## A CONTRIBUTION TO THE LIFE-HISTORY OF LITCHI CHINENSIS SONN.

By I. BANERJI AND K. L. CHAUDHURI (Department of Botany, Calcutta University)

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Litchi chinensis, a member of the family Sapindaceæ is a native of China. It is said to have been introduced into this country and is at present widely cultivated as a fruit tree for its sweet arillous fruits of extraordinary flavour.

The literature on the morphology of plants belonging to the family Sapindaceæ appears to be meagre. According to Schnarf,<sup>3</sup> Guérin in 1901, published a paper on the development of the endosperm and embryo in Cardiospermum Halicacabum. Later Perrot and Guérin worked on Alluandia (Didiera).

In India Abdur Rahman Khan² worked on the pollination and fruit formation of Litchi. He has mainly described the different forms of flowers and has recorded his observations on the nature of the flowers produced in the different branches during the flowering season. Recently Sen⁴ has made some morphological studies.

The interesting observations made by Khan<sup>2</sup> on the nature of flowering, and the dearth of any information regarding the embryology and cytology of the plant made it an important subject for study. A short note on some important observations was published in 1940 by one of us.<sup>1</sup>

## Material and Methods

The material for this study was obtained from plants growing in private gardens at Ballygunge and also at the garden of the Agri-Horticultural Society at Alipur. The material was fixed in the garden, and an exhaust pump was always used to ensure proper penetration of the fixing fluid. Allen's modified Bouin's fluid and Flemming's fluids were used for fixation. Dehydration, clearing and embedding were done in the customary way. Sections were cut 10 to 16 microns thick depending on the stage required for study. Heidenhain's iron-alum hæmatoxylin and Newton's gentian violet iodine were used for staining.

- (a) The flower and the inflorescence.—The flowers are functionally unisexual. The floral morphology of the different types of flowers is described below:—
- (i) Female flowers.—Calyx usually 4-lobed, dentate. Corolla absent. Disc fleshy, glabrous. Stamens 6, filaments short, pubescent, inserted inside the disc and around the pistil. Anthers turgid, non-dehiscent. Gynæcium pubescent. Ovary 2-lobed, style prominent. Stigma bifid with well-developed glandular hairs.
- (ii) Male flowers.—Calyx usually 4-lobed. Corolla absent. Disc glabrous, smaller than that in the female flower. Stamens usually 6, rarely 7 or 8. Filaments long, pubescent. Gynæcium in the form of a pistillode which is thickly covered with hairs.

Another type of male flower which varies slightly from the aforesaid type has been observed in some trees. In this, the filaments of the anthers are shorter than in normal male flowers. It has a pistillode which outwardly resembles a fertile gynæcium. Rudimentary ovules are present inside the pistillode. The style is short and the stigmatic lobes remain unopened.

All the flowers are pedicillate. The pedicel and the calyx lobes are hairy. In some of the female flowers the filaments become very short and bear two anthers. Of these, one anther lobe of each anther is bigger than the other. In other instances the filaments become branched and each of them bears an anther.

The inflorescence is a panicle. The flowers are borne on the axis of the 3rd order, rarely on the 4th, and occasionally on the main axis of the inflorescence. Generally speaking, the inflorescence is of a compound racemose type. The flowers occur in cymes, which are generally of one sex. The constitution of the cymes with regard to the sexuality of the flowers often varies during the flowering season. Generally variations are noted during the transitional stages of flowering, *i.e.*, when both male and female flowers occur together. The different types of cymes observed are as follows:

- (i) Cymes composed entirely of male flowers.
- (ii) Cymes composed entirely of female flowers.
- (iii) Cymes terminated by a male flower with the lateral flowers female.
- (iv) Cymes terminated by a female flower with the lateral flowers male.
- (v) Cymes terminated by a male flower with the lateral flowers of different sexes.

(vi) Cymes terminated by a female flower with the lateral flowers of different sexes.

All the branches of the inflorescence (with rare exceptions) begin flowering almost simultaneously. The flowering as noted by Khan<sup>2</sup> is characterised by the opening of flowers of different sexes at different periods. Flowering records of a few plants for two consecutive seasons (1940 and 1941) show that there are distinct periods of bloom for the male and the female flowers. These periods may occur alternately or two consecutive periods may overlap.

The majority of the trees produce male flowers first. The number of flushes' in the different trees appears to be variable and in some instances a transitional stage is also observed.

Similar observations have been made by Khan. It is interesting to note that the flowering period is very short and is composed of succession of flushes of male and female flowers. Table I shows the duration of flowering of the trees under observation during the seasons 1940 and 1941.

