

Morphological and anatomical development of the litchi fruit (*Litchi chinensis* Sonn. cv. Brewster)

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Morphological and anatomical development of the litchi fruit (*Litchi chinensis* Sonn. cv. Brewster).

Abstract — Introduction. The litchi tree produces fruit in terminal panicles in a development cycle of approximately 4 months. Data on its morphology and anatomy are scarce, although the fruit is of fundamental importance in the Sapindaceae systematic. This work was carried out to study fruit morphology and pericarp anatomy during the development of the cv. Brewster litchi. **Materials and methods.** Fruits were collected at different development phases on 12-year-old trees. The fruits were morphologically and anatomically studied. After fixation, the samples were sectioned in several anatomical planes, and mounted in temporary or permanent preparations. **Results.** The flowers were functionally male and female, with a superior bicarpelar ovary with two ovules, rarely three. Frequently, only a single fruit develops. The fruit is a heart-shaped drupe when ripe, fleshy and indehiscent. The pericarp is thin, leathery, rough, wrinkled and red colored, with aril covering all the seed. The fruit begins differentiation with the formation of the pericarp which consists of epicarp, mesocarp and endocarp. **Discussion.** The litchi fruit is elongated when young, becoming heart-shaped as it develops. It contains one or two seeds and each one is attached to the internal angle of the marginal sutures. When ripe, the litchi has a leathery and broken pericarp because of the presence of brachysclereides immediately below the epidermis, whose function may be protection against mechanical and physiological stresses and herbivorous action.

Brazil / *Litchi chinensis* / plant reproductive organs / fruit / plant anatomy / pericarp

Développement morphologique et anatomique du litchi (*Litchi chinensis* Sonn. cv. Brewster).

Résumé — Introduction. Chez le litchi, les fruits sont assemblés en grappes terminales après un cycle de développement d'environ 4 mois. Les informations sur la morphologie et l'anatomie du litchi sont limitées, bien que le fruit ait une importance fondamentale dans la systématique des sapindacées. Le travail présenté a eu pour objectif d'étudier la morphologie du fruit et l'anatomie du péricarpe durant le développement du fruit du cv. Brewster. **Matériel et méthodes.** Des fruits à différents états de développement ont été collectés sur des plants âgés de 12 ans. Ils ont été étudiés du point de vue morphologique et anatomique. Après fixation, les échantillons ont été sectionnés à différents niveaux anatomiques et montés sur lames pour observation microscopique. **Résultats.** Les fleurs sont unisexuées, avec un ovaire supère bicarpellaire, possédant deux ovules, rarement trois ; le plus souvent, seul l'un d'eux se développe en fruit. Le fruit est une drupe cordiforme, charnue et indéhiscente après maturation. Le péricarpe est mince, coriace, rugueux et rouge. La chair recouvre la totalité du noyau. Le développement du fruit commence avec la formation du péricarpe, constitué d'épicarpe, mésocarpe et endocarpe. **Discussion.** En début de croissance, le fruit est allongé puis il devient cordiforme à maturité. Chacun des noyaux, un par fruit, est attaché à l'angle interne du point de suture marginal. À maturité, le litchi se présente avec un péricarpe coriace et cassant à cause de la présence de brachysclérides sous l'épiderme. Ceux-ci assurent une protection contre les stress mécanique et physiologique, mais aussi contre les animaux herbivores.

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Brésil / *Litchi chinensis* / organe reproducteur végétal / fruit / anatomie végétale / péricarpe

1. Introduction

Litchi chinensis Sonn. is a species native to subtropical China [1]. It has small non-showy, generally whitish-green colored flowers [2], grouped in a terminal panicle [3]. It is a perennial plant, the production occurring during the warm months of the year [4].

The typical litchi ovary contains two ovules, one of which frequently presents an abnormality due to the absence or degeneration of the embryo sac [5]. The fruit possesses only one seed that varies in size and shape between cultivars [6]. The seed has a fleshy and succulent aril [7].

Fruits measure on average 50 mm in length and 40 mm in diameter when mature [8], and their color and form are similar to those of the strawberry [9]. Their development cycle is approximately 4 months long.

The litchi fruit is reddish colored with a pericarp composed of three regions: the epicarp, mesocarp and endocarp [10]. The endocarp is composed of a thin internal layer with a pinkish color indicative of the mature fruit [7, 11].

In the Kway May Pink cultivar, the endocarp is considered an abaxial epidermic zone of the pericarp, formed from small, thin-walled, non-suberized epidermic cells, and it is frequently separated from the aril when the fruit matures [10].

Information on the Sapindaceae is scarce [12]. The fruit is of fundamental importance for distinguishing genera of this family. In some cases, such as *Serjania* and *Paulinia*, the fruit shape determines the division of genera into sections. The morphological characteristics of the fruit are therefore of great importance for taxonomic classification [2].

The objective of this study was to describe the morphological characteristics of the female flower and fruit as well as the anatomical development of the pericarp of *Litchi chinensis* Sonn. cv. Brewster, as a contribution to taxonomic studies of the Sapindaceae.

