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FUNDAMENTAL STUDIES ON THE ASYMBIOTIC SEED GERMINATION OF *CALANTHE*

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エビネの種子発芽に関する基礎的研究

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Factors affecting the seed germination of *Calanthe* species were examined, using the seeds of *Calanthe discolor* LINDL. and *C. discolor* LINDL. x *C. sieboldii* DECNE.

The airtightness of culture vessels had little effect on seed germination and plantlet formation, but the growth of plantlet was better in the vessels with single-hole rubber stopper.

The size of vessels had no effects on the germination rate and number of days to germination, but the growth of plantlet was better in large vessels than in small ones.

The germination rate was the highest when the seeds were sown immediately after harvest, and decreased as they were stored for 2 months or more longer. The lowering of germinability could be prevented to some extent by storage under dry conditions, independently of the storage temperatures.

The viability of seeds could be perceived by the 2,3,5-triphenyltetrazolium chloride (TTC) test.

エビネ（*Calanthe discolor* LINDL.）およびエビネ×キエビネ（*Calanthe sieboldii* DECNE.）の種子を用いて，エビネの種子発芽に影響をおよぼすいくつかの要因について検討した．

容器の気密性は発芽率および幼苗率に影響しなかったが，幼植物の生長は密栓よりも通気栓ですぐれた．

容器の大きさは発芽率および発芽に要する日数に対して影響しなかったが，幼植物の生長にはかなりの影響を与えた．一般的に言えば大きい容器ほど良い結果を示した．

発芽率は取り播きで最高となり，貯蔵期間が長くなると低下した．

種子を乾燥状態で貯蔵することにより，ある程度は種子の活力低下を軽減できた．

種子の活力はＴＴＣ反応によりある程度予知できた．

Introduction

Calanthe is a genus of 150 species of terrestrial orchids, extending from South Africa and Asia to the Pacific Islands; while only a species has been found in the West Indies and Central America(2). In Japan, 17 or 19 species of *Calanthe* grow naturally, and we Japanese have enjoyed the beautifulness and the variations in color and shape of these flowers from old times

(about the 15th century)[2,4]. In recent years the culture of *Calanthe* comes into fashion. However there is undersupply for the demand of *Calanthe* seedlings because of the difficulty of the propagation by seeds and tissue culture. Nowadays, supply of the seedlings is mostly owing to gathering of native one or dividing of cultivated one.

Present study was carried out in order to make clear some factors related to the asymbiotic seed germination of *Calanthe*.

Materials and Methods

The seed and embryo of *Calanthe* are very small (Table 1). The plant materials used as seeds in this study are as follows:

(Sign)

A *C. discolor* LINDL. Self pollinated '74. 4. 29
Harvested '74. 11. 6

B *C. discolor* LINDL. Self pollinated '74. 4. 29
Harvested '74. 11. 6

C *C. discolor* LINDL. x *C. sieboldii* DECNE.
Cross pollinated '74. 4. 24
Harvested '74. 11. 6

D *C. discolor* LINDL. (var. *viridi-albi* MAXIMOWICZ) x *C. sieboldii* DECNE.
Cross pollinated '74. 4. 24
Harvested '74. 11. 6

After the pretreatment with 0.1M KOH for 10 min, the seeds were rinsed with about 1 litre of sterilized water, sterilized with the supernatant of calcium hypochlorite solution (5g of calcium hypochlorite per 250ml of sterilized water) for 3 min and sown on agar medium in the culture vessels. The medium contained the ingredients consisted of 3g Hyponex (7-6-19), 2g of Bacto-peptone, 35g of sucrose and 15g of agar per litre (H medium)[6], and according to circumstances 2g of activated charcoal was added (HA medium). The pH value of the media after autoclaving was around 5.0. The culture vessels were laid under 16 hour-light period illuminating with the plant grow lumps (about 400-500 lux) at 25°C.

The 2,3,5-triphenyltetrazolium chloride (TTC) test was performed for the germinability of the seeds. About 180 seeds of each lot placed on the slide glass in a moisten Petri dish were incubated at 20°C for three days in a dark room. After the incubation, 0.1% solution of TTC was added to the seeds, and the Petri dishes were transferred to the dark room at 30°C and kept for 24 hr.

