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Growth and Gemma-cup Formation in Relation to Archegoniophore Protrusion in *Marchantia polymorpha* L.

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Abstract

By aseptic culture of *Marchantia polymorpha*, gemma-cup and archegoniophore formation were studied in relation to the time course of growth under various culture conditions. Gemma-cup formation was accelerated by prolonged illumination at a relatively low light intensity and by sucrose added to culture medium. Environmental factors inducing archegoniophore were examined. A suppression of gemma-cup formation was observed about 20 days before archegoniophore protrusion.

Many physiological investigations on the growth of *Marchantia polymorpha* have been published. On gemma-cup formation, Voth (1941, 1943) pointed out the importance of calcium and other inorganic elements. Carter and Romine (1969), Kaufhold (1941) and Voth and Hamner (1940) described that short-day condition favored gemma-cup formation, though vegetative growth was better under long-day. Gametangiophore formation has been discussed in relation to culture conditions by many authors (Anthony, 1962; Benson-Evans, 1964; Courtoy, 1964, 1966; Dachnowsky, 1907; Kaufhold, 1941; Lilienstern, 1930; Lloyd and Steinmetz, 1937; Miller and Colaiace, 1969; Voth and Hamner, 1940 and Wann, 1925). They suggested that the gametangiophore formation was accelerted by or further depended on long-day cnodition under incandescent light or natural diffused daylight.

But there have been few studies on the time course of growth from a gemma up to a plant with sexual reproductive organ. Based on the results of examination of the growth process under various light intensities and photoperiods with changing sucrose concentration in the culture medium, I tried to clarify the dependency of sexual or asexual organ formation on culture conditions and also on the stage of growth.

Material and Methods

Thalli of *Marchantia polymorpha* were collected in the residential area of Morioka

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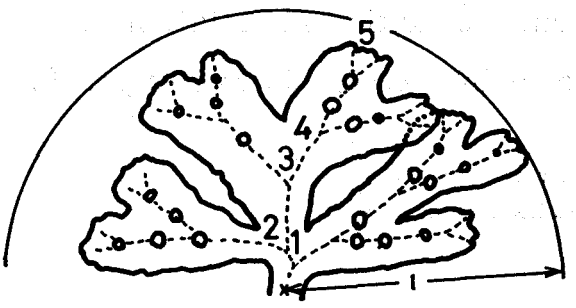

Fig. 1 A schematic drawing illustrating dichotomization times (D.T.) and thallus length. 1–5: diverging points of midrib. D.T. is five in this example. *l*: thallus length, always measured the longest lobe in each individual. Circles along the midribs indicate gemma-cups.

city. In order to use gemmae of the same genetic nature, one gemma was taken from a female plant and was cultured on sand with Voth's No. 5 inorganic nutrient solution (Voth, 1943), under natural diffused daylight. Gemmae taken from a gemma-cup on a plant grown on sand were sterilized with 0.1% Antiformin for five min, rinsed repeatedly with aseptic distilled water. The sterilized gemma was inoculated onto nutrient agar in a flask plugged with non-absorbent cotton. Later, when the aseptically produced gemmae became available, they were used as convenient material without sterilization.

The basal culture medium for aseptic culture was prepared after Kaul et al. (1962). Sucrose was added to the medium at various concentrtions. Each 100 m*l* of the nutrient solution with 1% Bacto agar Noble (Difco) in a 300 m*l* Erlenmeyer's flask was autoclaved at 120°C for 20 min. The basal medium without agar and sucrose was pH 4.4 to 4.5 before autoclaving.

Light source was incandescent lamps (Toshiba Co.). Light intensity was measured using selenium photocell Type-5 (Toshiba Co.). Temperature was kept at 23±2°C during the culture.

As the growth parameters, dichotomization times (D.T.), length of thallus, fresh weight, thallus area and the numbers of gemma-cups and archegoniophores were recorded. D.T. is defined as the number of midrib branching in a lobe which dichotomized most repeatedly in a plant as illustrated in Fig 1. Length of thallus (*l* in Fig. 1.) indicates the distance between the position of inoculated gemma and the terminal of the longest thallus lobe. Fresh weight was measured rapidly after careful removal of agar. Area of thallus was measured using Automatic Area Meter (Hayashidenko Co., Type AAM-5). When lobes overlapped or curled at the margin, they were cut and measured for exact estimation of the total area.

