Development of the Female Gametophyte in the Ovules on the Leaf blade of Ginkgo biloba

Sanae Soma*

オハツキイチョウの胚珠内の雌性配偶体の発達

相 馬 早 苗

Introduction

The ovuliferous structure of Ginkgo biloba consists of a stalk and two sessile ovules on the top. Ovuliferous structures produced on the leaf blade were discovered for the first time at the Buddhist temple Joutakuji in Shimoyama village Yamanashi Prefecture by Shiroi in 1891 (1). Fujii went there to observe them in October in the same year. The owner of this tree, however, did not want this special tree to be investigated. Then he looked for near by and fortunately he found another tree which also produced such ovuliferous structures different from the usual in the same village. He went there again in the next spring, and observed short shoots with many usual ovules, some leaf blades with ovules and the stalks with more than two ovules. At that time he also found the male tree with microsporangia on the leaf margin on the opposite side of Fuji River. These facts were reported by Fujii in 1896 (2). According to the information board under this male tree, Fujii discovered this tree in 1896. However, Fujii (2) and Sakisaka (3) described the date of discovery was the 17th of April 1892. So the report by Soma (4) needs correction. The illustrations of short shoots with ovules on the leaf blade and with the microsporangia on the leaf published by Fujii have been cited in many papers or books namely Carothers 1907 (5), Sprecheer 1907 (6), Chamberlain 1935 (7) and Bierhorst 1971 (8). The tree that Fujii investigated is in the precinct of Buddhist temple Hongokuji, and it is preserved as a natural monument of the country. It produces yearly many usual ovuliferous structures and some unusual structures in the short shoots. According to the view from the ground under the tree, the numbers of the short shoots which produce ovules on the leaf blade seem to be different year by year. In 1998 fortunately a lot of ovules on the leaf blade were produced. In the present investigation the development of the female gametophyte in the ovule on the leaf blade was observed with special reference to the sterility.

Materials and Methods

The tree, Ginkgo biloba used in this study is quite the same tree that Fujii investigated more than one hundred years ago. It is located at Shimoyama, Minobu-cho, Minamikoma-gun,

*そうま　さなえ　文教大学教育学部

Yamanashi Prefecture. Ovules were collected from 18th April to 31st August 1998. Because the location of the plant is rather far from author's address, materials were collected by the owner of the tree and sent to the author by parcel service (Yamato Transport).

Materials were fixed in 0.25% of glutaraldehyde in phosphate buffer at ph 7.2. Then the materials were kept in 70% ethanol in the refrigerator. Materials were then dehydrated by ethanol and tertiary butyl alcohol series and embedded in paraffin. Sections were cut 8 or 10μm thick. Sections were then stained in 0.05% toluidine blue-O in benzoate buffer at ph 4.4 (9). The stained materials were then dried and soaked in xylene for a short time and mounted in balsam.

Observations and Discussions

Ovules at the time of the shedding of pollens---

Usual ovules at the time of the shedding of the pollens were well investigated by Fabre-Duchartre (10), De Sloover-Colinet (11) and Freedman (12). Prior to the pollination a group of nucellar cells near the micropylar end of the ovule begin to degenerate so as to form a big cavity, the pollen chamber (Fig1,P). According to De Sloover-Colinet (11), as the pollen chamber develops a viscous droplet of fluid called pollination droplet is secreted outward through the micropyle.