Table 1. Size of seed and embryo of sevsral *Calanthe* species

(Average of 50 seeds)

	Seed		Embryo	
	Length(mm)	Width(mm)	Length(mm)	Width(mm)
C. amamiana FUKUYAMA	0.64	0.12	0.11	0.07
C. discolor LINDL.	0.75	0.12	0.14	0.07
C. izu-insularis OHWI et SATOMI	1.11	0.15	0.17	0.10
C. sieboldii DECNE.	0.73	0.15	0.12	0.08
C. tricarinata LINDL.	1.00	0.15	0.18	0.10
C. discolor LINDL. x *C. sieboldii* DECNE.	0.60	0.13	0.13	0.09
C. discolor LINDL. x *C. sieboldii* DECNE.	0.77	0.13	0.15	0.08

Results

1. Effect of airtightness of the culture vessels on the seed germination and the growth of seedlings

The seeds 'A', 'C' and 'D' were sown in the vessels and plugged with two kinds of stoppers, one of which was normal rubber stopper and the other was the single-hole rubber stopper stuffed with cotton. There was little difference in seed germination and plantlet formation between the airtight and aerated vessels (Table 2). However, the growth of plantlet, especially roots, was better in the aerated vessels than the airtight (Table 3).

2. Effect of the size of culture vessels on the growth of seedlings

The seeds 'B' and 'D' were sown uniformly on HA medium. The size of vessels had no effect on the germination rate and number of days to germination. The growth of seedlings in 200 and 300ml vessels was superior to that in 50 and 100ml vessels. Similar tendency was shown for the fresh weight of seedlings (Fig. 1).

3. Effect of storage conditions on the germination and the longevity of seeds

The seeds 'A' and 'C' were stored under the various conditions, i. e., (1) at 20°C, (2) at 20°C with dry condition, (3) at −15～−17°C (in the freezer) and (4) at −15～−17°C with dry condition.

The seeds were sown on HA medium in the test tube (20 x 200mm) at two months intervals. Percentage of the immatured seeds of 'A' and 'C' were about 20 and 5%, respectively. When the seeds were sown immediately after the harvest about 71.5% of the seed 'C' and 35% of the seed 'A' were germinated. The germination rate decreased gradually as the storage time was prolonged. The seed 'C' stored at 20°C with dry conditions, however, showed a temporal rise of the germination rate after the storage for 6 to 8 months. The both seeds stored at 20°C did not germinate after the storage for 8 months (Fig. 2).

The seeds stored at 20°C with dry conditions for 13 months was stained at high percentage by the TTC test, and the degree of staining was approximately the level 2 in the seed 'C' stored only at 20°C with dry conditions and the seed 'A' stored at the three conditions except in the normal conditions at 20°C. The viability of the seeds stored at 20°C without dry condition was lost rapidly, and most of the seeds died within a year (Fig. 4).

Table 2. Effect of airtightness of the culture vessels on seed germination and plantlet formation of *C. discolor* LINDL. and *C. discolor* LINDL. x *C. sieboldii* DECNE.

Kind of plug	*C. discolor*		*C. discolor* x *C. sieboldii*	
	Germination(%)	Plantlet(%)	Germination(%)	Plantlet(%)
Rubber stopper	33.7	83.9	69.7	56.1
Single-hole rubber stopper	35.0	79.8	71.5	69.8

Data were recorded at 176 days after sowing.

Table 3. Effect of airtightness of the culture vessels on the growth of seedlings of *C. discolor* LINDL. x *C. sieboldii* DECNE.

	No. of culture vessel					
	1	2	3	4	5	Av.
Degree of shoot growth						
Normal rubber stopper	1	1	1	1	1	1.0
Single-hole rubber stopper	1	1	2	1	3	1.6
Degree of root growth						
Normal rubber stopper	0	0	0	0	0	0
Single-hole rubber stopper	2	2	1	1	3	1.8

Degree of shoot and root growth were represented by index as follows:
0: No growth 1: Poor 2: Good 3: Excellent
Data were recorded at 84 days after sowing.