Results

Effects of photoperiod and sucrose concentration on the growth process

In the first experiment, effects of photoperiod and sucrose concentration were examined. Photoperiod was 16L/8D (LD) or 10L/14D (SD) and the light intensity was

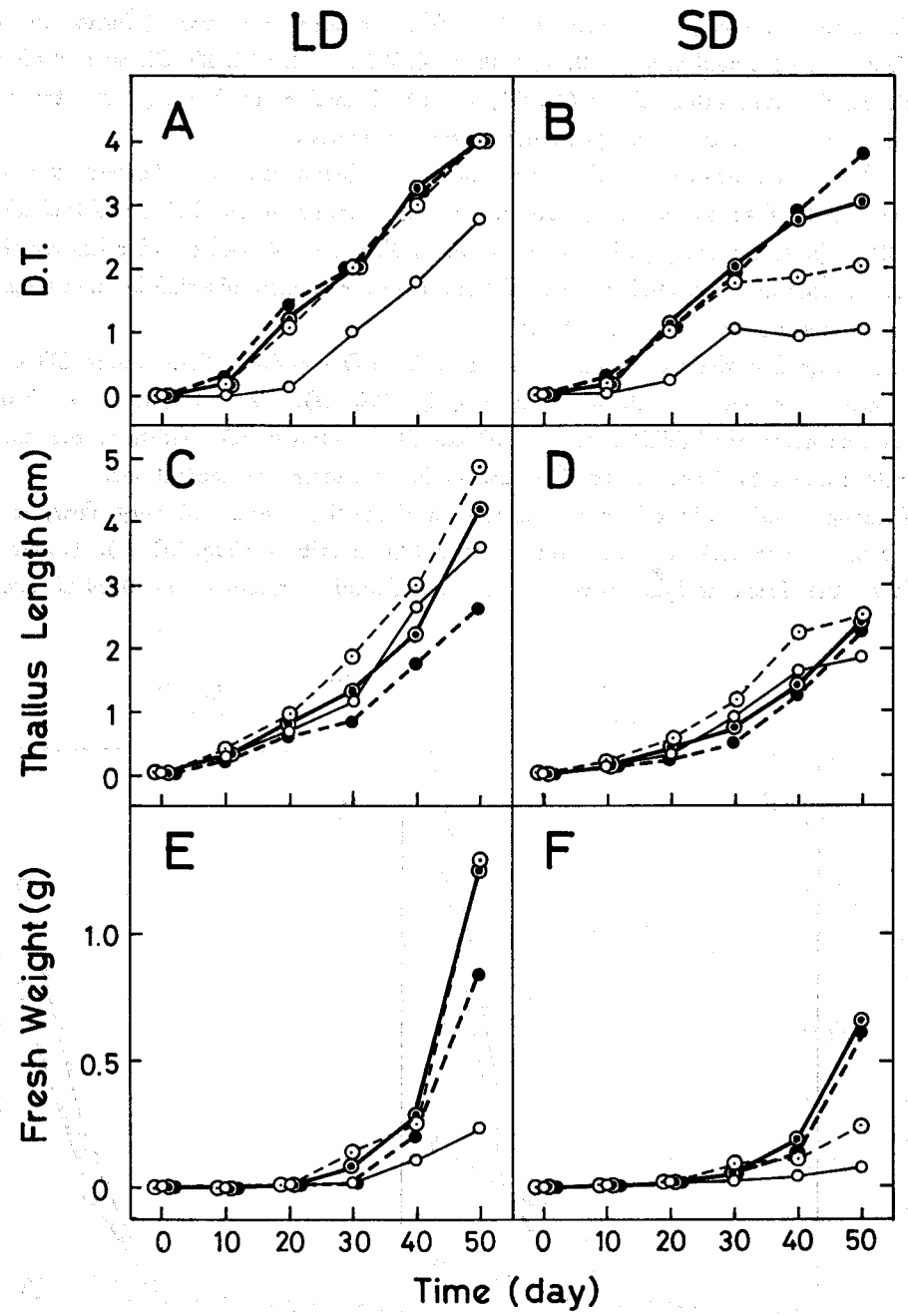

Fig. 2 Time courses of the increase in dichotomization times (D.T.) (A, B), thallus length (C, D) and fresh weight (E, F). LD (A, C, E) : under 16-hour photoperiods; SD (B, D, F) : under 10-hour photoperiods. Averages of six cultures. Symbols: ○——○, basal medium ; ⊙-----⊙, 0.03 M sucrose ; ⊙——⊙, 0.06 M sucrose ; ●-----●, 0.09 M sucrose.

1800 lx at the level of plant materials. The concentrations of sucrose (Kanto Chemical Co., G.R. grade) tested were 0, 0.03, 0.06 or 0.09 M. After 10, 20, 30, 40 and 50 days of culture, dichotomization times (D.T.), lenght of thallus, fresh weigt and the numbers of gemma-cups and archegoniophores were measured.