TABLE I

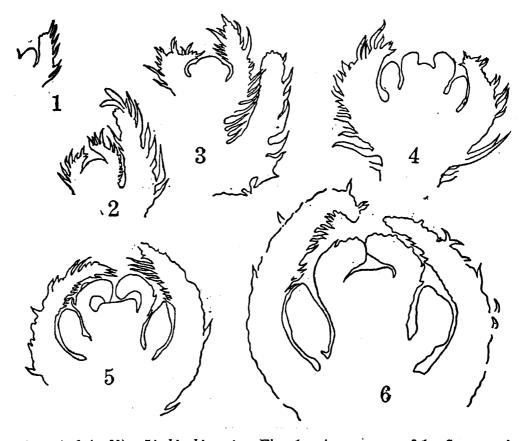
Litchi chinensis. Duration of Flowering in the Individual Trees

Tree No.	1940	1941
1	12th Feb. to 9th March (27 days)	Did not flower
2	15th Feb. to 10th March (25 days)	25th Feb. to 19th March (23 days)
3	20th Feb. to 4th March (14 days)	23rd Feb. to 11th March (17 days)
4	3rd March to 22nd March (20 days)	10th March to 22nd March (13 days)

Thus the average period of flowering in the year 1940 was 21.5 days and 14.3 days in 1941. Khan² found the average period to be 24.25 days in the year 1928. It must be admitted that these observations are of a preliminary nature and it is desirable that observations of a large number of plants extended over a few years should be made to get a clear idea as to the period of flowering and also on the influence of climatic factors on the production of flowers.

(b) The organogeny of the flower.—The flower primordium first makes its appearance in the axil of a bract (Text-Fig. 1). The primordium soon

enlarges and from its sides protuberances arise, which grow rapidly and develop into the calyx lobes (Text-Fig. 2). These are four in number as seen in transverse sections. The calyx lobes are characteristically hairy, the hairs are unicellular and are the prolongations of the epidermal cells. They are thick-walled, curved and subulate in form. At this stage the central primordium appears as a semicircular mass of tissue in longitudinal sections, so that it is really convex in form. The primordia of the anthers next become differentiated at the axil of the calyx lobes and away from the centre (Text-Fig. 3). The central primordial tissue grows slightly, but very soon two lateral protuberances develop and the tip becomes somewhat flattened (Text-Fig. 4). These protuberances are the carpel



Text-Figs. 1-6 ( $\times$ 50). Litchi chinensis.—Fig. 1. Appearance of the flower primordium. Fig. 2. The origin of the calyx. Fig. 3. The promordia of the stamens appear. Fig. 4. The growth of the stamens and appearance of the carpel primordia. Fig. 5. Further growth of the carpel primordia. Fig. 6. A later stage of the same, showing the presence of a gynophore.

initials which curve over and meet at the central region of the floral axis where the tissues subsequently become united. The later growth of the carpels takes place both in dorsal and ventral directions (Text-Fig. 5). Thus the solid style and the bilocular ovary is organised. The tissues encased by encircling carpels form the placenta. The stigma is bifid, the lobes are recurved and show the presence of glandular cells.

Simultaneously with the development of the carpels the tissue lying between the stamens and the ovary grows in length resulting in the formation of a short gynophore (Text-Fig. 6). A disc is also noted to develop between the bases of the growing filaments and calyx lobes. It assumes a massive form when fully developed.

(c) The development of the microspores and the tapetum.—The microspore mother cells soon after their differentiation are closely packed inside the anther loculus. At synizesis a contracted knot is produced which generally encloses the nucleolus in its meshes. The bivalent nature of the chromosomes becomes apparent at diakinesis (Text-Fig. 7). Division I is regular, though occasionally a few laggards have been noted. During interkinesis the chromosomes show the split for the II division. Cytokinesis takes place by the process of furrowing. No difference in the meiotic process was noted in the pollen mother cells of the functional female plants.

The differentiation of the tapetal layer in the microsporangium could not be traced. The tapetum is first recognised when the microspore mother cells have become organised. It consists of a single layer of cells, each cell containing a big nucleus and granulated cytoplasm (Text-Fig. 9). Along with the growth of the microsporangium the tapetal cells divide mitotically without the formation of a wall, as a result, the cells become binucleate. At this stage the cells increase in a radial direction and seem to protrude inside the anther cavity (Text-Fig. 10). This condition is generally maintained till the formation of the microspores when the tapetal cells grow laterally and become more or less rectangular in form as shown in Fig. 11. The tapetal cells degenerate after the pollen grains are fully differentiated.

