Studies on Spore Germination of Hepaticae 4*

Makinoa crispata (Steph.) Miyake

by Hiroshi INOUE**

井上 浩:** 苔類の孢子発芽 (4) マキノゴケ*

Received March 31, 1958

Makinoa crispata was first described by Stephani (1897) under the genus *Pellia*, based on Faurie's collection made in Japan. Two years later, Miyake (1899) studied on the sexual organs and the sporophyte, which resulted in his proposal of new genus, *Makinoa*, for this species. *Makinoa* is a famous genus for its remarkably large spermatozoid in the Bryophyte (Miyake 1899), and Nakai (1943) proposed the family Makinoaceae for this genus. Before Nakai, this genus had been included in the Dilaenaceae (Verdoorn 1932) or the Pallaviciniaceae (Evans 1939). Since Nakai's proposal of the family, Japanese bryologists have adopted it, but Müller (1951) placed the genus in the Dilaenaceae together with *Pallavicinia*, *Moerckia*, and *Calycularia*.

Makinoa comprises a single species, *M. crispata* (Steph.) Miyake, which has been known from Japan, Korea, Formosa and China (Hattori 1952). This species usually inhabits at much humid and more or less shady sites. The sporophytes appear from March to April. The present study was undertaken in order to determine the pattern of the sporeling of this species.

The spores for this study were collected at Yoshino-mura of Kochi Pref. (Mar. 24, 1955) and Kitago-mura of Miyazaki Pref. (Mar. 30, 1957). The spores were sown 10 days after the collection. The media for germination were used (1) Knop's solution, (2) Knop's agar (2%), (3) Meyer's solution, (4) sterilized *Sphagnum* mats moistened with Knop's solution. In any case the germination took place in 10 days after seedling, but except for the *Sphagnum* mats the development after 3-celled stage was mostly suppressed and the sporeling died. The complete pattern was observed in the *Sphagnum* mats.

Germination

The spores are spherical measuring 20–32 (mostly 24–27) μ , and are light to blackish brown with many reticulated ridges on the surface. The spore coat is uniformly thickened.¹⁾ In the endospore there are many chloroplasts and few oil-drops which

* Continued from J. Jap. Bot. 33: 6 (1958).

** Botanical Institute, Faculty of Science, Tokyo University of Education, Ohtsuka, Tokyo. 東京教育大学理学部植物学教室

¹⁾ Miyake (1899) gave the measurement of the spore as 20–25 μ , and Horikawa (1929) as 25.5–28 μ . The spore measurement is more variable, even in a single sporangium, as shown in this paper. Miyake's description on the spore coat, as "much thicker in one side than in the other half", seems to be that of died spore, in which the endospore is much shrunk and the spore coat, by surface view, seems to be irregularly thickened. But this is abnormal feature caused by the spore death.

are faintly stained by Sudan III. The chloroplasts are usually crowded surrounding the comparatively large nucleus (so that, by the surface view, there seems a green mass in the center of the endospore).

Before the germination, the chloroplasts disperse and the oil-drops mostly disappear. The spore much swells and elongates, so that the spore coat ruptures before the formation of the first wall (Figs. 1-3). Before the first transverse wall is formed, the nuclear division takes place; then, two crowded masses of the chloroplasts are observed in the germ cell (Fig. 3). The similar feature of the chloroplasts is observed also in the succeeding cell divisions (at least in earlier stage).

The first wall separates the germ cell into two unequal cells (Fig. 4).

The smaller cell grows nearly as large as, or a little larger than, the other (basal cell) and is divided with transverse wall or occasionally with longitudinal

Figs. 1-15. Various stages of sporeling in *Makinoa crispata* (Steph.) Miyake. For explanation see text. Figs. 1-6, $\times 360$; 7-12, $\times 160$; 13-14, $\times 96$; 15, $\times 160$.

one (Figs. 5, 6). The basal cell does not make further growth and division as well, and has brown spore coat throughout the developmental course of the sporeling. The pattern of the development may be somewhat modified by outer conditions. But, in any case, the apical cell with two cutting faces is formed before the 10-celled stage. (Figs. 7-9).

The first appearance of the rhizoid (Fig. 9) does not occur before about 6-celled stage (but in very few cases it was observed at the 4-celled stage). The rhizoid initial cell is usually a little smaller and contains fewer chloroplasts than the other cells. The wall of the rhizoid, at first, is faintly brownish. However, it soon becomes purplish brown. So, when rhizoids well developed, it is much embarrassed whether or not there is distinct wall between the rhizoid and its initial cell: the rhizoids are always continuous from their initial cells. The similar rhizoids are formed at random, but, when the dorsi-ventrality of the sporeling is confirmed, the rhizoids arise only from the ventral side (Figs. 13, 14). The chloroplasts in the rhizoid disappear.

The development of the thallus is caused by the activity of the apical cell with two cutting faces. The apical cell, however, becomes indistinct by the formation of meristematic apical cells (Fig. 15). The thallus formed by the meristematic cells becomes 4-7 cells thick in the central portion where the midrib of the thallus may develop.