In the plant grown on the basal medium, dichotomization delayed ten days compared with that on media containing sucrose, under either LD or SD condition (Fig. 2A, B). After a lag period of ten days, D.T. values of plants under LD condition incrased almost linearly. Under SD condition, however, dichotomization became atagnant earlier on lower sucrose concentrations.

It is clear that thallus elongated better under LD condition than under SD condition, except for one on 0.09 M sucrose (Fig. 2C, D). The elongation on 0.09 M sucrose was markedly inhibited under LD condition. Under SD condition, the elongation rate seemed to decrease after 40 days in lower sucrose concentrations.

Although fresh weight increase on the basal medium remained very slow, it was more rapid under LD condition than under SD condition (Fig. 2E, F). Under LD condition the fresh weight increase was considerably promoted on 0.09 M sucrose

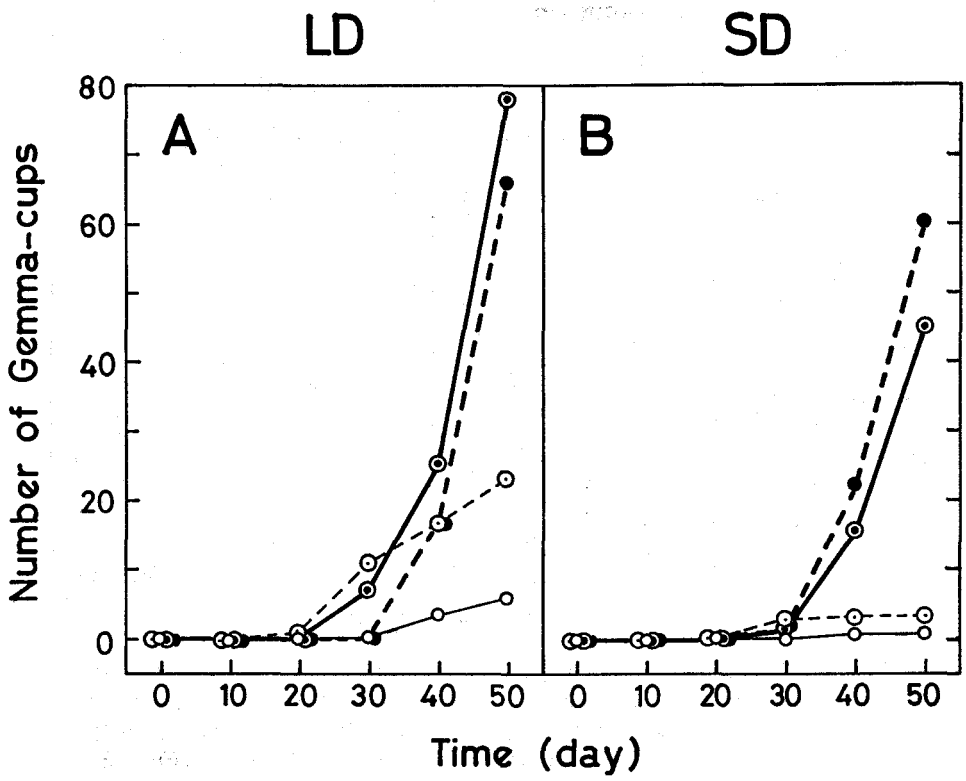

Fig. 3 Time courses of the increase in the number of gemma-cups. Averages of six cultures. For LD, SD and for symbols, see Fig. 2.

