Cymbidiumの生長点培養における器官形成(第4報)

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Organogenesis in the meristem cultures of cymbidiums

IV. Study on cytokinin activity in the extracts from the protocorms

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Summary

The cytokinin activity of ethanol extracts from the protocorms of *Cymbidium insigne* ROLFE, *C. pumilum* ROLFE and *C. goeringii* REICHB. F. was examined by tobacco callus assay. The previous works with the tissue cultures of these three species have shown that the difference in organogenesis, especially in the kinetin-requirement for shoot formation, exists between the former two and the latter.

The extract of *C. insigne* or *C. pumilum* showed, at 100 gram fresh weight equivalent per litre, approximately the same activity as that of 10 $\mu g/l$ kinetin. The cytokinin activity of *C. goeringii* was considerably lower than those of above two species. It is assumed that low cytokinin activity is responsible for difficulty in shoot formation in the aseptic culture of *C. goeringgi*.

Introduction

Previous works[6,7,8] in this serial research have demonstrated that the terrestrial Cymbidium (*Cymbidium goeringii*) differed from the semi-epiphytic species (*C. insigne* and *C. pumilum*) in the developmental process of protocorms obtained from shoot meristem on the medium prepared artificially. Namely, the protocorm-pieces of *C. insigne* and *C. pumilum* regenerated the new protocorms and produced the shoots within 4 to 6 weeks in the dark, whereas those of *C. goeringii* developed into the rhizomes and showed no tendency to form shoot. Most samples of the latter were, however, led to shoot formation by addition of 10 mg/*l* kinetin to the medium. In the case of *C. insigne* and *C. pumilum*, kinetin exerted no remarkable effect on the shoot formation.

There arise a question that whether the kinetin-requirement for shoot formation depends on the endogenous cytokinin level of the protocorm of the species.

In the present paper, a comparative study on the cytokinin levels of the protocorms was carried out with above three species.

Materials and Methods

Protocorms: Stock cultures used in this experiment were originally isolated in 1966 from the shoot meristems of *Cymbidium insigne* ROLFE, *C. pumilum* ROLFE and *C. goeringii* REICHB. F[5]. These cultures have been maintained on the medium of KNUDSON C with NITSCH microelement by cutting and transplanting (22°C, dark). All protocorms used for materials were about 3 weeks old after transplanting.

Preparation of extracts: The protocorms (f.w. 15 g) were minced and allowed to stand through overnight at 4°C in 60 m*l* of 80% ethanol. The solid material was removed by centrifugation. Then the extract was evaporated at 40°C under reduced pressure. Dry residue

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was dissolved in 150 m*l* of MURASHIGE and SKOOG medium containing 2 mg/*l* IAA (100 gram fresh weight equivalent per litre). Serial dilutions of 1 : 10 and 1 : 100 (10 GE/*l* and 1 GE/1) were prepared.

Bioassay : Cytokinin activities of the extracts were assayed using tobacco callus (*Nicotiana tabacum* var. Wisconsin No. 38). Callus tissue was kindly supplied by Assoc. Prof. S. MATSUBARA, Kyoto Pref. University. The test media (1% agar) were distributed in aliquots of 10 m*l* in test tubes (14×110 mm) and were autoclaved at 1.0 kg/cm² for 15 minutes. Ten test tubes were set up for each experimental medium. The basal media with and without kinetin (10 μg/*l*) were designated as the controls. Pieces of callus (each ca. 20 mg) were cut out from the stock culture, and each piece was placed on the agar surface in the tube. They were maintained at 22°C in darkness for 5 weeks. At the end of experimental process, cytokinin activity was estimated by the increase in fresh weight of cultured tissue.

Results and Discussion

The ethanol extracts from the protocorms of *C. insigne*, *C. pumilum* and *C. goeringii* were tested for their growth-promoting activities on tobacco callus at the concentrations of 1, 10 and 100 gram fresh weight equivalent per litre (Table 1 and Figure 1).

On the medium without the extract or kinetin, tobacco callus often became brown and friable, and the increase of fresh weight was slight.

With 10 μg/*l* kinetin callus growth was greatly stimulated and newly-formed tissues appeared as white and compact masses of cells. The increase in fresh weight of callus was almost four times as much as that on the medium without kinetin or the extract.

The extract of *C. insigne* or *C. pumilum* did not stimulate practically callus growth at a level of 1 GE/*l*. However, the growth-promoting effect of the extract was apparent at a level of 10 GE/*l* both in *C. insigne* and *C. pumilum* ; the increase in callus fresh weight was almost two times as much as that on the medium without the extract. The largest increase in callus fresh weight, observed at 100 GE/*l* in both species, was almost equal to that on the medium supplemented with 10 μg/*l* kinetin.

The extracts from the protocorms of above two species obviously stimulated callus growth but caused sometimes brownish tissues.

On the other hand, the extract of *C. goeringii* did not promote practically callus growth at the concentrations of 1 and 10 GE/*l*. The activity was very slight even at 100 GE/*l*.