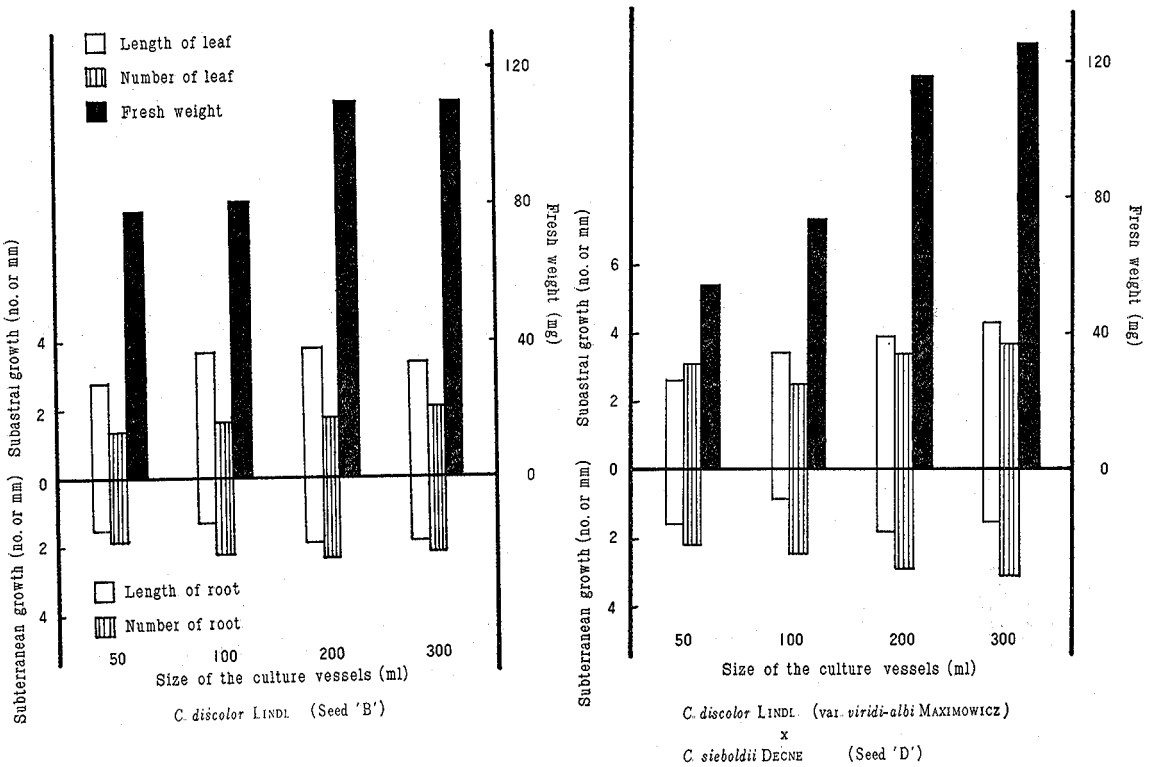

Fig. 1. Effect of the size of culture vessels on the growth of *Calanthe* seedlings. Data were recorded at 175 days after sowing.

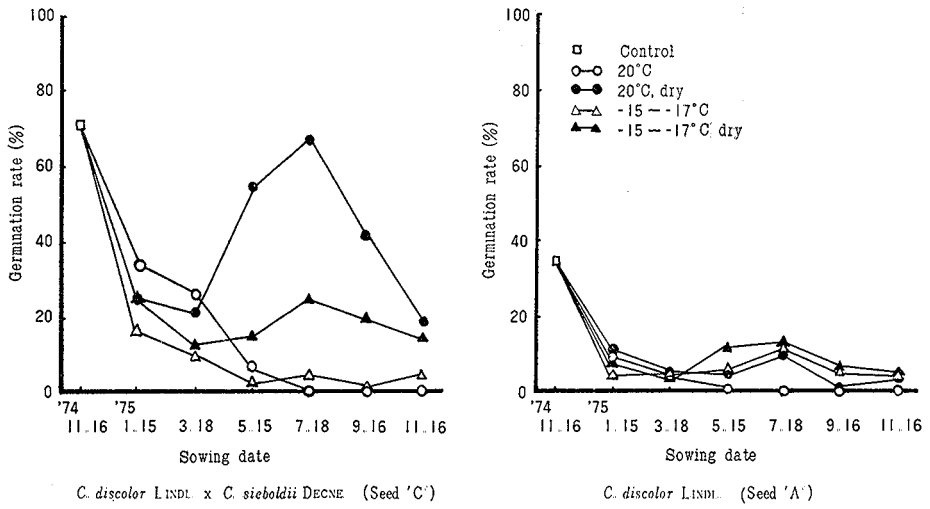

Fig. 2. Effect of storage conditions on the seed germination of *Calanthe*. Data were recorded at 200 days after sowing.

