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Regulation of gametogenesis of *Laminaria* and *Desmarestia* (Phaeophyta) by iron and boron[1)]

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Nutritional experiments on the gametogenesis of male gametophytes of *Laminaria angustata*, female and male gametophytes of *L. japonica*, and monoecious gametophytes of *Desmarestia ligulata* were carried out. Nitrate, phosphate, iodine, manganese, zinc and cobalt were not effective on *L. japonica*. However, gametogenesis of these species was induced remarkably in media containining high concentration of iron. On the contrary, boron inhibited gametogenesis. In media containing insufficient concentration of iron, gametogenesis could be observed by boron deficiency.

Key Index Words: boron; Desmarestia ligulata; *gametogenesis*; *iron*; Laminaria angustata; L. japonica; *nutritional experiment.*

Influence of some enviromental factors such as temperature (YABU 1964, LÜNING and NEUSHUL 1978, LÜNING 1980) and light (SAITO 1956a, b, HSIAO and DRUEHL 1971, LÜNING and DRING 1972 1975) on the development, growth and reproduction of the gametophytes of Laminariales have been investigated. Similar investigations have also been carried out on Desmarestiales (NAKAHARA and NAKAMURA 1971, MÜLLER and LÜTHE 1981, MÜLLER and MEEL 1982). The effects of different concentrations of nitrate, phosphate and iodine were investigated on gametogenesis of *L. saccharina* by HSIAO and DRUEHL (1972a, b) and various micronutrients were tested to establish a chemically defined medium for culturing of gametophytes and for producing of sporphytes on *Macrocystis pyrifera* by KUWABARA and NORTH (1980) and KUWABARA (1981). NAKAHARA (1984) investigated nutritionally the correlation between growth and nutrition of *Desmarestia viridis, D. ligulata, Laminaria japonica, Alaria crassifolia.*

A previous paper (MOTOMURA and SAKAI 1981) reported that axenic female gametophytes of *Laminaria angustata* grew only vegetatively into branched uniseriate filaments in a defined synthetic medium (ASP_{12} NTA). But oogenesis was induced in the same medium when higher concentration of Fe-EDTA was added, as well as in an enriched natural seawater medium (PESI).

In the present study, the effects of various chemical elements on gametogenesis were investigated in male gametophytes of *L. angustata*, female and male gametophytes of *L. japonica* and monoecious gametophytes of *Desmarestia ligulata.* The present results confirmed that the gametogenesis of these gemetophytes were induced by the medium containing high concentration of iron or boron-deficiency.

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Materials and Methods

The mature sporophytes of *Laminaria japonica* ARESCHOUG and *L. angustata* KJELLMAN were collected at Charatsunai, Muroran, Hokkaido, Japan in November 1980, and the axenic gametophytes were obtained by culturing zoospores which were washed with a capillary pipette method. The female and male gametophytes of *L. japonica* were preserved separately in $ASP_{12}NTA$ medium (PROVASOLI 1963) at 10°C, 14 : 10 hrs LD cycle and 2000-4000 lux provided by cool white fluorescent lamps. In the case of male gametophytes of *L. angustata*, they were preserved in iron-free $ASP_{12}NTA$ medium, because they usually formed antheridia in regular $ASP_{12}NTA$ medium. In this conditions, they grew only vegetatively into small tufts (1-3 mm diam. after one month's culture). The monoecious gametophytes of *Desmarestia ligulata* (LIGHTFOOT) LAMOUROUX (Strain No. MK-030) also grew vegetatively in $ASP_{12}NTA$ medium at 14°C, 14 : 10 hrs LD cycle, and 2000-4000 lux. These preserved gametophytes were used in the present study. Culture vessels used were screw-cap test tubes (18 mm×135 mm) containing 10 m*l* medium or 100 m*l* Erlenmeyer flasks containing 30 m*l* medium.

