C, G; 1 mg · liter^{-1} BA. D, H; 0.1 mg · liter^{-1} NAA and 0.5 mg · liter^{-1} BA.

The numbers (1 to 7) of abscissa mean the position of cells arranged in the order from outside toward inside of the protuberance tissue.

⊡ : represent anticlinal division; ⊞ : represent periclinal division.

E; epidermis.

Table 3. Effects of growth hormones on cell sizes[z].

Treatment	Cell sizes in μm[y] (days of culture) 7		14	
	L	W	L	W
Hormone-free MS (control)	40.7c	26.5b	29.3c	17.6b
NAA (1mg · liter^{-1})	28.9b	19.5a	26.3b	15.7a
BA (1mg · liter^{-1})	24.4a	19.3a	23.0a	15.6a
NAA (0.1mg · liter^{-1}) +BA (0.5mg · liter^{-1})	23.6a	18.2a	22.8a	15.3a

[z] At zero day, length and width of the cells were 57.6 and 31.6 μm in size, respectively.

[y] Values with different letters in the same column are significantly different at 5% level of probability according to Duncan's multiple range test.

L=Length; W=Width.

top area became higher in the rate and occurred from surface to deeper tissue in the protuberance (Figs. 4-E, F, G and H). There were differences in the direction of cell division between the outer and inner sides of the protuberance. The cells of outer layers divided into both anticlinal and periclinal direction, but the inner cells divided mainly periclinally. At the left and right side of the protuberance, cell division frequency was low compared to the central top area. The cell size at 14 days-culture-samples on the hormone-free treatment (control) became smaller compared with that of 7-days-culture-samples (Table 3).

In the control, no dividing cells were observed in the first layer at any side of the protuberance (Fig. 4-E). In the case of the left and right sides, cell division was limited only in the second and fourth layers respectively, and the cell divisions in the central top area were observed from the second to fourth layers and the division rate was low ranging from 1.8% to 2.0%.

In NAA treatment, cell division rate was higher in the three areas compared to control (Fig. 4-F). In the central top area, dividing cells were observed from the first to fifth layers and total number of anticlinally dividing cells were higher than that of periclinally dividing cells.

The cell division in BA treatment was also active, next to the combination of NAA and BA (Fig. 4-G). In this case, the number of periclinal cell division was higher than that of anticlinal division. The cells of the first two layers at the central top area divided mainly anticlinally but the cells inner than those layers divided only periclinally.

Cell division was most active in all the three regions when the explants were cultured on the medium with the combination of NAA and BA (Fig. 4-H). The cells of outer layers of the central top area, from the first to fourth, divided into both anticlinal and periclinal directions, at the rate ranging from 1.6% to 4.6%, but those cells located inner than the fifth cells divided only periclinally.

Discussion

There are many reports on micropropagation of *Cymbidium* using shoot tips or PLB as explants (Fonnesbech, 1972; Gu and Yan, 1989; Matsui et al., 1970; Morel, 1960, 1964, 1972; Sagawa et al., 1966; Ueda and Torikata, 1968; Wilfret, 1966; Wimber, 1963). Morel (1973) used cut sections of protocorms as explants and obtained protocorms from peripheral area but failed to obtain PLB from central core parenchyma of protocorms. In our previous study, we have reported that IT of PLB formed PLB indirectly through somatic embryogenesis under certain culture conditions, exogenous growth hormone, especially auxins, were essential for callus formation and this callus produced protocorms only when it was transferred onto the hormone-free MS medium (Begum et al., 1993). In contrast, the present study showed that OT of PLB produced new PLB directly from explants on both hormone-supplemented and hormone-free MS media (Table 1). This suggests that exogenous hormones are not essential for direct formation of PLB from OT.

The present study showed that PLB appeared almost at the same time on all the media supplemented with hormones (Table 1). The number of PLB was high on the media containing NAA and BA. It was also high on the media supplemented with NAA or BA alone at higher concentration. Although the number of PLB on the medium supplemented with 1 mg · liter^{-1} NAA was higher than the hormone-free MS medium, it was lower on the media supplemented with either BA singly or with combination of NAA and BA. This result suggests that PLB formation was enhanced by the addition of NAA to the basal medium (Table 1).

Gu et al. (1987) obtained higher number of PLB using shoot tips as explants on Vacin and Went medium supplemented with 1.0 mg · liter^{-1} BA. We also obtained higher number of PLB with 1.0 mg · liter^{-1} BA, although the highest number of PLB was observed in media containing NAA and BA (Table 1). Matsui et al. (1970) showed the effects of BA and NAA on the organogenesis of *Cymbidium*. They used PLB of *Cymbidium* × Mary Pinchess 'Del Mar' as explant and found that BA was the most effective hormone for PLB formation. This result did not agree with our results and this difference may be due to the different cultivar.