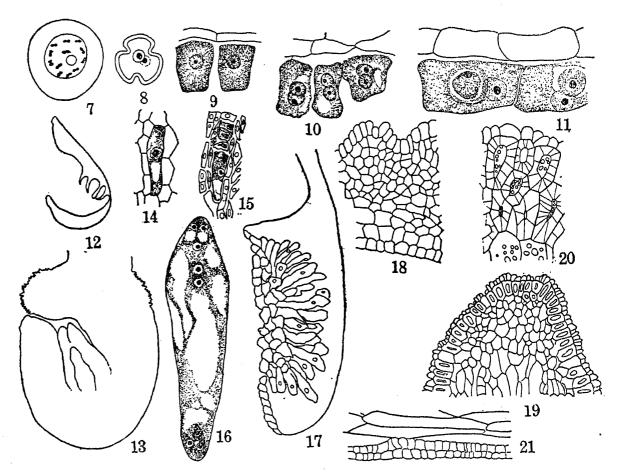
(d) The pollen grains.—Pollen grains obtained from the male flowers are barrel shaped when dry. Mounted in water or lactic acid, they become somewhat triangular in form, showing the presence of three germ pores at the corners. The exine shows the presence of three furrows in the centre of each of which a germ-pore appears to be present. The exine is granular at the surface and its colour light yellow. Comparative measurements of pollen grains obtained from each type of flower shows very little difference in size.

The pollen grains of the male flowers are binucleate at the time they are shed. The two nuclei lie very close together and appear to be almost identical in shape and size (Text-Fig. 8).

Attempts were made to germinate the pollen grains under laboratory conditions. Five per cent. cane sugar solution was used as a medium and

the cultures were kept under humid conditions. The pollen grains germinated within an hour. The male flowers produced normal pollen tubes, whereas the female flowers produced "short tubes".

(e) The development of the female gametophyte.—The ovule primordium first arises as a tiny protuberance from the placental tissue. It grows obliquely upwards and very soon assumes an anatropous form. The ovules are bitegmic. The outer integument is absent at the ventral side of the ovule where it is congenetially fused with the funicle. At this region a 'hump-like' obturator is present (Text-Fig. 12). It becomes differentiated simultaneously with the integuments but appears to grow faster. In the mature ovule the obturator lies just above the inner integument and close to the outer as represented in Text-Fig. 13.



Text-Figs. 7-21. Litchi chinensis.—Fig. 7. Diakinesis (P.M.C.).  $\times$  775. Fig. 8. A binucleate pollen grain.  $\times$  775. Fig. 9. Uninucleate tapetal cells.  $\times$  775. Figs. 10 and 11. Later stages of tapetal cells.  $\times$  775. Fig. 12. An early stage of the development of the ovule and obturator.  $\times$  60. Fig. 13. A later stage of the same, showing the origin of aril.  $\times$  60. Fig. 14. The megaspore mother cell.  $\times$  1250. Fig. 15. A linear tetrad of the megaspores  $\times$  750. Fig. 16. A mature E.S.  $\times$  490. Fig. 17. Initiation of aril development.  $\times$  225. Figs. 18-21. Illustrate the tissues found in the different regions of the fruit. Fig. 18.  $\times$  225. Fig. 19.  $\times$  75. Figs. 20 and 21.  $\times$  225.

The origin of the archesporial cells could not be definitely traced. The megaspore mother cell when first observed is comparatively large and occurs deep inside the nucellus (Text-Fig. 14). It passes through the usual stages of reduction division and produces a tetrad of megaspores (Text-Fig. 15) of which the chalazal one becomes functional and the others degenerate. Sometimes the three non-functioning megaspores persist upto the one-nucleate stage of the embryo-sac. The functional megaspore increases in size and by three successive divisions produce an eight-nucleate embryo-sac.

The mature embryo-sac is of the normal angiospermous type (Text-Fig. 16). The synergids are pear-shaped structures and show the presence of a vacoule at the lower end. The egg protrudes beyond the synergids. The secondary nucleus lies close to the egg and is comparatively big. The antipodals are ephimeral.

The endosperm is of the "nuclear type".

