STRUCTURE AND DEVELOPMENT OF SEED IN *CAMELLIA SINENSIS* (L.) O. KUNTZE

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Syngamy and triple fusion occur simultaneously. The remnants of the pollen tube persist for a long time. There are three antipodal cells which remain healthy up to the early stages of development of the seed. The endosperm is nuclear in the beginning but becomes cellular afterwards. The embryogeny conforms to the Solanad type. The mature embryo has two large cotyledons which grow backward on either side of the radicle. The testa is formed by the outer integument. The cells of the outer 10 or 12 layers excepting the epidermis differentiate into sclereids. The vascular supply extends almost to the tip of the outer integument. The inner integument becomes compressed and later degenerates. The pericarp is characterized by the presence of several idioblasts which are elongated, thick-walled and slightly branched.

Introduction

Kapil and Sethi (1963) have already published an account of the development of the male and female gametophytes in *Camellia sinensis*. A perusal of the earlier literature indicates that very little work has been done on the structure of the seed. Although Cavara (1899) dealt with endosperm formation, he made no mention of the early stages of its development. Similarly Wu (1960) described the embryogeny as of the Solanad type but emphasized the need for further verification. This paper therefore concerns events taking place after fertilization.

Material and Methods

Buds, flowers and fruits of *Camellia sinensis* were obtained by Professor B. M. Johri through the courtesy of Dr. D. N. Barua, Senior Botanist, Tocklai Experimental Station, Cinnamara, Assam. The ovaries were dissected out,

Fig. 1. (*ant*, antipodal cells; *ds*, degenerated synergid; *e*, egg; *end*, endosperm; *pemb*, proembryo; *pen*, primary endosperm nucleus; *pn*, polar nuclei; *pt*, pollen tube; *sn*, secondary nucleus; *z*, zygote; $♂_1$, $♂_2$, male gametes). A, B, micropylar portions of embryo sacs enlarged to show syngamy and triple fusion. ×634. C, fertilized embryo sac. ×140. D–F, two, four and sixteen-nucleate endosperm. ×280. G, l.s. young seed (testa removed) showing cellular endosperm. ×56. H, same at a later stage. ×24. I, the walls of the central cells of endosperm are disintegrating. ×21. J, magnified view of portion J from I to show healthy cells of endosperm lying close to the proembryo. ×317.