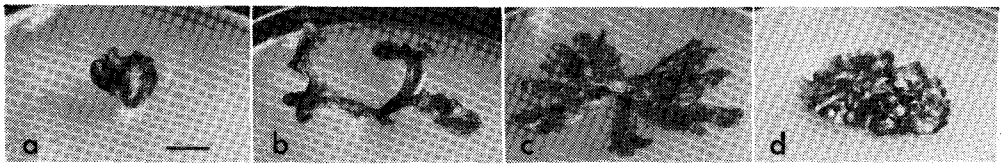

Fig. 4 50-day-old *M. polymorpha* under 10-hour photoperiods. a: on the basal medium. b-d: on medium with sucrose in a concentration of 0.03, 0.06 or 0.09 M respectively. Scale on a=1 cm.

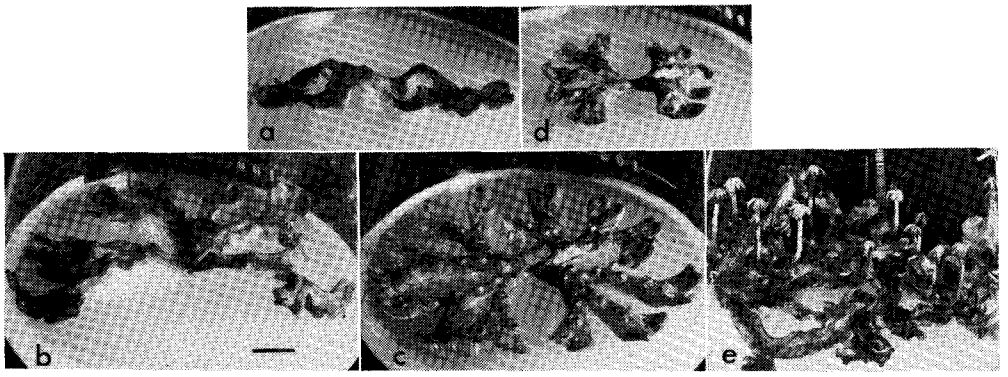

Fig. 5 *M. polymorpha* cultured under 16-hour photoperiods. a-d: 50-day-old plants; on sucrose concentration, see Fig. 4. e: 60-day-old plant on medium with 0.03 M sucrose, with elongated archegonial stalks. Scale on b=1 cm.

medium, though it was smaller than on 0.03 M or 0.06 M sucrose. Considering the suppressive effect of 0.09 M sucrose on the thallus elongation, it was indicated that thallus grew thick. The fresh weight increase on 0.09 M sucrose under SD condition was similar to that under LD condition.

On the basal control medium, the plants produced several gemma-cups under LD condition but only few under SD condition (Fig. 3). Under LD condition, gemma-cups appeared ten to 20 days earlier on 0.03 M sucrose than in higher sucrose concentrations. The number of gemma-cups on 0.03 M sucrose increased almost linearly, while it increased exponentially in higher sucrose concentrations. The time course of gemma-cup formation on 0.09 M sucrose under LD condition resembled very close to that under SD condition.

Archegoniophores, on the other hand, were produced only under LD condition on 0.03 M sucrose in this experiment. They were first observed on the 50th day of culture mostly as button-like appendages and the average number was 12.5 per plant.