(f) The development of the aril and the fruit.—In the mature ovule an obturator is present at the micropylar region. From this commences the development of a tissue which later forms the aril (Text-Fig. 17). In lorgitudinal sections of the fruit a similar outgrowth is seen on the other side of the funicle (Text-Fig. 13). Thus it appears that the primordium of the aril extends all around the seed. The growth of this tissue is not uniform and as a consequence portions of the aril are seen to overlap at the chalazal end of the seed. At first the tissue composing the aril consists of closely packed parenchymatous cells. Soon the cells increase in size, become elongated and show the presence of intercellular spaces. It should be noted that the development of the aril and the fruit do not proceed simultaneously in every instance as in many well-developed fruits the aril was noted to be in a rudimentary condition.

As has been noted by Khan² fruits are generally developed from one loculus of the ovary. In rare instances, however, both the loculi develop into fruits. The orientation of the fertile loculus during the development of the fruit is a noteworthy feature. The fertile loculus, during its development, bends outwards and towards the axis of the flower and ultimately comes to lie in the same plane as the latter. The sterile loculus and the style being deflected from their original position remain at the base of the fruit.

The mature fruit of Litchi is crimson coloured. It is indehiscent nutlike and tuberculed. Anatomical studies of the developing fruits show that the tubercules develop from the wall of the ovary from which the hairs had fallen off after fertilisation. Changes in the outer wall of the ovary are noted

during the later stages of the development of the female gametophyte. The epidermis becomes wavy in appearance due to localised division of certain cells in the hypodermal region. The epidermal cells also divide to accommodate the tissues below (Text-Fig. 18). In this way the tubercules are produced. Along with the growth of the tubercules and the development of the fruit, the cells composing it also become differentiated. The tissues found in the different parts of the pericarps of the fruit are as follows:—

- (1) Epicarp.—The tubercules contain the tissues of the epicarp. Four distinct types of tissues are noted: (i) The epidermis composed of rectangular cells with slightly thickened outer walls. (ii) Hypodermal sclerotic cells, with characteristic transverse striations. These cells occur in one, two or three layers and show the presence of tanin inside: (iii) Ordinary parechymatous cells with slightly lignified walls and dense cell contents similar to the above. (iv) Thin-walled parenchymatous cells occur at the base of the tubercle. Special cells containing "resinous or latex-secretions" occur mostly at the sides (Text-Figs. 19 and 20).
- (2) Mesocarp.—The mesocarp tissue is somewhat loose in consistency. There does not appear to be any line of demarcation between the epicarp and the mesocarp. In the initial stages this tissue is composed of ordinary paranchymatous cells which later becomes separated on account of the development of large intercellular spaces.
- (3) Endocarp.—Two distinct tissues have been noted. (i) The outermost one (i.e., next to the aril) is composed of two layers of rectangular cells which are considerably thickened and lignified. (ii) The tissue above it is composed of thick-walled elongated prosenchymatous cells with tapering walls (Text-Fig. 21).

The vascular supply of the fruit is confined to the tissues of the mesocarp. If the tubercules of a ripe fruit are carefully removed a white membranous structure is seen enveloping the aril. The impressions of vascular strands are noted on this structure which seems to be composed of the tissues of the endocarp. Sometimes the tissues of the mesocarp with adhering vascular bundles are seen attached to this tissue.

## Summary

The paper gives an account of some aspects of the life-history of Litchi chinensis.

1. The flowers though apparently bisexual behave functionally as male and female. In the female flowers the filaments are short and the

anthers do not burst. In the male flowers on the other hand, the pistil does not attain full development and remains as a pistillode.

- 2. The inflorescence is a panicle. It bears male and female flowers alternately. Normally female flowers appear after the male flowers without any break. The limits of the flowering periods may or may not overlap. The flowering period as a whole is very short.
- 3. The development of the floral parts occurs in the following sequence calyx, andrœcium and gynæcium. The development of the gynophore is first noted during the differentiation of the gynæcium.
- 4. Development of the pollen grains is of the simultaneous type. Meiosis in the pollen mother cells of the functionally male and female flowers does not show any difference or irregularity. Cytokinesis takes place by the process of furrowing. The tapetal cells are binucleate.
- 5. The pollen grains of both the types of flowers appear to be similar in shape and size. The exine is granular and shows the presence of three furrows, in the centre of each of which a germ-pore appears to be present. The pollen grains are binucleate at the time they are shed.
- 6. The development of the female gametophyte is of the normal type. The mature embryo-sac is eight-nucleate. The antipodals are ephimeral.
- 7. The aril develops from the funicle and the tissue of the obturator takes part in the process.
- 8. The pericarp consists of an epicarp, mesocarp and endocarp. The cells of the epicarp develop into tubercules and show tissue differentiation. The mesocarp appears to be continuous with the epicarp. The endocarp is composed of two different types of tissues.

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