The basal medium based on $ASP_{12}NTA$ was prepared by omitting each objective nutrient ($NaNO_3$, Na_2 glycerophosphate and various trace elements composed in PII metals), and test media were then made up by the additions of different concentrations of the objective nutrient. Effects of $NaNO_3$, Na_2 glycerophosphate, iodine (as KI), boron (as H_3BO_3), iron (as EDTA 1 : 1 molar, PROVASOLI 1968), manganese (as $MnCl_2$), zinc (as $ZnCl_2$) and cobalt (as $CoCl_2$) on the gametogenesis of *L. japonica* gametophytes were tested. On *L. angustata* male gametophytes, the effects of iron and boron were tested in different concentrations. As the test medium of boron, iron-free $ASP_{12}NTA$ medium was used. The effect of iron on the gametogenesis of *D. ligulata* was examined by culture grown in both $ASP_{12}NTA$ basal media containing 0-4.0 mg-B/*l*.

In these experiments, small tufts of gametophytes were cut by glass knife into small fragments of filaments, and such fragments were inoculated into each test medium. Culture for experiment of 2 species of *Laminaria* gametophytes were conducted at 10°C on a 14 : 10 hrs LD cycle of 2000-4000 lux cool white fluorescent light, and the experiment of *Desmarestia* was carried out at 10°C on a 10 : 14 hrs LD cycle under the same illumination. All experiments were carried out aseptically and the results were obtained after two weeks from inoculation. The percentages of oogonium-bearing female gametophytes of *L. japonica* were determined by random counting more than 200 gametophytes. Furthermore, the gametogenesis of *Laminaria* male gametophyte was shown in table as + (effective), ±(slightly effective) and −(no effective) by examining the degree of antheridium formation on several male gametophytes under light microscope.

Results

The gametogenesis of *Laminaria japonica* female and male gametophytes were not promoted by the addition of $NaNO_3$ (0, 100, 200, 300, 400 mg/*l*) and Na_2 glycerophosphate (0, 30, 60, 90, 120 mg/*l*) to each basal medium. Similarly, conspicuous effects were not observed by the addition of trace elements such as iodine (0, 50, 100, 200, 300 μg-I/*l*), manganese (0, 0.2, 0.4, 0.6, 0.8 mg-Mn/*l*), *zinc* (0, 2, 5, 10, 20 μg-Zn/*l*) and cobalt (0, 5, 10, 20, 30 μg-Co/*l*) to the respective basal medium.

On the other hand, Fe-EDTA induced remarkably the gametogenesis of *Laminaria*. In two weeks after inoculation, the percentages of oogonium-bearing female gametophytes of *L. japonica* were 0.9% in iron-free medium, 92.5% in 0.5 mg-Fe/*l*, 98.6% in 1.0 mg-Fe/*l*, 99.4% in 2.0 mg-Fe/*l* and 74.0% in 5.0 mg-Fe/*l* (Fig. 1) and many eggs were observed (Fig. 3H). The antheridium formations of *L. japonica* and *L. angustata* were also induced greatly by adding iron to the basal medium, and many antheridia were

Table 1. Effect of iron chelated with EDTA on antheridium formation of *L. angustata* and *L. japonica*

Concentration (Fe-mg/*l*)	Antheridium formation	
	L. angustata	*L. japonica*
0	−	−
0.5	+	−
1.0	+	+
2.0	+	+
5.0	+	+

+=effective, −=not effective.

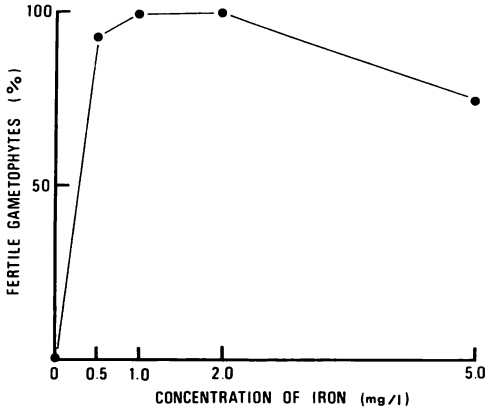

Fig. 1. Effect of iron on the egg formation of *L. japonica* female gametophytes.