The plants cultured under SD condition (photographs in Fig. 4) had smaller sizes than comparable plants under LD condition (Fig. 5). Thalli waved conspicuously on the control medium, and waved in the lesser extent on 0.03 M sucrose, whereas in the higher sucrose concentrations they crept keeping contact with agar surfaces. It is seen in the photographs (Fig. 5) that gemma-cups were produced abundantly on 0.06 M

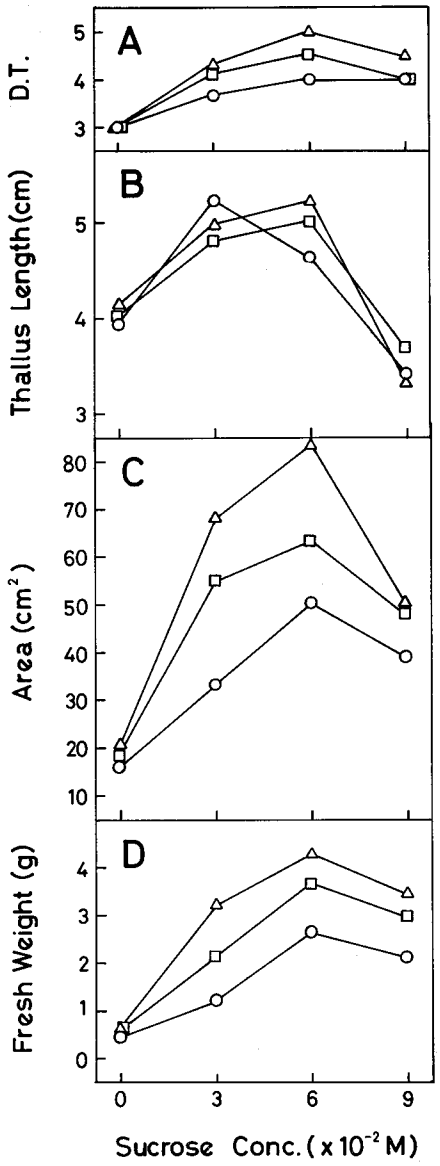

Fig. 6 Vegetative growth under different light intensities and sucrose concentrations for 40 days of culture. A: dichotomization times (D. T.). B: thallus length. C: area. D: fresh weight. Averages of eight cultures. Symbols: ○—○, 2000 lx; △—△, 3500 lx; □—□, 5000 lx.

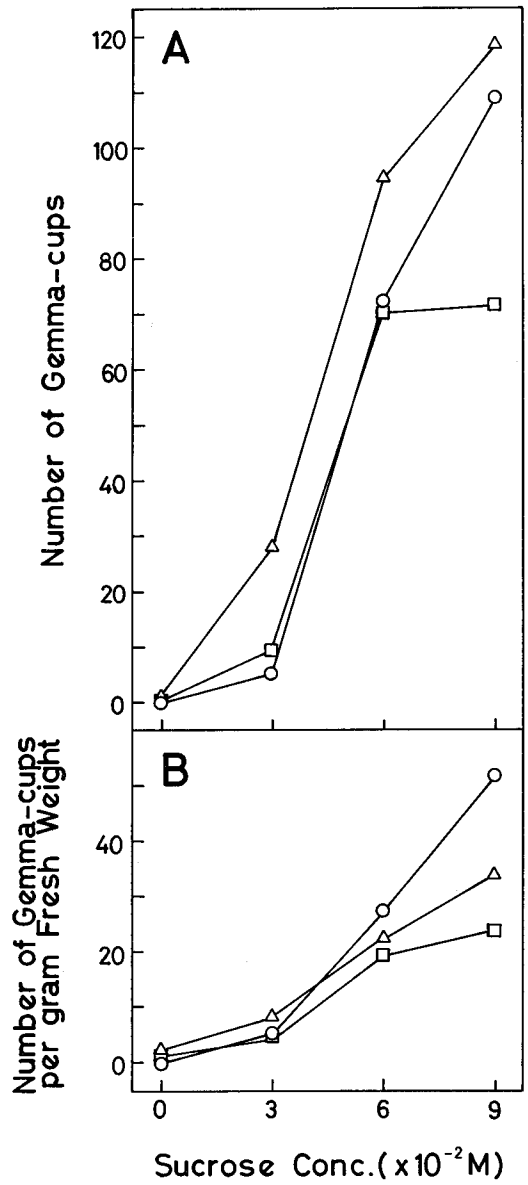

Fig. 7 Gemmma-cup formation during 40 days of culture. A: the number of gemma-cups; averages of eight cultures. B: the number of gemma-cups produced per gram fresh weight, calculated from the data described in Fig. 6D and Fig. 7A. Symbols: see Fig. 6.

sucrose under LD condition and that archegoniophores were produced on 0.03 M sucrose at apices of the thallus bearing only few gemma-cups. Stalks elongated to a few centimeters tall (Fig. 5e).

