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# Crossing experiments between four local forms of *Agarum cribrosum* Bory (Phaeophyta) from Hokkaido, northern Japan

By

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## Introduction

So far as we know, few studies of cultural experiments on *Agarum* have been reported. KANDA (1941) reported in detail the development and morphology of gametophytes of *A. cribrosum* from Muroran. VADAS (1969) studied the ecology of *Agarum* occurring in San Juan Island near Washington, in the study some environmental factors controlling the maturation of gametophytes and the development of sporophytes in *A. cribrosum* and *A. fimbriatum* were examined under cultural conditions. Crossing experiments in the members of the Laminariales have been carried out in the genus *Laminaria* (SCHREIBER, 1930; SUNDENE, 1958; YABU, 1964; SVENDSEN and KAIN, 1971) and in the genus *Undaria* (SAITO, 1966, 1972; MIGITA, 1967). In these works the occurrence of intra- or interspecific fertilization has been reported to discuss the taxonomic relationship.

It was shown in the preceding paper (I. YAMADA, 1974) that among the plants of *Agarum cribrosum* occurring in Hokkaido and adjacent regions, four local forms, f. *cribrosum*, f. *rugosum*, f. *rishiriense* and f. *yakishiriense*, could be distinguished morphologically. In the present investigation, interfertility between these four local forms is examined to elucidate the taxonomic relationship between them. Crossing experiments were carried out at the Institute of Algological Research of Hokkaido University at Muroran from November, 1969 to March, 1971.

## Material and Method

The plants used in the experiments were collected at five localities in Hokkaido (Table 1). They were all washed ashore plants.

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TABLE 1. Materials used for the crossing experiments

| Material | Locality | Date |
|---|---|---|
| f. *cribrosum* | Enrumu-misaki, Samani, Hidaka Prov. on the Pacific coast. | Dec. 7, 1969 |
| f. *rugosum* | Charatsunai, Muroran on the Pacific coast. | Nov. 14, 1969 |
| f. *rugosum* | Reuke, Rumoi on the Japan Sea coast. | Feb. 14, 1970 |
| f. *rishiriense* | Oshidomari, Rishiri Isl. on the Japan Sea coast. | Nov. 29, 1969 |
| f. *yakishiriense* | Higashi-hama, Yakishiri Isl. on the Japan Sea coast. | Dec. 19, 1969 |

1. Stock cultures of gametophytes.

At the collecting place fertile pieces of a blade were washed with sterilized sea water and then allowed to dry for some hours. When again soaked in sea water they released zoospores. A few drops of water with the zoospores were pipetted into test tubes ($2 \times 18$ cm with a screw-cap) containing 10 ml of ESI medium (TATEWAKI, 1966). Within 48 hours these cultures were transferred to a culture room, which was maintained at 14°C in a 14-hr photoperiod under 1000 lux with cool white fluorescent lamps. After 20–30 days most of the gametophytes from zoospores consisted of 20–40 cells, the male gametophytes were slightly slender and more richly branched than the female ones. At this stage the gametophytes were isolated and placed separately in new test tubes. These cultures were unialgal but not bacteria-free. All of the isolated female and male gametophytes remained sterile and continued growth vegetatively. They became profusely branched and took a more or less spherical form. Female and male gametophytes derived from the same mother plant were split into small pieces which rapidly grew up into new plants. The genetically identical materials of each form obtained were used in the crossing experiments.

2. Maturation of gametophytes.

When some pieces of female and male gametophytes transferred to a condition of 10°C and 14-hr photoperiod with fresh medium, they became fertile after 10 days. Within 20 days almost all branches of female and male gametophytes formed oogonia and antheridia respectively. In the

cultures of female gametophytes only, most of egg cells degenerated or became abnormal sporophytes when germinated, but a few developed into normal sporophytes parthenogenetically. On the other hand, when mixed female with male gametophytes derived from the same form, most egg cells developed into normal sporophytes and a few developed abnormally or degenerated.

3. Crossing

Crossings between four forms were carried out by transferring several sterile female and male fragments of gametophytes derived from the two different forms to a culture vessel containing 200 ml of medium, and they were cultured at 10°C in a 14-hr photoperiod. As a control, fragments of female gametophytes only were cultured in the same condition. As a yardstick, the germination rate of normal sporophytes 20 days after transferring was used to detect the presence of interfertility.

## Result and Discussion

The result of the crossings 20 days after the start of the experiments is presented in Table 2, and the degrees of interfertility are summarized in Table 3. Young sporophytes obtained 20 days after reached to about 300 $\mu$m in height. In this stage, pure sporophytes and hybrids were mostly similar to each other and differences in the growth and morphology could not be recognized.

In the cultures of female only, the germination rates of normal parthenogenetic sporophytes were below 10%. On the other hand, when both female and male from four forms were mixed, the rates were 32–96% (73% in average). Accordingly, we have judged that the four forms of *Agarum* collected from different localities of Hokkaido are interfertile. This supports the opinion that the four forms in Hokkaido including f. *yakishiriense* should be referred to the same species as stated in the preceding paper (I. YAMADA, 1974).