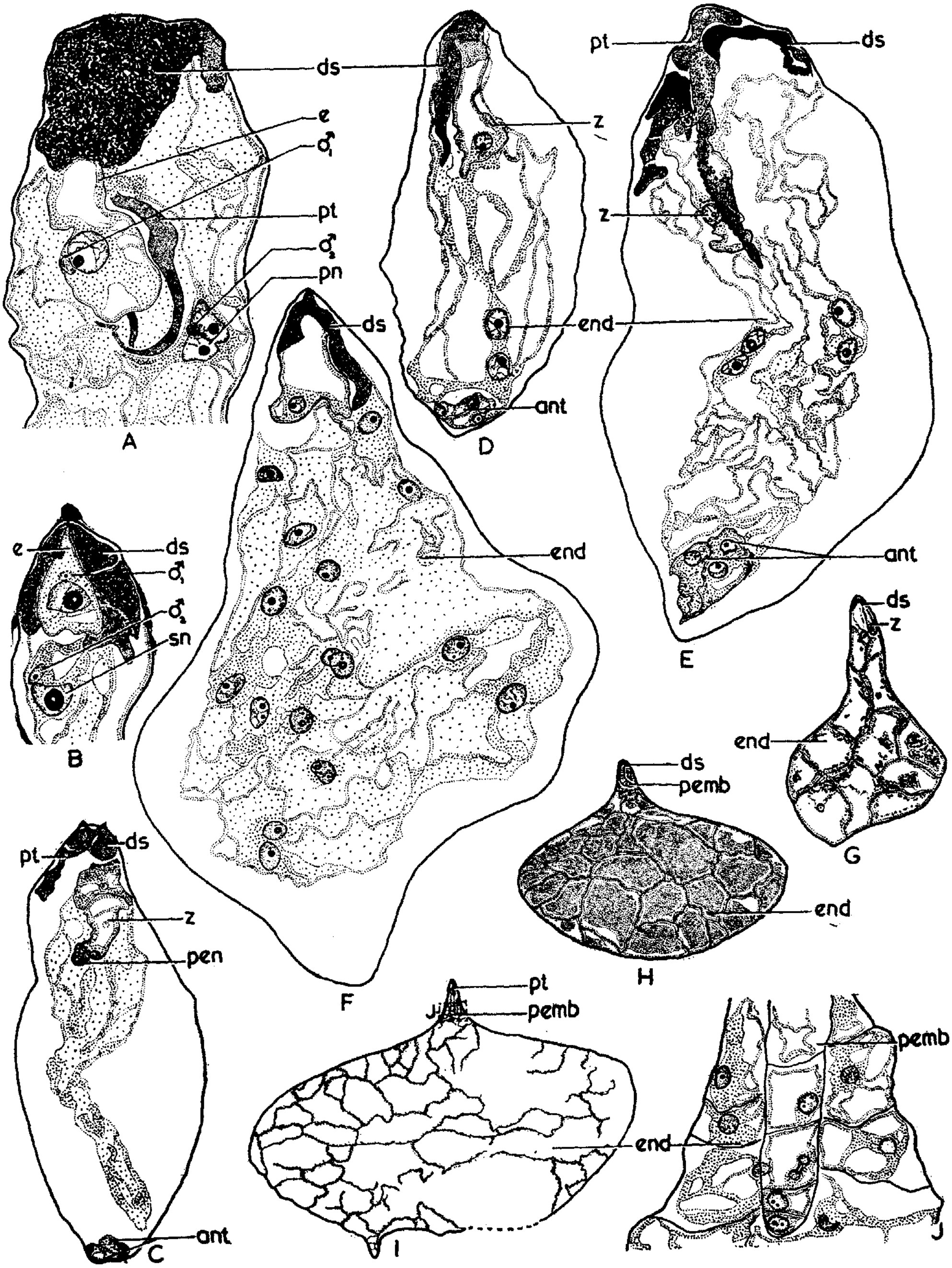
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FIG. 1.

trimmed from the top, fixed in formalin-acetic-alcohol and then stored in 70 per cent ethyl alcohol. The seeds were also trimmed before fixing. After the usual dehydration and infiltration, the material was imbedded in paraffin wax of 56–58° C melting point. The mature seeds and young fruits were treated with 20 per cent hydrofluoric acid in 70 per cent ethanol for two to three months before processing them for dehydration. The paraffin-imbedded material was exposed to a mixture of glacial acetic acid 10 cc, 60 per cent ethyl alcohol 90 cc, and glycerine 30 cc. The sections were cut at thicknesses of 14–20 μ, and stained with safranin and fast green. Since the seed coat becomes very hard at maturity, it was removed and the embryos were imbedded separately. Sections of the testa were made with a sliding microtome.

Observations

1. *Fertilization*

Syngamy and triple fusion take place simultaneously (Fig. 1A). In one preparation one of the male gametes was lying close to the egg nucleus while the second was near the secondary nucleus (Fig. 1B). Sometimes the pollen tube is very long and reaches almost the middle of the embryo sac (Fig. 1E). Its remnants persist even when the filamentous proembryo has been formed (Fig. 1I). Although the pollen tubes have been seen to enter almost all the ovules in a locule, only one ovule develops into a seed. This is probably due to the early degeneration of the embryo sac or failure of syngamy and triple fusion. The synergids usually disorganize before fertilization and only their remnants persist as darkly stained masses. Occasionally they increase in size, are hooked, and either both or only one of them remains healthy for some time. There are three antipodal cells which are discernible till the early stages of development of the endosperm (Fig. 1D, E); an exceptional embryo sac showed as many as six antipodal cells.