Effects of light intensity and sucrose concentration under LD condition

In this experiment, effects of light intensities (2000, 3500 or 5000 lx) were tested under 16L/8D (LD) condition. The concentrations of sucrose (Nakarai Chemical Co., DGD-21) in the culture medium were 0, 0.03, 0.06 or 0.09 M. After 40 days of culture, the area of thallus was measured in addition to the five growth parameters in the first experiment.

On the basal medium, all the growth parameters except thallus length were small at any light intensities compared with those on the sucrose-containing medium (Fig. 6). The average value of D.T. of the plant cultured on the basal medium was 3.0 (Fig. 6A). The highest value of D.T., 5.0, was observed on 0.06 M sucrose at 3500 lx. This culture condition gave maximum increase in area (Fig. 6C) as well as in fresh weight (Fig. 6D). Effects of light inetnsity on the thallus elongation was slight (Fig. 6B). Light intensity hardly affected the level of the inhibition in thallus elongation by 0.09 M sucrose.

Gemma-cups were scarcely produced on the basal medium (Fig. 7A). Gemma-cup formation was accerelated by 3500 lx light, which was considered to be a result of vigorous vegetative growth. Relatively poor gemma-cup formation on 0.03 M sucrose was a similar result to that in the first experiment. Contrary to the inhibition in vegetative growth by 0.09 M sucrose, gemma-cup formation was enhanced in this concentration of sucrose at 2000 or 3500 lx. The number of gemma-cups produced per gram fresh weight (Fig. 7B). was almost proportional to sucrose concentration, with the tendency that lower light intensity was more effective.

Archegoniophores were produced rarely on the basal medium or under the lower light intensity (2000 lx) (Table 1). They were produced most abundantly on 0.03 M sucrose at 5000 lx as in the first experiment. Further, there found the tendency that the high intensity light hastened archegoniophore formation except for the case on

Table 1 Effects of light intensity and sucrose concentation on the archegoniophore formation under 16-hour photoperiods. Means in the number of archegoniophores of eight cultures are expressed with standard errors.

Sucrose Concentration (M)	Light Intensity (lx)		
	2000	3500	5000
0 (Basal Medium)	0	0.50±0.33	0.71±0.71
0.03	0.63±0.32	5.83±2.01	8.13±2.33
0.06	0.25±0.16	5.13±1.42	3.38±1.39
0.09	0	2.71±1.84	6.67±2.25

0.06 M sucrose.

Results of the two experiments are summerized as follows. Both vegetative growth and formation of two types of reproductive organ were promoted under LD condition. Under LD condition, the low intensity light (2000 lx) and the high sucrose concentration (0.09 M) hastened gemma-cup formation, the high intensity light (5000 lx) and the low sucrose concentration (0.03 M) hastened archegoniophore formation, and the light of medium intensity (3500 lx) and the medium sucrose concentration (0.06 M) gave the maximum vegetative growth.

Discussion

The acceleration of vegetative growth of *M. polymorpha* by sucrose observed in this study agrees with the result of Kaul et al. (1962) in a culture study of *M. nepalensis* and also with that of Chopra and Sood (1973) in *Riccia crystallina*. The growth of *M. polymorpha* on the medium without sucrose was very slow, even at the high light intensity in this study. This may be related to low photosynthetic activity due to decreased CO_2 quantity in culture vessels.

Mache and Loiseaux (1973) described that growth and photosynthesis of *M. polymorpha* were saturated by a low light intensity of 2000–3000 lx, and were inhibited by an excess of light. This agrees approximately with the present result that 3500 lx was the optimum light intensity and 5000 lx was suppressive for vegetative growth.

