# EMBRYOGENESIS IN THE TEA PLANT[1]

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## Introduction

The tea plant, ***Thea sinensis*** (L.) Sims, belongs to the Theaceae and also to the open-pollinated group according to the mode of reproduction. In reviewing Johansen's (1950) comprehensive survey of the embryology in all groups of seed plants, there has not yet any report for this family. However, Schnarf (1931) summarized some results contributed by Braun (1860), Buschmann (1914), Cavara (1899) and Cohen-Stuart (1916) in his *Vergleichende Embryologie der Angiospermen.* With no exception in all the tea varieties, it takes a long time from blossom to seeds. Every year in September, October and November, one can see the flowers, flower buds and the fruits from the last year's blossom grow on the same bush. The writer was very much interested in the development of tea plant embryo and completed the most part of his work in 1959 when he was in the Pin Chin Tea Experimental Station.

## Material and Methods

The materials used in this study consisted of three plants of cultivated tea bush. Two plants, named Dah-Yeh-Oolong and Tainung No. 8, designated I and II were crossed in 1958. Another combination between Kao-Lu, designated III and Plant I was also made. In these two crosses, plant I was used as a maternal parent. Material was collected from the plants at 24, 48 and 72 hours and then 6, 12, 18, 24, 30, 60, 90, 120, 150, 180 and 210 days after pollination. The material was fixed in medium chromic acid, thoroughly washed and stored in 70% ethyl alcohol. Paraffin method was used to section the young ovary or the dissected ovule by 12 micra (Johanson 1940). Safranin and fast green combination was used.

## Results

Results were obtained with most part of the I×II combination for the

(1) This work was done under the direction of Mr. C. T. Wu, director, Pin Chin Tea Experimental Station, to whom I am indebted for suggestions and financial support. I am also indebted to Dr. H. W. Li, Director, Institute of Botany, Academia Sinica for reading the manuscript.

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surrounding tissues of the embryo, fertilization and proembryo, and development of young embryo of the tea plant.

*The Surrounding tissues of the Embryo*

The mature ovule of tea plant was anatropous surrounded with the inner integument and the outer integument, and both of these integuments were composed of eight to ten layers of cells. The megagametophyte was ellipsoidal and surrounded by a single layer of nucellar cells. The egg apparatus, antipodals and polar nuclei were normally laid out in this sac. Vascular tissue was found to have arisen from the bundles of the placenta and extended well into the funiculus. In this connection, the reader was referred to the writer's paper of 1954 for more detailed description.

*Fertilization and Proembryo*

The pollen tube passed through the micropyle and entered the magagametophyte and finally reached the destination where the egg and the synergids were laid. The synergids were not destroyed by the pollen tube, but remained intact and persisted for a period of time after fertilization, then gradually shrunk in size and ultimately disappeared (Figure 1). For the most part, fertilization took place approximately 72 hours after pollination. The first division of the endosperm nucleus took place shortly after fertilization. As a result of such successive division, the endosperm assumed a cellular appearance. The zygote divided by a transverse wall (Figure 2). Both the terminal and basal cell followed soon transversely, so that the proembryo consisted of four cells (Figure 3). Further transverse division of these cells yielded the eight-celled embryo arranging in a linear order (Figure 4).

*Development of the Young Embryo*

Some distal cells on further development gave rise to the embryo itself and consisted of a small growing sphere (Figure 5–8). Contemporaneously, the basal cells elongated and underwent a number of transverse divisions, filamentous suspensor of 10–14 cells being formed. The cell which was situated close to the micropyle, became conspicuously enlarged (Figure 5, 7). The spherical embryo continued to enlarge with further divisions of its constituent cells. As the cotyledons began to develop in the distal region, the spherical embryo became transformed into a somewhat flattened cordate body and lost its attached suspensor (Figure 9). Both the cotyledons and the hypocotyl began to elongate at that time; the nascent shoot apex consisted of a small-celled region, situated in the depression between the cotyledons (Figure 10, 11). The root apex was meanwhile becoming organized, and incipient vascular tissue could be seen in the hypocotyl between the shoot and root apices (Figure 12, 13).