2. *Endosperm*

The fertilized embryo sac (Fig. 1C) broadens considerably. The primary endosperm nucleus divides much earlier than the zygote and its first few divisions are free nuclear (Fig. 1D–F). At the 16-nucleate stage the endosperm nuclei are evenly distributed all over the embryo sac (Fig. 1F). At the time the zygote is about to divide, thin septa appear in the endosperm

FIG. 2. (*lp*, leaf primordia; *rc*, root cap; *sus*, suspensor). A, zygote. ×317. B–D, two, three and four-celled proembryos. ×317. E, twin proembryos. ×317. F–I, stages in the development of filamentous proembryo. ×317. J, K, later stages in embryogeny. ×317. L, M, young and old globular proembryos. L, ×317; M, ×280. N–Q, stages leading to the formation of a dicotyledonous embryo. N–P, ×56; Q, ×32. R, portion R of the embryo in Q enlarged to show proliferation of suspensor cells. ×280. S, T, l.s. mature embryos; three pairs of leaf primordia are seen just below the level of the shoot apex in T. ×8. U, mature embryo in surface view. ×2.

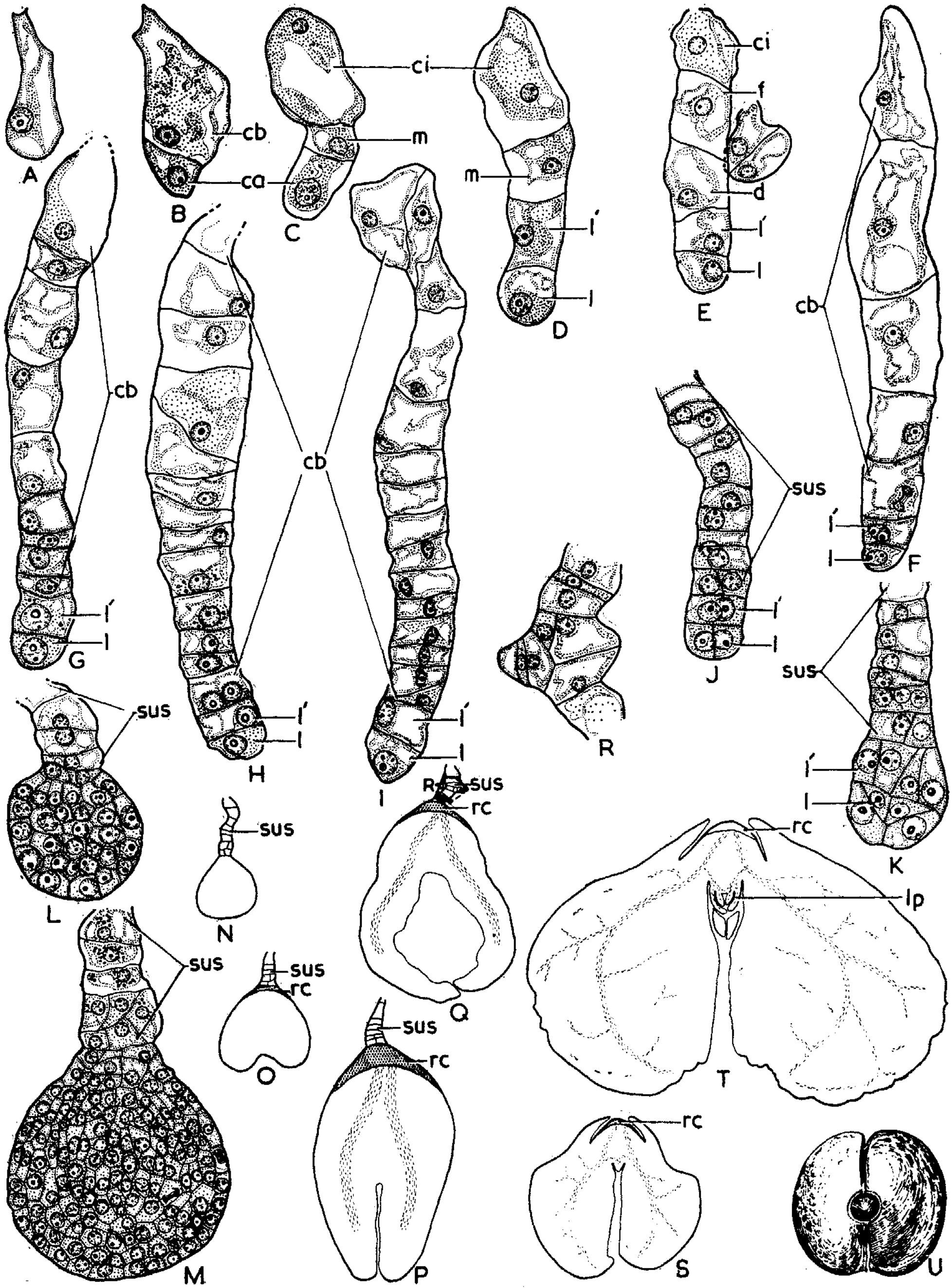

FIG. 2.