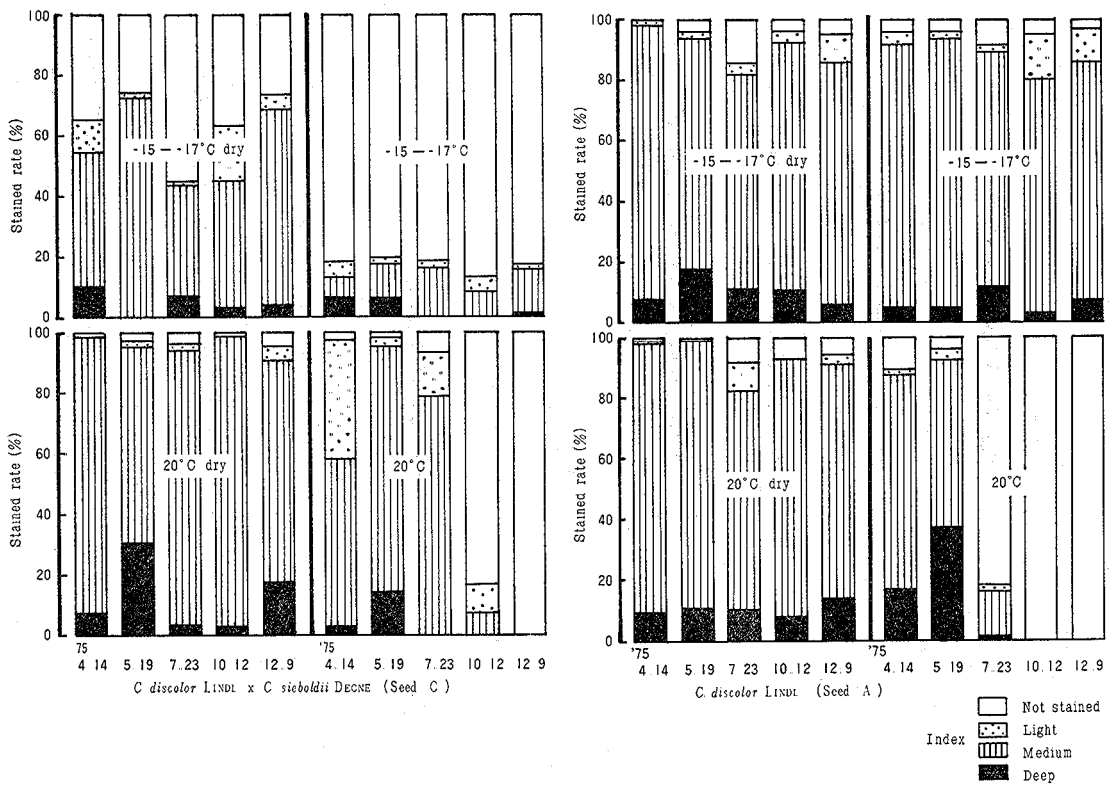

Fig. 3. The stained rate of the stored *Calanthe* seeds in the TTC test.

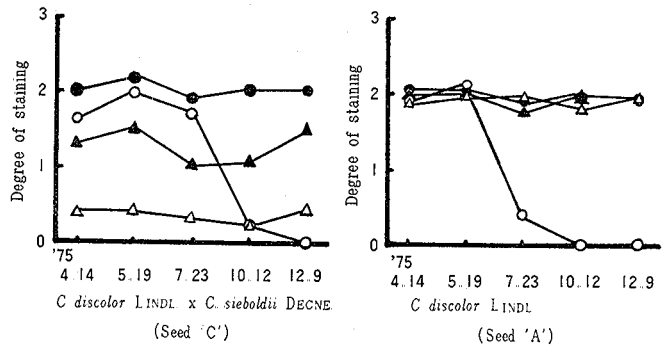

Fig. 4. The change of staining of the stored *Calanthe* seeds in the TTC test.

Index
0: Not stained
1: Light
2: Medium
3: Deep

Discussion

In general, asymbiotic seed germination of *Calanthe* is difficult as in the case of other terrestrial orchids. The germination rate differs depending upon the species and the circum-

stances surrounding the seeds. The seeds of *C. discolor* LINDL. and *C. tricarinata* LINDL. will not germinate by any procedure tried so far, but those of *C. sieboldii* DECNE., *C. bicolor* LINDL. and *C. furcata* BATEMAN germinate easily, i. e., about 0.1%, in some case as high as 30% of these seeds may germinate[2]. The germination rate, however, may differ in compliance with crossing and clone or individuals in the same species.