## Discussion and Conclusion

The initial study of embryo is often morphological. It provides a record of development from the first division of the point where the embryo could maintain itself as a free-living individual and show us what the embryos of particular species look like. The zygote of tea plant tides over a long resting period. Its first division takes place at 120 days after fertilization. This phenomenon was also noted by Cohen-Stuart (1916) and Cavara (1899) in Theaceae but no determination was made how long it would be. Successive division then soon follows in the next sixty days and the embryo transforms itself into a very small white dot by the visual view. It is of interest to note that the young embryo lost its suspensor at 180 days old. Does it mean that the embryo can earn a living by itself? The course of development of tea plant embryo may be summarized as follows:

| Stage (Days after pollination) | Development of the embryo |
|---|---|
| 3–120 | resting stage of zygote |
| 120 | zygote gets first division; two, four and eight-celled proembryo formed, arranging in a linear order |
| 150 | spherical young embryo attached with elongated and basal cell enlarged suspensor |
| 180 | flattened cordate to forked young embryo with elongated hypocotyl and cotyledons; suspensor disappeared |
| 210 | young embryo with well organized shoot and root apices and incipient vascular tissue between them |

Degenerated young embryos have been observed both in I×II and I×III combinations, (Figure 14), but no reasonable explanation was offered.

Braun (1860) reported some polyembryos in *Camellia japonica*. Adventitious embryos in *Thea sinensis* afforded another examples of polyembryony (Cavara 1899). No polyembryo had been met in this investigation, although the writer did discover three twin seedlings out of 500 germinated seeds of Hwangkan variety in the previous year.

Schnarf (1929), Johanson (1945), and Maheshwari (1950) distinguished five principal types of dicotyledon embryo. Beyond these, Johanson (1950) added the Piperad type. The cellular configuration of the proembryo at each stage, and the part played by each of these cells in the organogenic development constituted the basis for a classification of embryonomic types (Wardlaw 1955). Cavara (1899) reported that the proembryo of *Thea sinensis* consisting of 12 cells, arranging in a linear order. The distal one divided longitudinally first and

followed by the transverse division; thus, by another division, formed an octant young embryo. His result proved that the basal cell (cb) of the two-celled proembryo had no function in the organization of the embryo proper. The development of *Daucus carota* (Umbelliferae, of Solanad type) embryo is comparable to that of the *Thea sinensis*. Both of them have a linear eight-celled proembryo and the difference between these two species is that the former has a short suspensor and the later an elongated one. In the description of Solanad type, Johanson (1950) stated that the terminal cell (ca) of the two-celled proembryo divided by a transverse wall; the basal one (cb) played only a minor part or none in the subsequent development of the embryo and therefore the basal cell usually formed a suspensor of two or more cells. It can be inferred that the development of *Thea sinensis* embryo belongs to the Solanad type. Further studies are needed to varify this inference.

# 茶樹胚胎之發育

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1. 茶樹人工雜交授粉後72小時，即可看到授精現象(圖一)。

2. 卵細胞授精後，渡過一甚長之休眠期，其第一次分裂發生於授粉後120天，隨即繼續分裂形成四細胞及八細胞之初期胚胎(圖二、三及四)。此後30天內，初期胚胎上端之若干細胞不斷分裂形成球形之幼胚，其下端若干細胞則形成一細長之懸體，懸體末端之細胞特別肥大(圖五、六、七及八)。再過30天，即授粉後180天，可用肉眼於胚珠內壁近種臍處看到白色小點之幼胚；此時子葉，胚莖胚根之頂端及中莖之維管束等已漸次形成(圖九、十及十一)。210天幼胚之發育，更見完善(圖十二及十三)。

3. 茶樹初期胚胎至球形幼胚期內之詳細發育過程，尙需進一步研究由此可以追究茶樹胚胎之發育屬於何種型式。由於卵細胞授精後第一次分裂爲橫的分裂及 Cavara (1899) 氏稱茶樹幼胚是由初期胚胎頂端的一個細胞分裂形成，或可推論茶樹胚胎發育之型式屬於茄科型 (Solanad Type)。

4. 授粉後150天取樣之胚珠中，發現一、二已經崩壞之幼胚，其原因未明。(摘要)

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## Explanation of Plates

### Plate I

Figure 1. Transverse section of ovule collected at 72 hrs after pollination, showing the resting zygote, I×II,×400.