and the newly formed cells show a deposition of some granular substance along their periphery (Fig. 1G). This material gradually spreads into the cells (Fig. 1H) but subsequently disappears. The nuclei become indistinct whereas the cells in the central portion forming the bulk of the endosperm appear unhealthy and begin to disintegrate. However, those lying in the proximity of the proembryo and at the chalaza are quite healthy. They are thin-walled, have prominent nuclei and vacuolate cytoplasm (Fig. 1I, J). There is little or no endosperm in the mature seed. One embryo sac, probably abnormal, showed great heterogeneity in the size of the endosperm nuclei; the larger ones had apparently been formed by the fusion of smaller nuclei.

3. *Embryo*

The zygote undergoes a long period of rest and divides only after the endosperm is well advanced. It enlarges and divides transversely to form the terminal cell *ca* and the basal cell *cb* (Fig. 2A, B). Both the cells now undergo another transverse division to produce a four-celled, filamentous proembryo (Fig. 2C, D). Several transverse divisions in the derivatives of the basal cell result in a 14 to 16-celled uniseriate suspensor (Fig. 2E–I). However, the cells lying at the basal end show some vertical divisions. The suspensor is discernible even when the cotyledons have enlarged and frequently its cells contain starch grains.

The tiers *l* and *l′* derived from the terminal cell divide by vertical walls to produce two cells in each tier (Fig. 2J). Fig. 2 (K–M) shows stages in the development of a globular proembryo. Further differentiation leads to the formation of the cotyledons (Fig. 2N–Q). The mature embryo has two large, profusely vascularized cotyledons which grow backward on either side of the radicle (Fig. 2S, T); they are rich in starch and fatty substances. The shoot apex is enclosed by foliar primordia (Fig. 2T, U).

Although only one embryo usually develops from the zygote, in one case a two-celled problematic structure was seen lying close to the filamentous proembryo (Fig. 2E). In another preparation a knee-shaped embryonal protuberance was observed to arise from some cells of the suspensor (Fig. 2Q, R).

4. *Seed and Testa*

The cells of the hypostase become further thickened after fertilization, take a deep red stain with safranin and persist at the chalaza. At the

FIG. 3. (*ii*, inner integument; *oi*, outer integument; *sc*, sclereids; *vs*, vascular supply). A, l.s. ovule at the mature embryo sac stage. ×173. B, longisection of seed coat at filamentous stage of proembryo; vascular strands traverse the outer integument. ×152. C, same at dicotyledonous stage of embryo; the inner integument appears as a compressed layer of flattened cells. ×115. D, portion of mature seed coat showing several layers of sclereified cells differentiated in the outer zone of the outer integument; the inner integument has degenerated. ×108.