Matsui et al. (1970) also found that combinations of high concentration of BA and low concentration of NAA were the most suitable for shoot formation. As regards shoot formation, the results of the present study showed similar findings (Table 2).

Ueda and Torikata (1968) reported that Knudson C medium (with Nitsch's micro element) supplemented with 0.1 mg · liter^{-1} NAA produced shoots in *Cymbidium goeringii* within 8 weeks. But in the present study, it was revealed that NAA highly suppressed and retarded shoot formation. Kim and Kako (1983) obtained similar result as to ours, when shoot tips were used as explants. These results speculate that NAA may respond to different *Cymbidium* in different way.

Thus it may be concluded that NAA does not interfere for the PLB formation from OT but it highly suppressed shoot formation from PLB.

The rate of cell division was highest in the combination of NAA and BA followed by BA treatment. NAA acted effectively for cell division but it was less effective than BA. Hormone-free MS medium showed the lowest cell division activity (Fig. 4). These cell division activities in each treatment agree well with rates of PLB formation as discussed before and shown in Table 1.

Histologically it was observed that the shoot apical meristem of PLB was developed within 28 days on both hormone-free and hormone-supplemented media (Figs. 3-A, B, C and Fig. 2-F). However, shoot formation took a long time in NAA supplemented media. This might be due to the late initiation of leaf primordia and development of leaves (Fig. 3-B). Kim and Kako (1984b) found that in case of shoot tip culture, BA promoted cell division at the distal end of explants and resulted in increase of leaf number, and NAA suppressed leaf initiation and promoted swelling of the proximal end of explants. From these observations, NAA seems to retard the plastochron.

Anatomical observations revealed that the cell divisions of *Cymbidium* PLB are limited to the peripheral layers (Morel, 1972, 1974). This means that L-1 and L-2 layers (epidermal layers) of PLB explants have a capacity to produce new PLB (Stewart and Derman, 1970a, b). According to Morel's observation, the first cell divisions occur in the sub-epidermal layer and give rise to a small aggregate of cells. These cells divide very actively and develop a protocorm within a few days (Morel, 1974). The present study showed a similar pattern of the development of PLB from OT (Fig. 2), but the epidermis of OT explants was ruptured after 7 days of culture and it was not involved in PLB formation.

We have reported in our pervious study (Begum et al., 1993) that IT of PLB, consisting of vascular bundle and ground tissue produces protocorms through somatic embryogenesis. Initial cell division occurred in parenchyma cells of vascular bundles. Thus somatic embryos are L-3 origin. In case of OT, the explants consist of epidermal and parenchymatous cells. A small group of dividing cells was observed in sub-epidermal cell layers, which formed a protuberance. This protuberance developed into PLB. So it is concluded that OT differentiates new PLB from L-2 and L-3 layers.

There are fundamental differences in hormone requirement between IT and OT. In case of IT, globular embryos did not require any hormones to develop into protocorms. Supplements of either NAA, BA or the combination of NAA and BA totally suppressed the protocorm formation. But in case of OT, both hormones (NAA and BA) favored PLB formation.

This may be suggested that the PLB of *Cymbidium* × Thanksgiving, cultivar 'Nativity' proliferate through organogenesis or somatic embryogenesis, depending on both the nature of the explants and exogenous hormones.

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シンビジウムのPLB外部組織の培養によるPLBと苗条の形成

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摘　要

シンビジウムのPLB外部組織片（OT）を外植体とし，これをBAとNAAの各濃度で（0.1～1.0 mg・liter^{-1}）単独でまたは組み合わせて添加したMS培地で培養し，PLBと苗条の形成に対する影響を調べた．

1．OTからのPLB形成は，直接的な器官形成の過程を経て起こり，PLBの形成過程とその苗条への発達過程ではともに植物ホルモンの添加は不要であった．

2．OTからのPLB形成に対し，NAAはホルモン無添加の場合よりも促進的に作用し，本実験の処理濃度の範囲内では抑制作用を示すことはなかった．一方BAは単独でも促進作用を示し，NAAの共存下ではさらに強い促進作用を示した．

3．OTで形成されたPLBからの苗条形成の過程においては，NAAは単独では強い抑制作用を示した．一方BAは促進的に作用し，NAAの共存下ではさらに強い促進作用を示した．

4．PLB形成過程を組織学的に調べた結果，OTの表皮細胞層は培養7日頃から退化し始め，21日後には崩壊した．一方表皮直下の1群の下表皮細胞が培養7日後までには細胞分裂を起こし，外植体の上方向に組織を盛り上げ，14日後に球状突起となり，21日後にはPLBを形成した．培養7日と14日後の培養初期における細胞分裂活性は，植物ホルモンの影響を受け，NAAとBA両者の添加で最も高く，ついでBA，NAA，ホルモン無添加の順となり，これはPLBの形成率と一致した．また培養組織の細胞分裂の方向や部位にはホルモンによる相違がみられ，これについて考察を行った．