observed (Table 1 and Fig. 3 G, I). The male gametophytes of *L. angustata* and the female and male gametophytes of *L. japonica* cultured in iron-free $ASP_{12}NTA$ medium did not differentiate the reproductive cells, and grew only vegetatively into branched uniseriate filaments (Fig. 3 A, B, C). Whereas, PESI medium (TATEWAKI 1966), enriched seawater medium, induced the gametogenesis of *Laminaria* conspicuously (Fig. 3 D, E, F).

In contrast to the effect of Fe-EDTA, boron showed a unique effect on the gametogenesis of *Laminaria*. The percentages of oogonium-bearing gametophytes of *L. japonica* were 55.1% in boron-free medium, 7.4% in 1.0 mg-B/*l*, 27.0% in 2.0 mg-B/*l*, 10.0% in 3.0 mg-B/*l* and 16.7% in 4.0 mg-B/*l* (Fig. 2), and several eggs were observed on female gametophytes in boron-free $ASP_{12}NTA$ medium (Fig. 3 K). Many antheridia were also formed conspicuously on *L. angustata* and *L. japonica* male gametophytes (Table 2 and Fig. 3 J, L). This result on *L. angustata* was obtained in the iron-free medium. In the case of test media containing boron and insufficient iron, however, most gametophytes of *Laminaria* continued only vegetative growth.

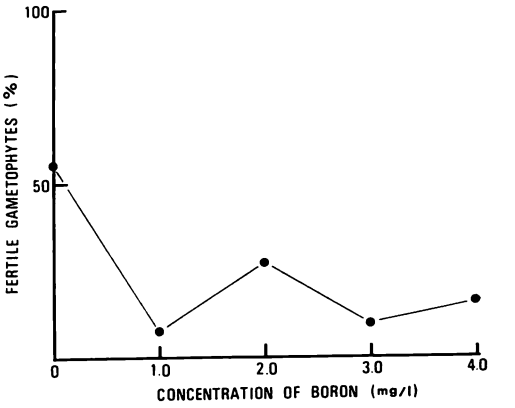

Fig. 2. Effect of boron on the egg formation of *L. japonica* female gametophytes.

Table 2. Effect of boron (as H_3BO_3) on antheridium formation of *L. angustata* and *L. japonica*

Concentration (B-mg/*l*)	Antheridium formation	
	L. angustata	*L. japonica*
0	+	+
1.0	+	+
2.0	±	−
3.0	−	−
4.0	−	−

+=effective, ±=slightly effective, −=not effective.

In the case of gametogenesis of *Desmarestia ligulata*, iron and boron showed similar effects to the *Laminaria* species. In the test medium containing 4.0 mg-B/*l*, the gametophytes of *D. ligulata* continued to grow vegetatively into uniseriate filaments when 0-0.5 mg-Fe/*l* was added (Fig. 4 A, B). But gametogenesis was induced slightly at 1.0 mg-Fe/*l* (Fig. 4 C). At 2.0 mg-Fe/*l*, gameto-