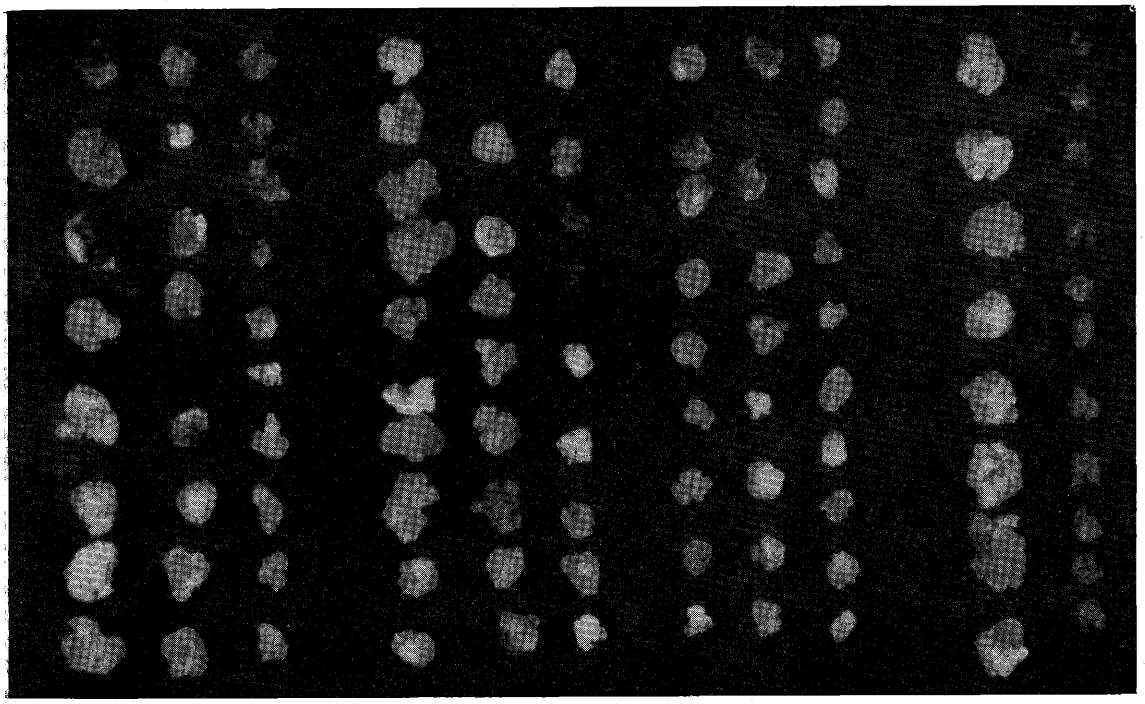
The results of the present experiment with the crude extract may suggest that the endogenous cytokinin-level in the protocorm of *C. goeringii* is considerably lower than that in the protocorm of *C. insigne* or *C. pumilum*.

As demonstrated in the previous works[7,8], the addition of kinetin to the culture medium was required for the shoot formation in the aseptic culture of *C. goeringii*. It may be suggested

Table 1. Effects of different concentrations of ethanol extracts from the protocorms of *C. insigne*, *C. pumilum* and *C. goeringii* on the growth of tobacco callus.

Additives		Average fresh weight per piece (mg)
—		119
C. insigne	1 GE/*l*	121
	10	257
	100	434
C. pumilum	1	123
	10	268
	100	429
C. goeringii	1	124
	10	126
	100	149
kinetin	10 μg/*l*	442

GE/*l*: gram fresh weight equivalent per litre.



A B C D E F G H I J K

Fig. 1. Effects of different concentrations of ethanol extracts from the protocorms of *C. insigne*, *C. pnmilum* and *goeringii* on the growth of tobacco callus.
A, B, C; *C. insigne*, 100, 10, 1 GE/*l*, D, E, F; *C. pumilum*, 100, 10, 1 GE/*l*, G, H, I; *C. goeringii*, 100, 10, 1 GE/*l*, J; 10 μg/*l* kinetin, K; no kinetin.

that the low level of endogenous cytokinin is responsible for the difficulty in the shoot formation from the protocorm (or rhizome) of *C. goeringii* cultured on the medium of KNUDSON Cma with NITSCH microelement. Histological study on the rhizome-shoot differentiation of this species[8] supports this concept. In the rhizome cultured on the medium of KNUDSON C with NITSCH microelement, there were observed a poor meristematic activity in the apical region and rapid aging of the cells in the leaf primordia and subapical region. The addition of kinetin to the culture medium appeared to promote the meristematic activity and prevent the rapid aging of the cells to cause shoot differentiation[8].

When the seeds of *C. goeringii* germinate in nature, they form the rhizomes which grow into the ground. The rhizome, in which abundant mycorrhizal fungi are always observed, produces aerial shoot after a period of underground life.

The possibility is considered that the mycorrhizal fungi take part in supplying cytokinin and other substances necessary for shoot formation to the orchid. Further works are required to examine these possibilities.

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プロトコーム抽出液のサイトカイニン活性について

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摘　要

Cymbidium insigne, C. pumilum（キンリョウヘン），*C. goeringii*（シュンラン）の三種を用い，プロトコームのエタノール抽出液のサイトカイニン活性をタバコカルス（*Nicotiana tabacum* var. Wisconsin No. 38）によるバイオアッセイで調べた。*C. insigne, C. Pumilum* では共に 10 GE/*l* 以上の濃度で活性を示し，100 GE/*l* で 10 μg/*l* kinetin とほぼ等しい活性が見られた。*C. goerringii* は前二種に比しサイトカイニン活性は著しく低かつた。

C. insigne, C. pumilum（熱帯産，半気生）と *C. goeringii*（温帯産，地生）との間にはサイトカイニン形成能力に遺伝的な差のあることが考えられ，無菌培養における shoot 形成の難易の差を生ずる一つの原因と思われる。