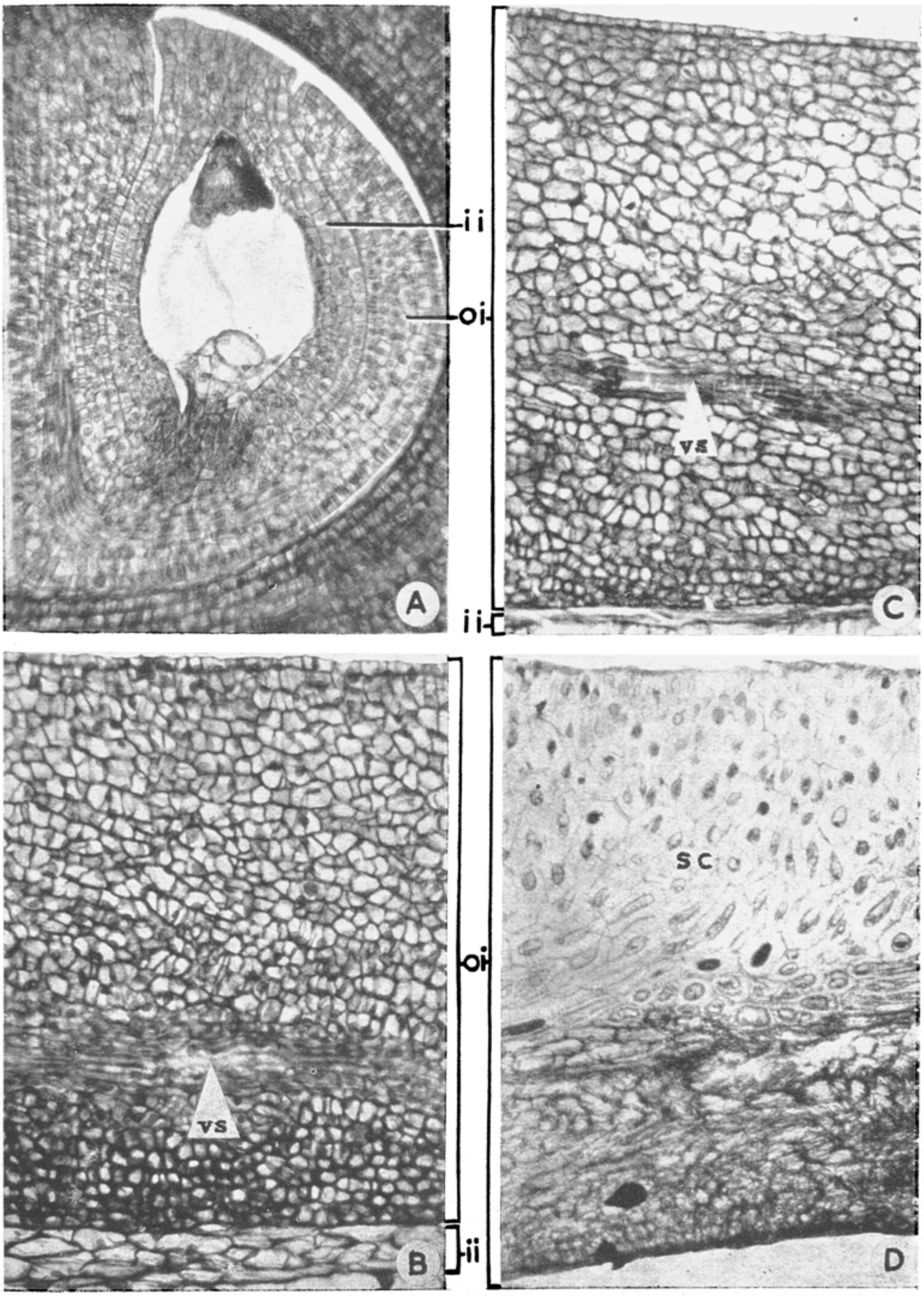

FIG. 3.