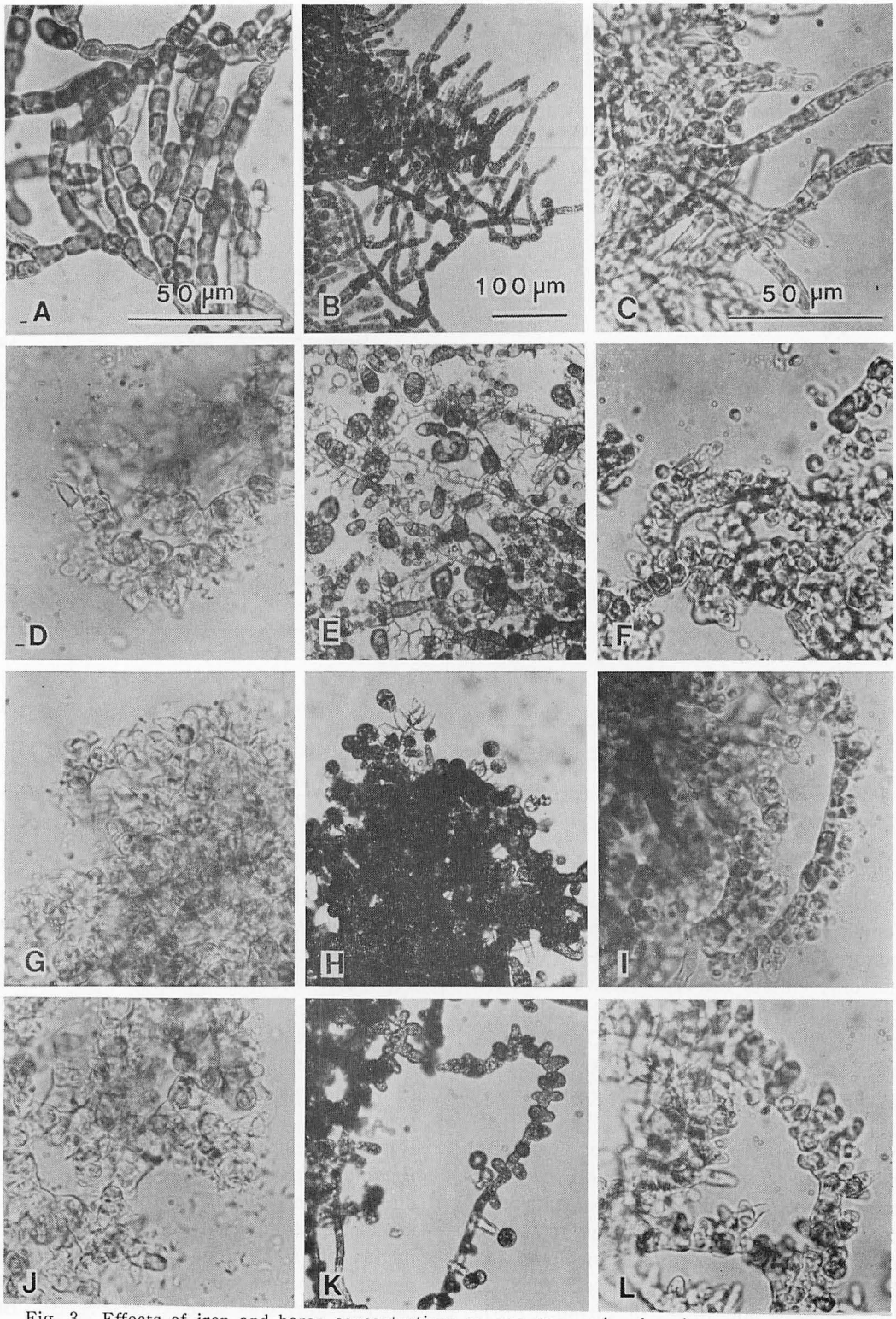

Fig. 3. Effects of iron and boron concentrations on gametogenesis of male gametophytes of *L. angustata* (left), female gametophytes (middle) and male gametophytes (right) of *L. japonica* after 14 days: A-C=Fe-0 mg/*l*; B-2.0 mg/*l*; D-F=PESI medium; G-I=Fe-2.0 mg/*l*; B-2.0 mg/*l*; J-L=Fe-0 mg/*l*, B-0 mg/*l*: Scale=50 μm (left and right) and=100 μm (middle).