The oil-bodies, which are not stained by Sudan III, are formed in the cells at about the 10-celled stage (but occasionally observed also in earlier stage). They are much small and spherical (about 1μ), about 10 in number per cell, and white in color. They are evidently the same with those of the adult plant except for their size.

Discussion and Conclusion

The characteristic features in the sporeling *Makinoa crispata* are (1) the first cell division which takes place after the expansion of the exospore, (2) the basal cell which is always distinct and does not develop further, (3) the much crowded chloroplasts surrounding the large nucleus, (4) the deep brown rhizoid which is in direct continuation from the basal cell of rhizoid and appears after the 4-celled stage, at least, (5) the development of the young thallus which is derived from the apical cell with two cutting faces, and (6) the apical cells of the young thallus, which form the meristematic apical region.

Schiffner (1901) and Horikawa (1929) discussed the systematic position of *Makinoa* and the latter author stated the close relation to the Metzgerioideae and the Leptothecaceae (Pallaviciniaceae) after the extensive studies of the plant. The related genera whose sporeling pattern were studied are *Metzgeria* (Goebel 1915, 1930), *Pallavicinia* (Haupt 1918, Wolcott 1942, Kachroo 1956), and *Moerckia* (Mader 1929). In those genera, except for *Metzgeria*, the first cell division may take place within the exospore and before the expansion of the exospore several cells are formed.

In *Metzgeria* the first wall is formed after the rupture of the exospore as *Makinoa*, but the protonema is filamentous. Compared with them, *Makinoa* has a distinct pattern of the sporeling. Another related genus to *Makinoa* is *Calycularia*, whose literature is not seen, and which may have also another pattern (unpublished data); that is, the first cell division takes place within the exospore.

Goebel (1930) included *Makinoa* in the 'Aneuraceen'. The sporeling pattern in *Riccardia* (*Aneura*) was studied by Leitgeb (1874-1882), Showalter (1925), and Goebel (1930). According to their studies, the exospore ruptures before the formation of the first wall by the elongation of the endospore as in *Makinoa*. However, the earlier stage of the development in *Riccardia* takes much different course, and filamentous protonema develops and the apical cell has two cutting faces as in *Makinoa* (Campbel 1905). The pattern in *Metzgeria* is allied to that in *Riccardia*.

Between the Pelliaceae and the Makinoaceae are some essential distinctions in the sexual organs and the sporophytes (Miyake 1899). Besides those differences, the sporeling pattern of the Pelliaceae (Wolfson 1928, Goebel 1930) and of the Makinoaceae are remarkably different.

Summary

The sporeling pattern of *Makinoa crispata* is described and discussed. The first cell division takes place after the rupture of the exospore; the second or third division is longitudinal and the sporeling develops into several-celled primary plant whose apical cell has two cutting faces; the apical cell forms the meristematic apical region which becomes 4-7 cells thick at middle; the first rhizoid develops normally after the 6-celled stage and is characteristically purplish brown as that of the adult thallus; the oil-bodies in the earlier stage of development are similar to those of adult thallus except for their size. This pattern may be named as *Makinoa*-type. The relationship between the Makinoaceae and other related families is discussed from the view point of the sporeling pattern.

The writer is much indebted to Dr. S. Hattori of the Hattori Botanical Laboratory and to Prof. H. Ito of Tokyo University of Education for their very suggestive advice.

References

- Kachroo, P., Proc. Nat. Inst. Sci. India 22: 6 (1956). Hattori, S., J. Hattori Bot. Lab. 8: 21 (1952). Müller, K., Die Lebermoose Europas (ed. 3), especially 179-200 (1951). Nakai, T., Quaestiones characterum naturalium plantarum. Tokyo (1943). Wolcott, G. B., Bryologist 45: 29 (1942). Evans, A. W., Bot. Rev. 5: 49 (1939). Verdoorn, Fr., Mann. Bryol. especially 413-443 (1932). Goebel, K., Organographie der Pflanzen (ed. 3), 2. Bryophyten-Pteridophyten. Jena (1930). Horikawa, Y., Sci. Rept. Tohoku Imp. Univ. Ser. 4 (4): 37 (1929). Mader, A., Planta 8: 742 (1929). Wolfson, A. M., Amer. J. Bot. 15: 179 (1928). Showalter, A. M., Bull. Torrey Bot. Club 52: 157 (1925). Haupt, A. W., Bot. Gaz. 66: 524 (1918). Campbel, D. H., The structure and development of mosses and ferns. London (1905). Schiffner, V., Österr. Bot. Zeitschr. 51: 82 (1901). Miyake, K., Bot. Mag. Tokyo 13: 1 & 21 (1899). Stephani, F., Bull. Herb. Boiss. 5: 49 (1897). Leitgeb, H., Untersuchungen über die Lebermoose III. Jena. (1877).