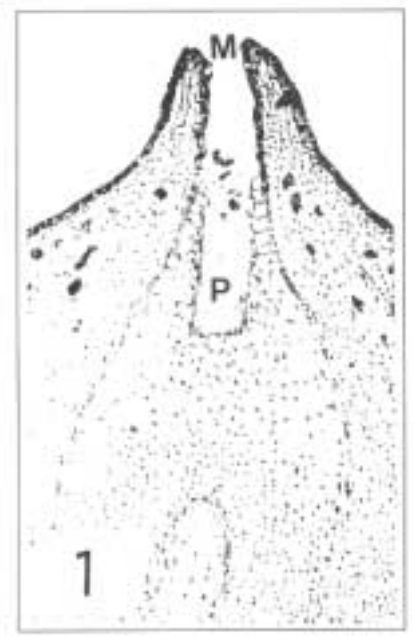


Fig. 1

There were one to several white ridges on the adaxial surface of some leaf blade (Fig. 2a). Only a few of them had some distinguishable characters of the ovule and many of these ridges were only masses of cells on the surface of the leaf blade. The vertical sections of a white ridge indicated by an arrow in Fig.2a showed a small micropyle (Fig. 2b). However, there was no cell degeneration under the micropyle. Therefore, this small ovule had no pollen chamber yet at the time of the shedding of the pollens, and probably it did not produce pollination droplet. Moreover, it was difficult to distinguish the integument and the nucellus. However, in another section of the same ridge shown in Fig.2b there was a symptom of the formation of the megaspore (Fig. 2c). According to Chamberlain (7) the development of the female gametophyte begins at the time of pollination. There were three white ridges on the leaf blade shown in Fig.2a, and two of them were only masses of cells and did not have any characters of ovule. It is in the vague whether these two white ridges might have been delayed to develop to the ovule or would remain as ridges until autumn. In fact white ridges were

still observed on some leaves even at the end of August.

Growth of the ovules---

Ovules on the leaf blade collected a week later showed a remarkable growth and development (Fig. 3a). Growth of the ovules on the leaf blade were observed and reported already (13). Around the end of June many ovules on the leaf blade were shed, and many of the usual ovules on the stalk were also shed. These abscission phenomena of ovules in June were already observed by Sakisaka (3). The reason why ovules were easily shed has not been understood. In the case of the ovules on the leaf blade some of them were shed, and others remained until autumn. It might be the competition among the growing ovules to remain on the leaf surface. In many cases a single ovule on a leaf blade were observed in autumn.

Growth of the female gametophyte---

The ovules on the leaf blade collected on the 21st of July were smaller and in many cases more slender in form (Fig. 4a) comparing with the usual ovules on the stalk collected at the same time. The vertical sections of the ovules on the leaf blade showed that the cellular gametophytes had archegonia near the surface of the gametophyte (Fig. 4b). The archegonium at this stage had one big cell, the central cell with a big nucleus. The position of the archegonium was quite different from the usual one. In the usual cases two archegonia appear on the surface of the gametophyte near the micropyle on both sides of the tent pole. One of the two ovules shown in Fig.5a was sectioned. There were two archegonia attached to each other in the gametophyte (Fig. 5b, 5c). One archegonium was located under the other. Each of them had a big nucleus. Even in the usual cases some of them have three to four archegonia in one gametophyte, Fabre-Duchartre (10).

Female gametophyte before fertilization---

The ovules collected on the 31st of August of which the second stony layer of the integument was removed is shown in Fig.7a. Two protuberances indicated by arrows were on the surface of the remaining inner layer of the integument. Two tent poles were recognized underneath the two protuberances, the one located on the side of the ovule being much smaller than the other on the top of the ovule. There were totally four archegonia in this ovule, and two archegonia which were located on both sides of the smaller tent pole were oriented obliquely to the other two archegonia. At the 31st of August, just before the fertilization in the usual ovules, the ovules on the leaf blade were still smaller than the usual, and the color of the ovules turned to yellowish (Fig. 6a, 8a). The integument of this time consists of three layers, namely outer fleshy layer, the second stony layer and the thin inner layer. This is quite the same as the usual one. The innermost layer of the integument has a somewhat withered appearance, and this is also the same feature as the usual one. The color of the innermost layer of the integument were different between the micropylar and the charazal halves. In some ovules, the dark brown portion of the innermost layer of the integument was much smaller in the micropylar portion as shown in Fig.7a. The sections of the ovule on the 31st of