In this study, the seeds of *C. discolor* and *C. discolor* x *C. sieboldii* were germinated easily, particularly when the seeds were sown immediately after the harvest (Fig. 2). The germinability of the seeds, however, decreased gradually, depending upon the storage conditions with the lapse of storage time. The results of the TTC test was in accord, to some extent, with the germinability (Figs. 3 and 4). However, deep staining with the TTC test did not always correlated with germinability of *C. discolor* seeds (Figs. 2-4). These results suggested that the requirements for seed germination differ among species, clone and even individuals.

It is well known that the storage conditions affect the longevity of orchids seeds[3,5]. In *Calanthe* the desirable storage condition has not been determined. The results obtained in this study suggest that the dry condition is favourable to the longevity of *Calanthe* seed, and that the seed can not maintain the germinability at high level more than 12 months even if they are stored in such condition. The lower temperature about −15～−17°C did not prevented the seeds from the loosing of germinability. Ito reported that the pollen of *C. furcata* and *Dendrobium nobile* can be stored successfully for 2 years or more at ultra-cold temperature (−79°C)[1]. This methods may be applied to the *Calanthe* seeds. The reason why the germination rate is rised from 6 to 8 months in the seed 'C' particularly are remained unclear.

The airtightness of the culture vessels had little effect on seed germination and plantlet formation, but affected badly for the growth of plantlet. This suggest that the plantlets demand much air supply for the growth. If the vessels were left in the airtightness, the plantlets will fade and die. It is thus recommended that when the plantlets formed roots (Fig. 14), they should be transplanted to a new subculture medium in aerated vessels. In that case the plantlets may grow into the good and strong seedlings with wide and thick leaves in dark green, providing good seedlings for potting. HA medium is recommended for the subculture medium because activated charcoal prevents the roots of plantlet from upwards apart from the medium (Fig. 15).

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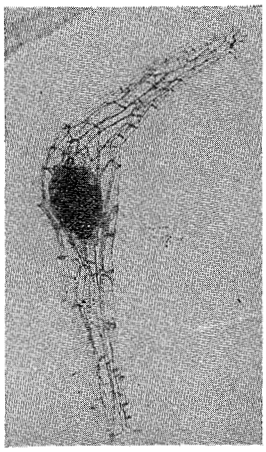
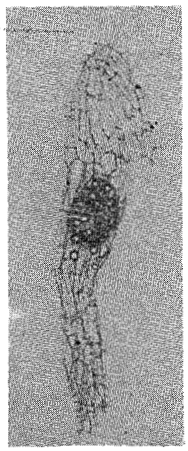


Fig. 5



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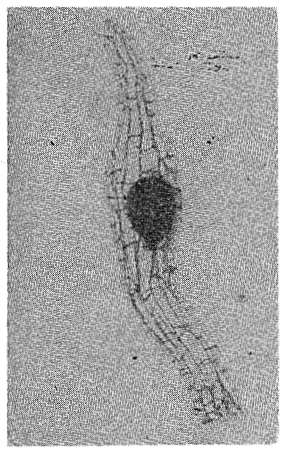

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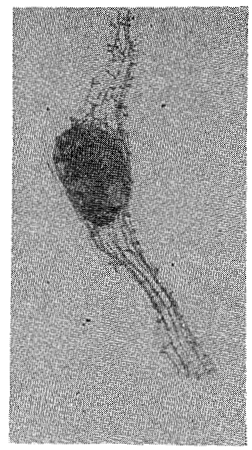

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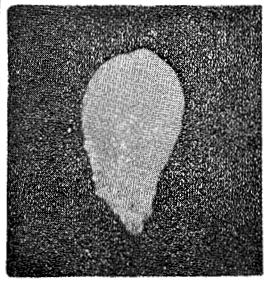