Figure 2-4. Transverse section of ovules collected at 120 days after pollination, showing the proembryos, I×II,×400.

Fig. 2, Two-celled proembryo.

Fig. 3, Four-celled proembryo.

Fig. 4, Eight-celled proembryo, the dotted line helps to count the cells.

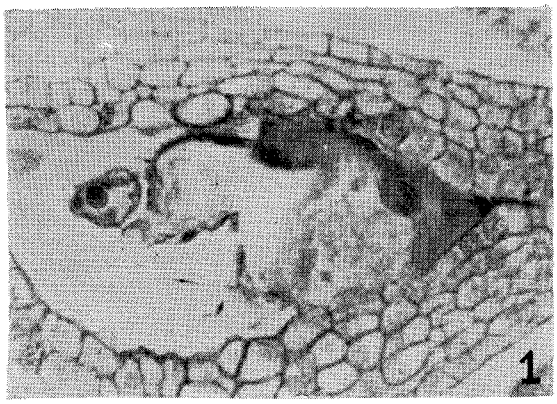
Figure 5-8. Transverse sections of ovules collected at 150 days after pollination, showing the young embryo.

Fig. 5, Young embryo with elongated and enlarged basal cell of the suspensor, longitudinal section, I×II,×100.

Fig. 6, Young embryo, consisting of a small growing sphere, I×II,×400.

Fig. 7, I×III, similar to Fig. 5.

Fig. 8, I×III,×600, similar to Fig. 6.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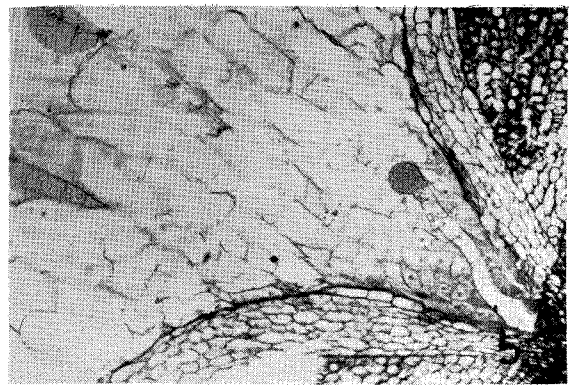