August showed dry desiccated archegonia (Fig. 6b, 8b). Though the desiccation of one of the archegonia were observed even in the usual ovules on the stalk sometime, all archegonia were desiccated in the ovules on the leaf blade.. The reason why the desiccation of the archegonia namely the central cells in the gametophyte of the ovules on the leaf blade occurred just before the time of fertilization in usual ovules were not known. However, it may be considered that the nutrient supply to the ovule on the leaf blade might be much meager than to the one on the stalk. Two small ovules were recognized on a single stalk in spring. Then one of them became desiccated at the early stage of the development, and a stalk sustaining a single ovule to develop might supply enough nourishment. The absence of the male gametophyte in the pollen chamber of the ovule on the leaf blade might be an another reason for the early desiccation of the central cells of the archegonia.

Acknowledgement

The auther thanks a lot to the owner of this tree, Rev. Tomotaka Akiyama for providong materials. This study owes a lot of his kindness.

Literature

(1) Shiroi,K. 1891 in Japanese (銀杏の奇樹) Bot.Mag.Tokyo 5, 341-342
(2) Fujii,K. 1896 On the different views hither-to proposed regarding the morphology of the flowers of Ginkgo biloba L. Bot.Mag.Tokyo 10, 7-8, 13-15, 104-110.
(3) Sakisaka,M. 1927 On the morphological significance of seed-bearing leaves of Ginkgo. Bot.Mag.Tokyo, 41, 273-278
(4) Soma,S. 1997 On the microsporangium on the leaf in Ginkgo biloba. Ann. Rep.Fac. Educ.Bunkyo Univ. 31, 1-7
(5) Carothers,E. 1907 Development of ovules and female gametophyte in Ginkgo biloba. Bot.Gaz. 43, 116-130
(6) Sprecher,A. 1907 Le Ginkgo biloba L. These Univ. de Geneva
(7) Chamberlain,C.J. 1935 Gymnosperms, Structure and Evolution. Univ. of Chicago Press, Chicago
(8) Bierhorst,D.W. 1971 Morphology of vascular plants. Macmilan, New York
(9) O'Brien, T.P. and Mc Cully,M.E. 1981 The study of plant structure. Principles and selected methods. Termarcarphi. PTY Melbourne
(10) Favre-Duchartre M. 1956 Contribution a l'etude de la reproduction chez le Ginkgo biloba. Rev.Cytol. et Biol. Vegetales Paris 17, 1-214
(11) De Sloover-Colinet,A. 1963 Chambre pollinique et gametophyte male chez Ginkgo biloba. Cellule 64, 129-145
(12) Friedman,W.E. 1987 Growth and development of the male gametophyte of Ginkgo biloba within the ovule (in vivo). Amer.J.Bot. 74, 1797-1815
(13) Soma,S. 1998 On the ovules on the leaf blade in Ginkgo biloba at Hongokuji tample. Ann.Rep.Fac.Educ.Bunkyo Univ. 32, 140-147

Fig. 1 Vertical section of the ovule at the shedding of the pollens.
M: micropyle P: pollen chamber (from De Sloover-Colinet 1963)

Fig. 2a White ridges on the leaf blade collected at 18th of April.
Arrow: ridge with ovular structure

Fig. 2b Vertical section of the arrowed ridge.
M: small concave like a micropyle

Fig. 2c Other vertical section of the same ridge.
Arrow: symptom of the megasporogenesis

Fig. 3a Enlarged ridges on the leaf blade collected at 25th of April.

Fig. 4a Slender ovule on the leaf blade collected at 21st of July.

Fig. 4b Vertical section of the ovule shown in Fig.4a.
C: central cell of the archegonium
N: nucleus of the central cell

Fig. 5a Two ovules on one leaf blade collected at 21st of July.