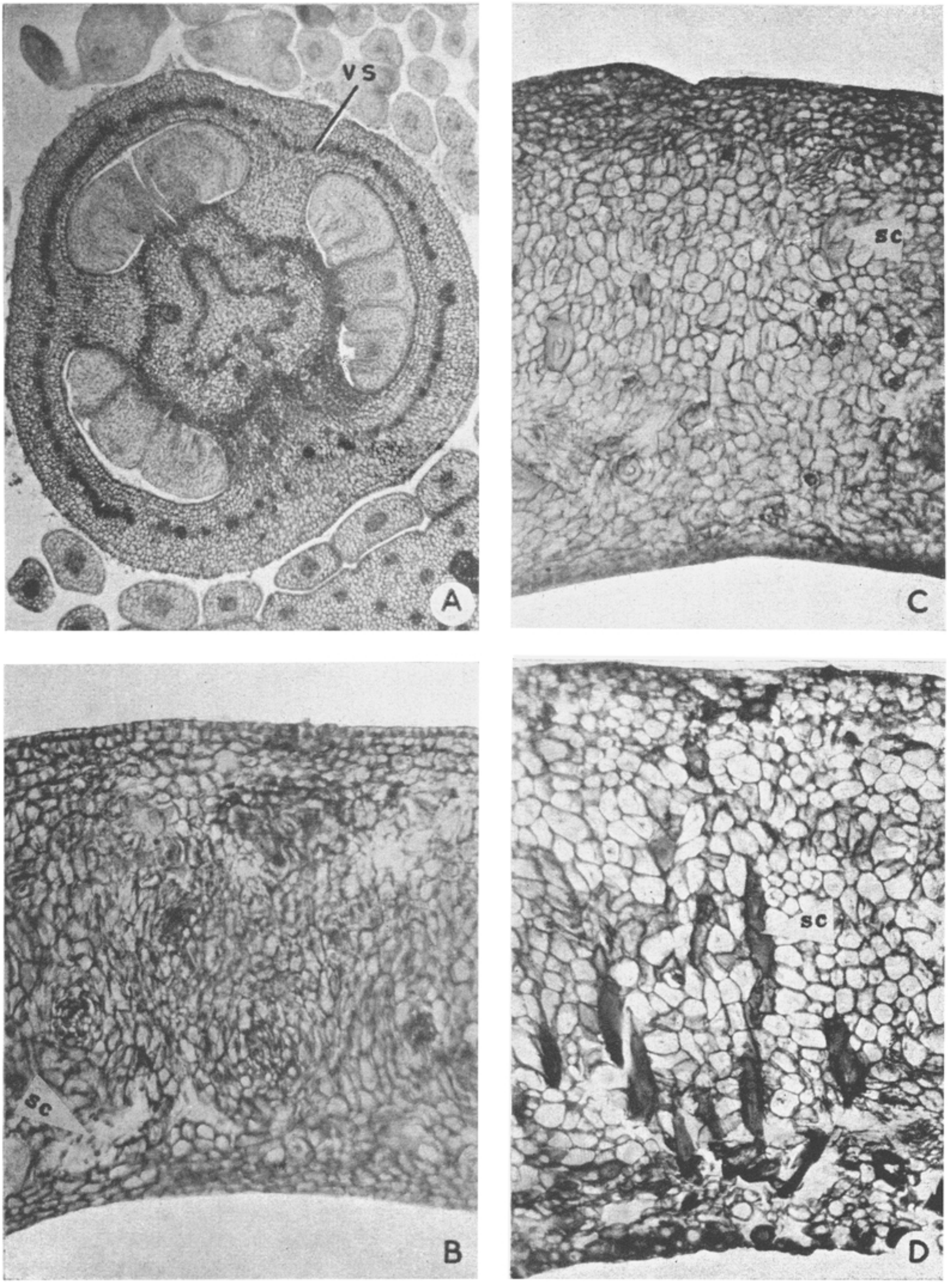

FIG. 4.

archesporial cell stage the outer integument consists of three or four layers of parenchymatous cells whereas the inner shows two or three layers. Except for an increase in the number of cell layers, no other conspicuous changes occur in them till the maturation of the embryo sac (Fig. 3A). Subsequent differentiation of the tissues is confined to the outer integument. Its outer epidermis becomes stretched and flattened. The parenchymatous zone below the epidermis is separated into two portions by the ramifying vascular strands which consist of spiral tracheids and elongated parenchymatous cells (Fig. 3B). The cells of the outer region show granular contents and begin to develop sclerenchymatous thickenings; some of them also contain crystals of calcium oxalate. The thickenings increase further so that the lumen of the cells is considerably reduced (Fig. 3D). The cells of the inner zone also become slightly thickened and contain tannin. Contrary to this, the cells of the inner integument elongate, become vacuolate and begin to degenerate (Fig. 3C at *ii*).

5. *Fruit and Pericarp*

The fruit is green and rough and dehisces from the apex by splitting into three valves.