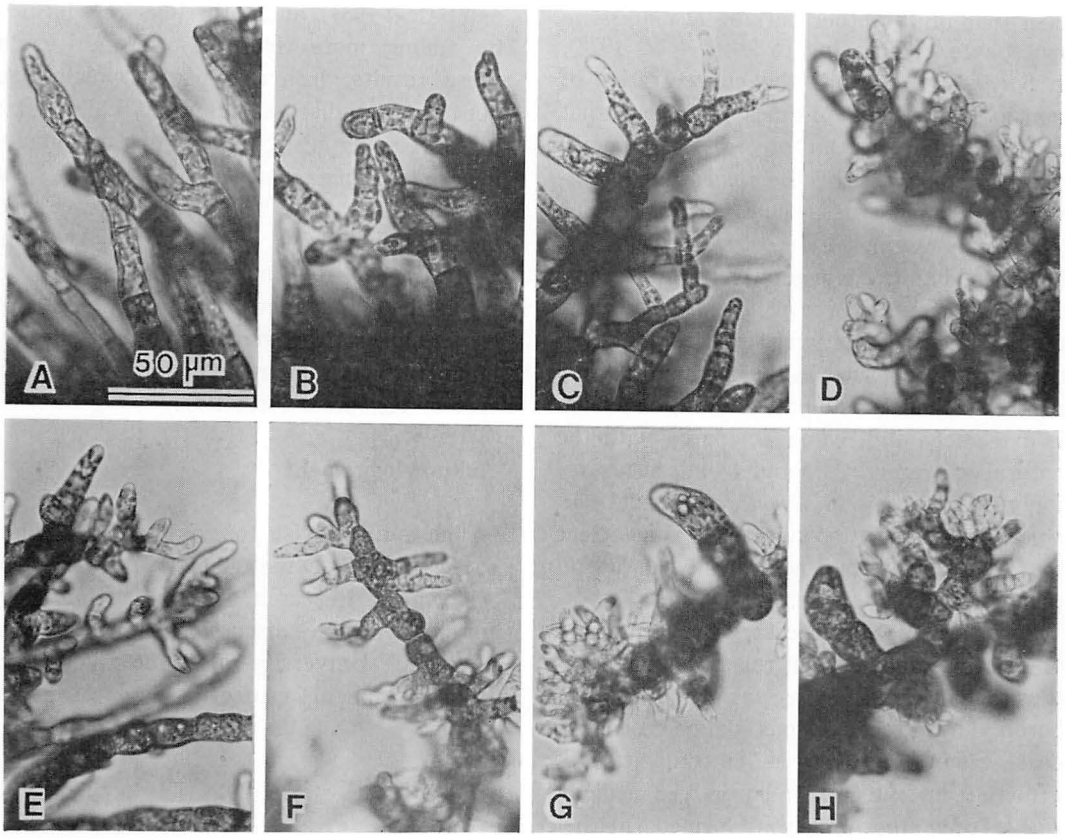

Fig. 4. Effects of iron and boron concentration on gametogenesis of monoecious gametophytes of *D. ligulata* after 14 days: A-D=B-4.0 mg/*l*; E-H=B-0 mg/*l*; A, E=Fe-0 mg/*l*; B, F=Fe-0.5 mg/*l*; C, G=Fe-1.0 mg/*l*; D, H=Fe-2.0 mg/*l*: Scale=50 μm.

genesis, especially antheridium formation, was induced strongly (Fig. 4 D). Whereas, the gametogenesis was induced a little in the medium lacking in both boron and iron (Fig. 4 E), and was induced significantly in this medium added 0.5 mg-Fe/*l*, 1.0 mg-Fe/*l* and 2.0 mg-Fe/*l* (Fig. 4 F, G, H).

Discussion

In a previous paper (MOTOMURA and SAKAI 1981), the authors reported that the oogenesis of *L. angustata* was induced strongly by the addition of iron (Fe-EDTA at 2.0 mg-Fe/*l*) to synthetic medium based on $ASP_{12}NTA$, and was slightly inhibited by the addition of boron. The oogonium formation of this alga was not promoted in the media containing various concentration of nutrients such as nitrate, phosphate, manganese, zinc and cobalt. The present study confirmed that the formation of oogonia and antheridia in two *Laminaria* species and one *Desmarestia* species can be regulated by the addition or removal of iron and boron in a laboratory culture.