Fig. 9

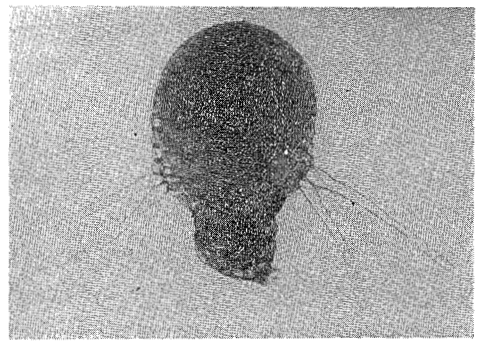
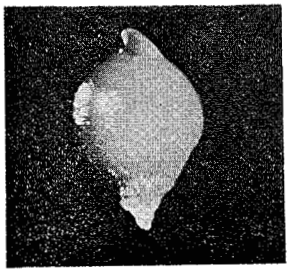
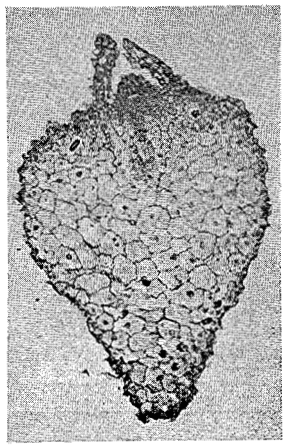


Fig. 10





(a) (b) (c)

Fig. 11

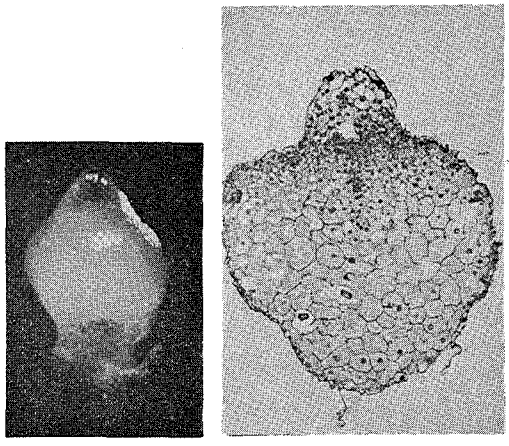


Fig. 12

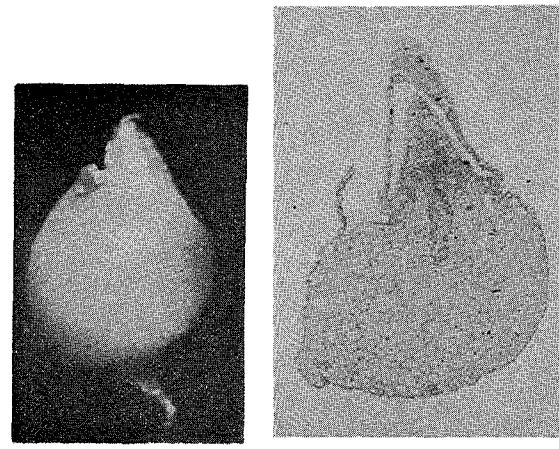


Fig. 13

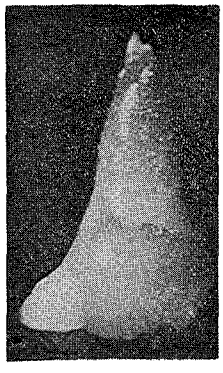
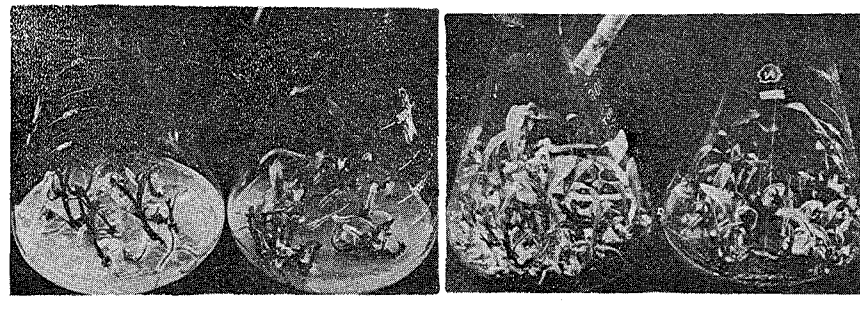


Fig. 14



(a) (b)

Fig. 15

Figs. 5～15. Stages in the germination of *Calanthe* seed and seedling development. (5) Substantial seed, (6) Immatured (blasted) seed, (7) Seed swollen and obvious suspensor, but still covered with testa, (8) Seed swollen more, (9) Embryo emarged from testa, (10) Embryo with five rhizoids, (11) Young protocorm (a) and longitudinal section (b). Vascular bundle was formed at this stage (c), (12 and 13) Young protocorms which external appearance changed from the spindle-shaped (see Fig. 11-a) to the swollen in the lower part-shaped, (14) Plantlet showing one root, (15) Subculture of seedlings: (a) H medium; the roots grown upwards, (b) HA medium; the roots submerged agar medium.