Fig. 5b The vertical section of the ovule shown in Fig.5a.
One central cell is located under the other.
N: nucleus of the cemtral cell

Fig. 5c Other section of the same ovule.
N: nucleus of the under central cell

Fig. 6a Ovules before fertilization collected at 31st of August.

Fig. 6b Vertical section of the big ovule in Fig.6a.
Two archegonia were recognized, both were desiccated.

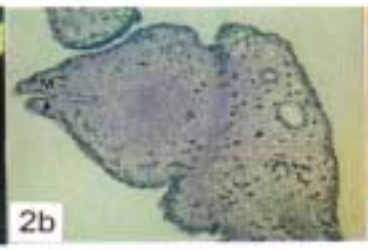
Fig. 7a The ovule with an inner layer of the integument.
Two protuberances shown by arrows were recognized, one is much smaller than the other. Each protuberance had two archegonia, thus 4 archegonia were recognized.

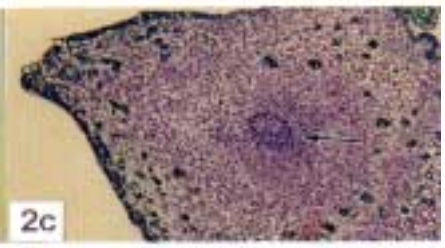
Fig. 8a One ovule on the leaf blade, this is round but smaller than the usual one.

Fig. 8b The vertical section of the ovule shown in Fig.8a.
4 archegonia were recognized, All of them were desiccated.

(スケールは１ mm)

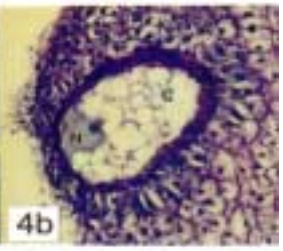

2a


2b

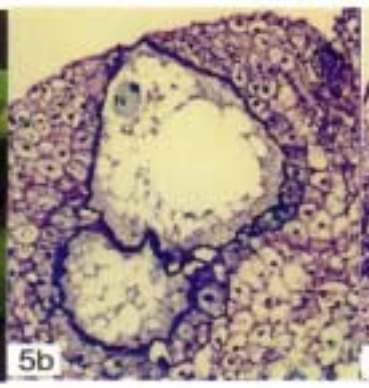

2c

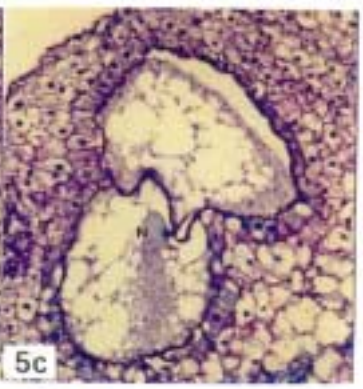

3a


4a

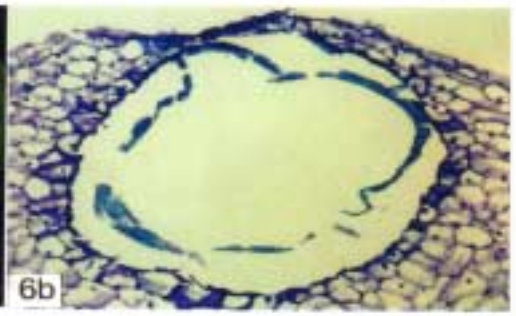

4b


5a


5b

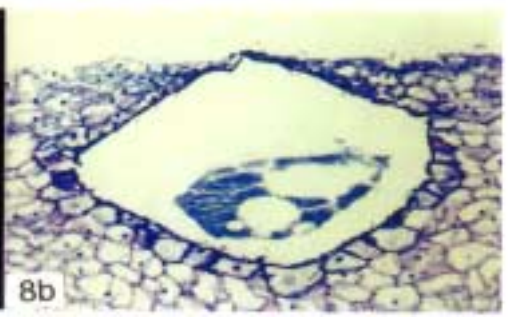

5c


6a


6b


7a


8a


8b