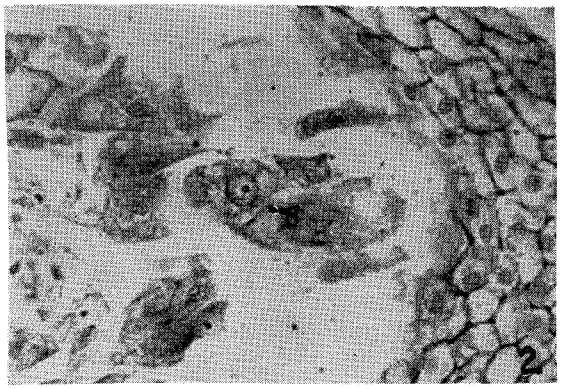
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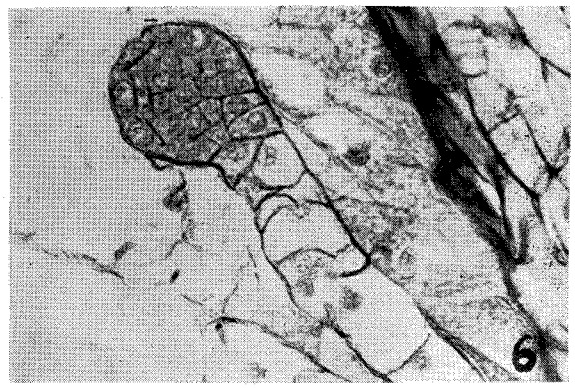
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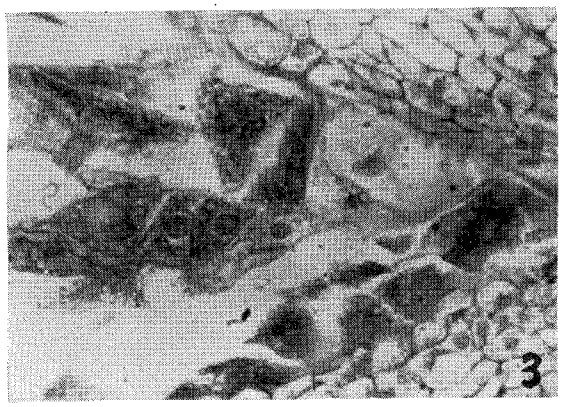
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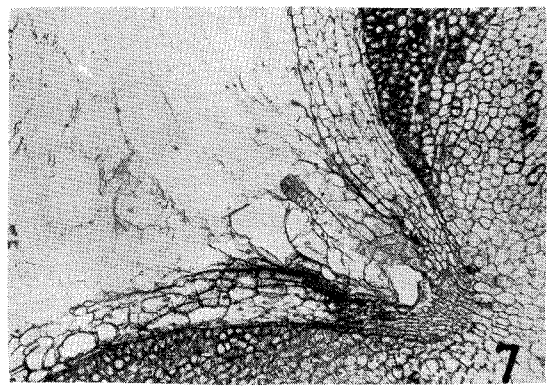
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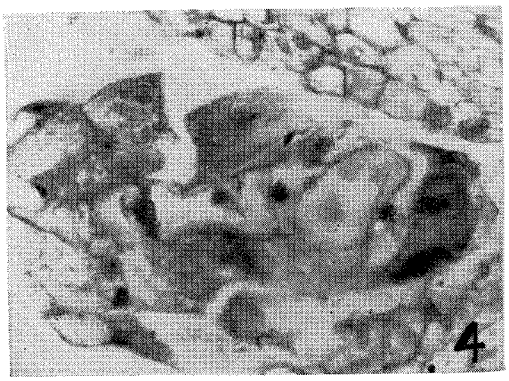
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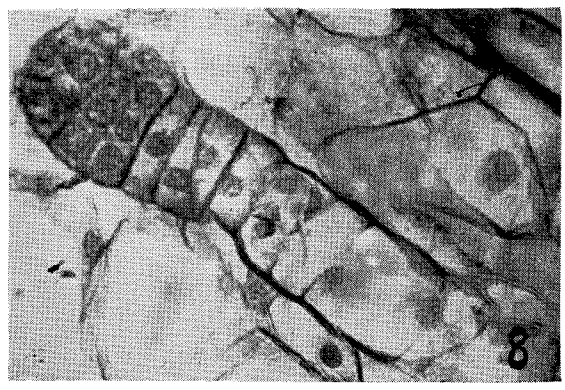
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## Plate II

Figure 9-11. Transverse sections of ovules collected at 180 days after pollination, I × II, × 100.

Fig. 9, Flattened cordate young embryo, longitudinal section.

Fig. 10, Young embryo, longitudinal section, showing the elongated hypocotyl and cotyledons.

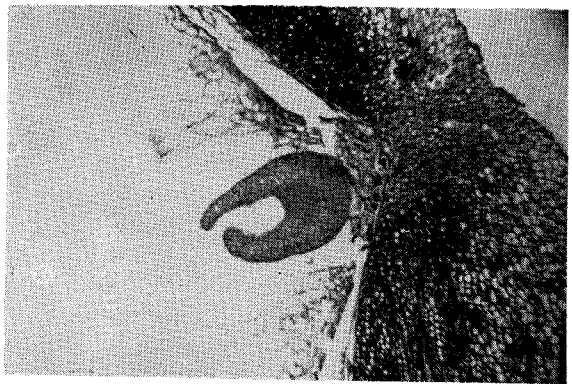
Fig. 11, Similar to Fig. 10, a little bit older.