It is well known that many algae require concentration of iron for growth and also believed that iron acts as a co-factor for many enzymatic reactions. However, the specific function of iron in the algae is still obscure. In some brown algae, such as *Laminaria* and *Sargassum*, it is found that the concentration of accumulated iron is considerably different between fertile part and other part of the same individual plant. HOSODA (1972) reported that the concentration of iron in the fertile part was lower

than that of the other part of *L. longissima* sporophyte. According to ISHII *et al.* (1978), in *Sargassum horneri*, the concentration of iron in lamina was 20 times as much as that of receptacle. In the present experiment, the gametophytes of *Laminaria* and *Desmarestia* which were cultured in regular (containing 0.1 mg-Fe/*l*) or iron-free ASP_{12} NTA meidum grew vegetatively, and reproductive structure never occurred. However, their gametogenesis was induced by high concentration of iron in the medium (0.5-5.0 mg-Fe/*l*). This amount of iron in the medium is extremely high value compared with the natural seawater. Although iron is necessary for gametogenesis of *Desmarestia* and *Laminaria* in a laboratory culture, it is not clear that much iron has accumulated or not in their reproductive cells.

Boron seems to be necessary for the growth of diatoms, and it is not related to photosynthesis, since *Cylindrotheca fusiformis* required boron during the periods of heterotrophic dark growth as well as autotrophic light growth (LEWIN 1966). In boron-free medium, *Fucus edentatus* embryos formed neither apical hairs nor secondary rhizoids (MC LACHLAN 1977). In *Ulva* and *Dictyota*, boron promoted growth and reproductive maturity (NASR and BEKHEET 1970). And NAKAHARA (1984) reported that boron deficiency in the medium induced strongly the gametogenesis of *Desmarestia ligulata* and *D. viridis*. On the other hand, in higher plants, boron deficiency involves alternations in cellular membranes and ion uptake through membrane (POLLARD *et al.* 1977, MOORE and HIRSCH 1983).

According to the present study, the gametogenesis of *Laminaria* and *Desmarestia* was observed when 0-1.0 mg-B/*l* was added to the basal medium based on ASP_{12}NTA medium (containing 0.1 mg-Fe/*l*). In the regular ASP_{12}NTA medium containing 2.0 mg-B/*l*, the gametogenesis of these algae was induced by adding higher concentration of iron (0.5-2.0 mg/*l*). Especially in *L. angustata*, oogonia were formed in this medium by adding more than 1.0 mg-Fe/*l* (MOTOMURA and SAKAI 1981), and antheridia were formed by adding more than 0.5 mg-Fe/*l*. From these results, iron and boron, which have not fully been discussed physiologically, seem to show antagonistic effects to the gametogenesis of *Laminaria* and *Desmarestia*, but it is not clear that iron is accelerative and boron is inhibitory. The ratio of the concentration of iron and boron in the medium would be significant for gametogenesis of these algae. Iron requirement for antheridium formation seems to be lower level than that of oogonium formation.

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本村泰三・阪井與志雄: *Laminaria, Desmarestia* の配偶子形成に対する鉄とホウ素による制御

ミツイシコンブ雄性配偶体，マコンブ雌性・雄性配偶体，ウルシグサ配偶体の生殖細胞分化について，人工合成培地 $ASP_{12}NTA$ を基本培地に用い無菌条件のもとで，培地中の種々の栄養素について栄養実験を行った。その結果，(1)培地中の鉄濃度が低い場合には単列糸状の栄養生長を繰り返すが，2.0 mg/*l* の鉄を含む培地中では顕著に生殖細胞が分化する。(2) 培地中の鉄濃度が低い場合でも培地中からホウ素を除去することにより，生殖細胞が分化する。以上のことから，コンブ・ウルシグサの配偶体の成熟は，培地中の鉄とホウ素の濃度により制御できることが明らかになった。(051 室蘭市母恋南町 1～13，北海道大学理学部付属海藻研究施設)