Lobes of a thallus were sometimes very different in length, probably because of apical deminance among lobes (Davidonis and Munroe, 1972). For estimation of the developmental stage of this plant, therefore, the employment of just one growth parameter like thallus length, fresh weight or the other is not satisfactory. In fact, under LD condition, elongation was inhibited and fresh weight increase was slow on 0.09 M sucrose, while D.T. value on this medium was almost equal to those on 0.03 M or 0.06 M sucrose. D.T. value, therefore, may be a better and easily observable scale of the develomental stage of this plant.

Hedger et al. (1972) suggested that inhibition in thallus elongation on 0.05 M sucrose was caused by the osmotic effect. Inhibition in thallus elongation on 0.09 M sucrose observed in the present study may be explained by the osmotic effect. While, the promoted gemma-cup formation by 0.09 M sucrose shown in Fig. 3 and 7 revealed independency of gemma-cup formation from the probable inhibitive effect by high sucrose concentration.

Short-day condition has been reported to be an important factor for gemma-cup formation from the experiments under more or less different culture condition: non-aseptic culture under natural diffused daylight (Voth and Hamner, 1940), aseptic culture at a high light intensity such as 1200 f.c. (Carter and Romine, 1969) or aseptic sand culture under incandescent light at 1300–6000 lx (Kaufhold, 1941). The prsent result,

however, demonstrated that the gemma-cup formation was favored by the daily prolonged illumination, rather than by short-day condition.

During over 70 days of culture in prelimnary experiments, archegoniophores were never produced when illuminated with only fluorescent light, but were produced under LD condition when illuminated with natural diffused daylight or incandescent light. Environmental factors inducing archegoniophore formation, which have been repeatedly discussed by many authors, are confirmed in this experiment concerning the following points: long-day condition and illumination by natural diffused daylight or by incandescent light accelerate archegoniophore formation, and it is hastened under the high light intensity. Courtoy's conclusion (1964, 1966) on the accelerative effect of 1 % (about 0.03 M) sucrose added to media was also confirmed.

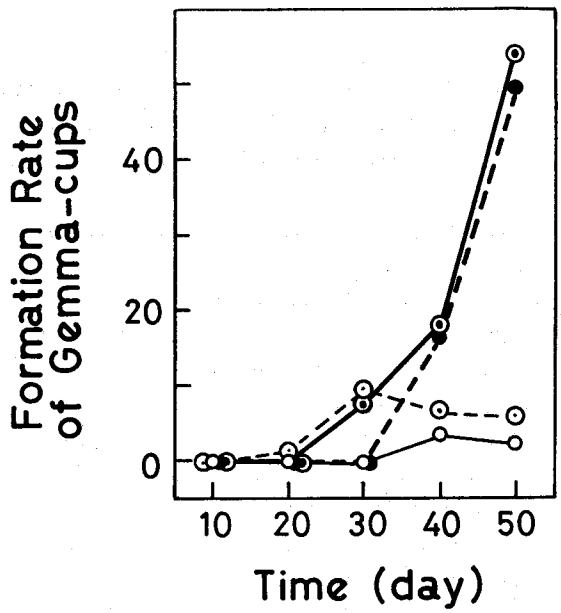

Fig. 8 Formation rate of gemma-cups. The number of gemma-cups produced during each ten days was calculated from the data in Fig. 3A. Symbols: see Fig. 2.

When an archegoniophore protrudes at apex of a lobe, the lobe ceases to grow. However, the very protrusion itself did not affect the vegetative growth of thallus during the culture period in the present study, because archegoniophores appeared only in the last period of culture. The gemma-cup formation began after 20 days of culture and the formation rate on 0.06 M sucrose rose rapidly thereafter. While, the gemma-cup formation rate on 0.03 M sucrose, where archegoniophores were observed most abundantly on the last day of culture, remained almost constant (Fig. 8). Therefore, a kind of suppression of gemma-cup formation seemed to be acting in a plant on 0.03 M sucrose about 20 days before archegoniophore protrusion. This suppression was more conspicuous in the culture at the high intensity of light.

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