The germination rates in self-fertilization, as seen by Table 3, were high in f. *cribrosum* and f. *rugosum* from Muroran, whereas low in f. *rugosum* from Rumoi, f. *rishiriense* and f. *yakishiriense*. Furthermore, the rates were high when males of the former two forms were mixed respectively with the other forms, but it is generally low when males of the latter three forms were mixed. It is interesting that the former two forms occur on the Pacific coast and the latter three on the Japan Sea coast. From these results it is supposed that Pacific forms of f. *cribrosum*

TABLE 2. Frequency of the occurrence of sporophytes in each combination of crossings between four forms of *Agarum cribrosum*

| ♀ | ♂ | E | ab-S | n-S | n-S % |
|---|---|---|---|---|---|
| f. *cribrosum* | | 165 | 69 | 0 | 0 |
| " | f. *cribrosum* | 6 | 23 | 296 | 91.4 |
| " | f. *rugosum* (M) | 6 | 18 | 346 | 93.5 |
| " | f. *rugosum* (R) | 8 | 12 | 248 | 92.5 |
| " | f. *rishiriense* | 83 | 50 | 158 | 54.3 |
| " | f. *yakishiriense* | 43 | 60 | 130 | 55.8 |
| f. *rugosum* (M) | | 255 | 83 | 3 | 0.8 |
| " | f. *cribrosum* | 5 | 40 | 299 | 86.9 |
| " | f. *rugosum* (M) | 17 | 1 | 147 | 89.1 |
| " | f. *rugosum* (R) | 62 | 59 | 168 | 58.1 |
| " | f. *rishiriense* | 55 | 49 | 49 | 32.0 |
| " | f. *yakishiriense* | 50 | 36 | 198 | 69.7 |
| f. *rugosum* (R) | | 32 | 90 | 0 | 0 |
| " | f. *cribrosum* | 4 | 6 | 221 | 95.7 |
| " | f. *rugosum* (M) | 3 | 9 | 171 | 93.4 |
| " | f. *rugosum* (R) | 17 | 90 | 106 | 49.8 |
| " | f. *rishiriense* | 3 | 6 | 142 | 94.0 |
| " | f. *yakishiriense* | 11 | 46 | 183 | 76.3 |
| f. *rishiriense* | | 95 | 90 | 1 | 0.5 |
| " | f. *cribrosum* | 6 | 19 | 263 | 91.3 |
| " | f. *rugosum* (M) | 27 | 68 | 325 | 77.4 |
| " | f. *rugosum* (R) | 23 | 57 | 190 | 70.4 |
| " | f. *rishiriense* | 48 | 63 | 195 | 63.7 |
| " | f. *yakishiriense* | 50 | 68 | 272 | 68.7 |
| f. *yakishiriense* | | 33 | 175 | 17 | 7.6 |
| " | f. *cribrosum* | 7 | 44 | 182 | 78.1 |
| " | f. *rugosum* (M) | 7 | 38 | 330 | 88.0 |
| " | f. *rugosum* (R) | 8 | 45 | 162 | 75.3 |
| " | f. *rishiriense* | 10 | 49 | 148 | 71.5 |
| " | f. *yakishiriense* | 24 | 111 | 192 | 58.7 |

E, ab-S, n-S: Numbers of ungerminated egg cells (E), abnormal sporophytes (ab-S), normal sporophytes (n-S); n-S%: Percentage of normal sporophytes (n-S/E+ab-S+n-S).

M, R: Materials collected from Muroran (M) and Rumoi (R).

TABLE 3. Degrees of the occurrence of normal sporophytes in crossings between four forms, summarized from Table 2.

| ♀ \ ♂ | f. *cribrosum* | f. *rugosum* (Muroran) | f. *rugosum* (Rumoi) | f. *rishiri-ense* | f. *yakishiri-ense* |
|---|---|---|---|---|---|
| f. *cribrosum* | A | A | A | D | D |
| f. *rugosum* (Muroran) | A | A | D | E | C |
| f. *rugosum* (Rumoi) | A | A | E | A | B |
| f. *rishiriense* | A | B | B | C | C |
| f. *yakishiri-ense* | B | A | B | B | D |

A, 80–100 %; B, 70–80 %; C, 60–70 %; D, 50–60 %; E, less than 50 %.

and f. *rugosum* may differ from f. *rishiriense*, f. *yakishiriense* and Japan Sea form of f. *rugosum* in the cultural condition required for fertilization. Detailed cultural experiments in each of the four forms are needed to elucidate the condition for maturation.

Our gametophytes in the experiments, as mentioned before, matured at 10°C in a 14-hr photoperiod under 1000 lux but remained sterile at 14°C. According to VADAS (1969), the maturation of gametophytes of *A. cribrosum* from San Juan Island occurred mostly rapidly at 5 and 10°C under 100 ft-c (1075 lux) and 200 ft-c (2150 lux), and at 15°C also normal maturation occurred but was inhibited by high light intensity. This seems to show that there may be some differences in the condition for maturation between our materials and the plant from San Juan Island.

As regards the morphology of the young sporophytes, SUNDENE (1958) pointed out that the morphological difference between true form and hybrids in the younger stage of *Laminaria digitata* could not be perceived, and the transplantation into the sea from laboratory would possibly determine the genetical characteristics of the hybrids.

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## Summary

Crossing experiments between four local forms of *Agarum cribrosum* (f. *cribrosum*, f. *rugosum*, f. *rishiriense* and f. *yakishiriense*) were carried out with the materials collected from five different localities of Hokkaido.

At the rate of 32–96% (73% in average) they were interfertile. This supports I. YAMADA's proposal in 1974 that the four forms including *A. yakishiriense* belong to *A. cribrosum* in the specific rank.

From the difference in the germination rates of sporophytes among the four forms in self-fertilzation and others, it is suggested that there may be some differences in physiological properties according to the forms, especially in the condition for maturation of gametophytes.


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