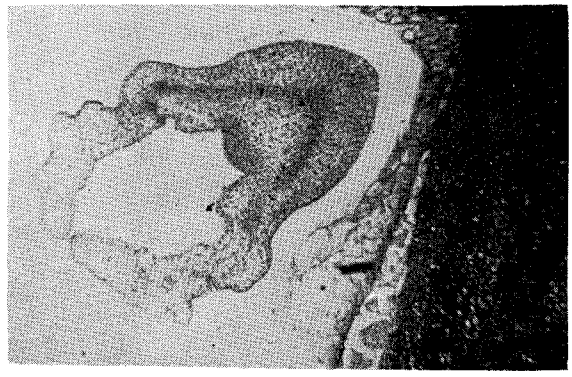
Figure 12-13. Transverse sections of ovules collected at 210 days after pollination, I × II, × 100.

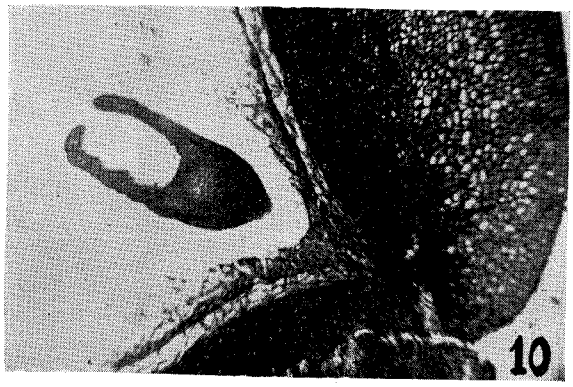
Fig. 12, Young embryo, longitudinal section, showing the organized shoot and root apices and incipient vascular tissue between them.

Fig. 13, Similar to Fig. 12, a little bit older.

Figure 14. Transverse section of ovule collected at 150 days after pollination, showing the degenerated young embryo, longitudinal section, I × III, × 400.



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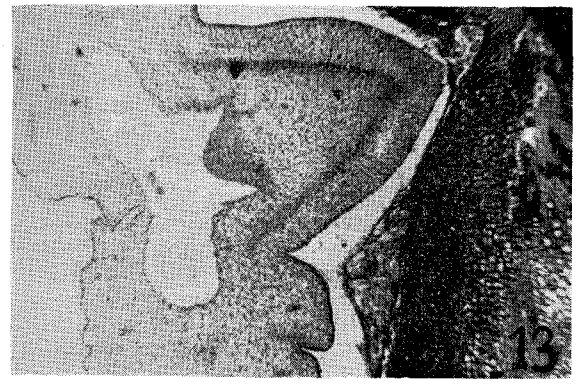
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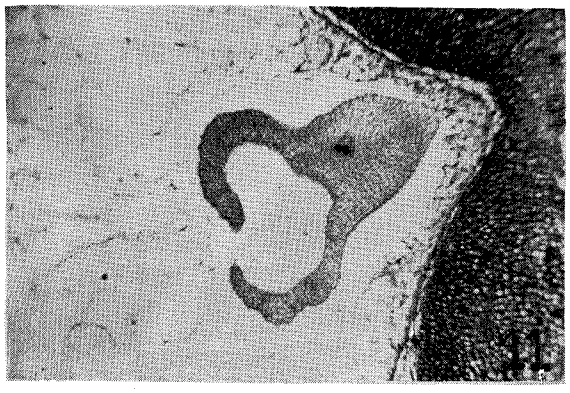
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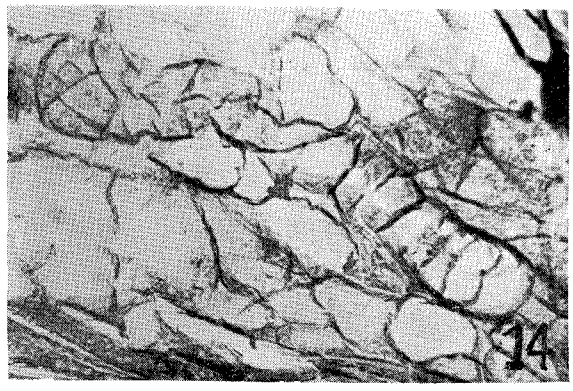
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