At the megaspore mother cell stage, the ovary wall consists of 16–18 layers of parenchymatous cells. Those of the outer as well as inner epidermis are more or less elongated and in the tissue enclosed by them there is a ring of vascular bundles. Numerous hairs are present on the wall (Fig. 4A), but they soon fall off and are absent in the mature fruit. At the time of fertilization, the parenchymatous cells become slightly thickened. They show chloroplasts and a large number of crystals of calcium oxalate. The cell layers continue to increase and some of the cells in the periphery become transformed into thick-walled cells with a wide lumen containing the cytoplasm and nucleus. A similar change takes place in the inner layers (Fig. 4B). The sclereids are scattered throughout the parenchymatous tissue (Fig. 4C) and their arms extend into the adjacent cells. When mature, they are extremely thickened, and their cytoplasm and nuclei are not seen except for a thin streak representing the lumen. The cells of the innermost two or three layers also become sclerotic but the sclereids are rounded and have a wider lumen (Fig. 4D).

FIG. 4. (*sc*, sclereids; *vs*, vascular supply). A, t.s. ovary; the wall consists of 16–18 layers of parenchymatous cells traversed by vascular supply. ×48. B, pericarp after fertilization; sclereids have developed in the outer as well as inner part. ×119. C, same at a later stage; sclereids have appeared in the middle part also. ×68. D, fruit wall showing numerous sclereids. ×68.

Discussion

Cohen Stuart (1916) reported the presence of three antipodal cells but sometimes he noted 10 or 11 cells which by the density of their cytoplasm and strong staining of their nucleoli seemed to belong to the embryo sac (*see also* Schnarf 1931). My observations show that there are only three antipodal cells and there is no increase in their number at any time even though they often remain healthy until after fertilization. Cohen Stuart (1916) seems to have misinterpreted the upper cells of the hypostase as antipodal cells.

According to Cavara (1899) the division of the primary endosperm nucleus commences when the male nucleus penetrates the egg indicating thereby that triple fusion precedes syngamy. Cohen Stuart (1916) was unable to make any observation on this point since in his preparations the egg apparatus was always enveloped by a black and opaque mass of protoplasm. I find that syngamy and triple fusion take place simultaneously, and the pollen tube persists up to the filamentous stage of the proembryo.

Cavara (1899) states that there are several endosperm nuclei which become separated by "linee granulari di citoplasma che non segregano una membrana cellulosica." Cohen Stuart (1916) has presented a few stages showing a cellular endosperm but makes no mention of the earlier development. Wu (1960) writes: "The first division of the endosperm nucleus took place shortly after fertilization. As a result of successive divisions, the endosperm assumes a cellular appearance." He thus makes no definite statement whether the endosperm is *ab initio* Cellular or Nuclear. My observations prove that the first as well as a few subsequent divisions of the primary endosperm nucleus are nuclear, and the endosperm becomes cellular only at a later stage.

The development of the embryo corresponds to the Solanad type as also recorded by Wu (1960). A feature of special interest is the backward growth of the cotyledons on either side of the radicle. In *Floerkea proserpinacoides* (Limnanthaceae) Maheshwari and Johri (1956) observed not only the backward extension of the cotyledons but also a bifurcation of their tips so that in a cross-section four lobes are seen at the upper end. Cavara (1899) reported multinucleate suspensor cells but I failed to observe such a condition in any of my preparations.

In 1860 Braun reported some supernumerary embryos in *Camellia japonica*. According to Cavara (1899) in *Camellia sinensis* several adventitious embryos arise from the placental cells as well as the inner integument. He also noticed twin embryos occupying the micropylar end with their suspensors twisted around each other. However, Cohen Stuart (1916) argues that two embryos could arise as a result of fertilization of an embryo sac having double the number of nuclei, by two pollen tubes. Subba Rao (1943;

quoted in Venkataramani 1950) found a small secondary embryo in between the cotyledons of a normal tea seed but without any organic union. Venkataramani (1950) states that a germinating tea seed gave rise to three radicles; and when he removed the hard shell he found three embryos, each having its own radicle, plumule and cotyledons. Wu (1960) spotted three twin seedlings out of 500 germinated seeds of the Hwangkan variety. During my study in one preparation I observed a slight proliferation of the cells of the suspensor* whereas another showed a two-celled proembryo lying close to the filamentous proembryo.

Acknowledgements

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* In *Actinidia chinensis* (Crété 1944) and *A. polygama* (Vijayaraghavan 1963), members of the closely related family Actinidiaceae, such proliferations lead to the formation of supernumerary proembryos.