2. Materials and methods

Litchi fruits (*Litchi chinensis* Sonn. cv. Brewster) at different development stages were collected from 12-year-old plants, in the orchard at the Federal University of Viçosa, Minas Gerais, Brazil, located at 20° 45' S and 42° 51' W.

The fruits were placed in sealed glass flasks, kept in a polyurethane box containing crushed ice, and brought to the laboratory. Part of the samples was used for the morphological study and the remainder was used for the anatomical study.

Fruit wet weight, length (axis parallel to the peduncle) and diameter (perpendicular to the length at the average fruit circumference) were measured in the caliper.

Two 50 mm² samples were extracted from the basal, equatorial and apical regions of the fruits and fixed in a mixture of formaldehyde, acetic acid and ethanol at 50% and 70% (FAA 50% and 70%) [13] and ethanol at 70% [14] for anatomical studies of the pericarp. The fixed samples were placed under vacuum, sectioned by hand in different anatomical planes, and mounted on temporary and semi-permanent preparations.

Permanent slides were prepared from fixed samples that were dehydrated in a series of ethanol solutions of increasing concentration and then transferred to a solution of ethanol and xylol. The samples were placed in a paraffin-saturated xylol solution for 12 h at room temperature and were block mounted by transferring them to paper moulds containing 92% paraffin and 8% wax. The paraffin blocks were mounted in wooden blocks and cut into 10 µm sections in a rotatory microtome with steel and/or glass blades. Paraffin was then removed by soaking in xylol and a series of ethanol solutions of decreasing concentration.

Slides were colored by soaking in 1% aqueous safranin for 8 h and then washing in a series of ethanol solutions of increasing concentration. The slides were then transferred to an ethanolic solution of Fast Green for 5 s and rinsed in ethanol and

xylol. The slides were mounted in Canadian Balsam [13] for future observation.

Permanent slides were mounted using synthetic polymerized resin, J.B4. Ethanol 100% in liquid resin at the proportion of 1:1 was added to the paraffin infiltration process. The samples were kept in this solution at room temperature for 8 h. They were then transferred to an infiltration solution, "A medium" containing liquid resin and "activador powder", with 100 mL resin for 0.9 g of activador powder. The samples remained in this solution for 24 h at room temperature.

A "B medium" made up of "A medium" and hardener (25 mL "A medium" for 1 mL of hardener) was used for blocking. After blocking, the mould plate was kept in a chamber at 40 °C until the resin hardened. The resin blocks were sectioned at 5–8 μm thickness in a rotating microtome. The sections were stained with Toluidine Blue 0.025 $\text{mg} \times \text{L}^{-1}$ in aqueous solution [15] for later observation.

The drawings and diagrams on the morphological and anatomical aspects were made with the help of a Wild m4A stereomicroscope with a light chamber and Carl Zeiss Jena optical microscope.

Fruits were collected at 21 different times, with each collection composed of four fruits and four repetitions.

3. Results

3.1. Fruit morphology and development

The 'Brewster' inflorescence (*figure 1*) has male (*figure 2*) and female (*figure 3*) flowers. The female flower has a pistil with two upper lobules, short style and forked stigma (*figure 3*). The ovary is upper bicarpel, with thick, complete walls and a very reduced central space. A large septum divides the ovary into two locules (*figure 4*). Each locule contains a bitegumented anatropous ovule, with a short funiculus and ample calaza, and an obturator near the micropyle.



Figure 1. Branches of *Litchi chinensis* Sonn. with blossom in panicles.

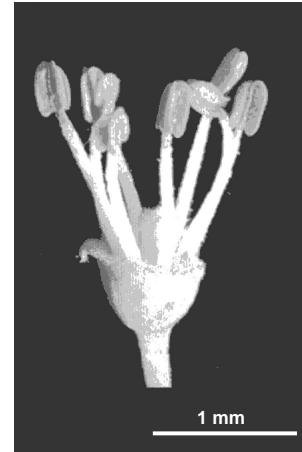


Figure 2. Male flower of *Litchi chinensis* Sonn.

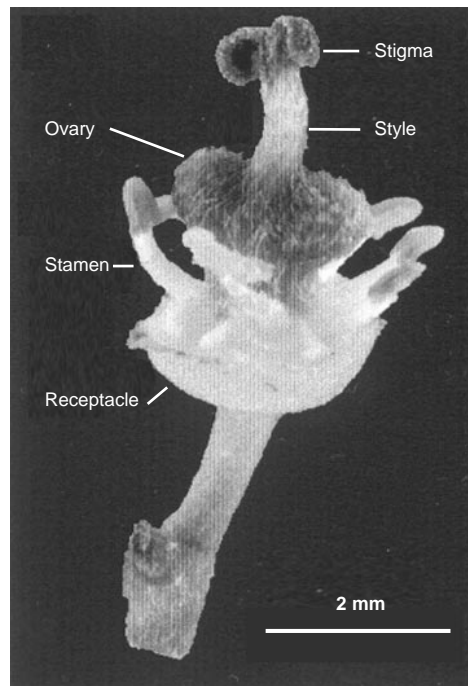


Figure 3. Female flower of *Litchi chinensis* Sonn.

Figure 4.
Longitudinal section of a female
flower of *Litchi chinensis*
Sonn., showing the ovary.

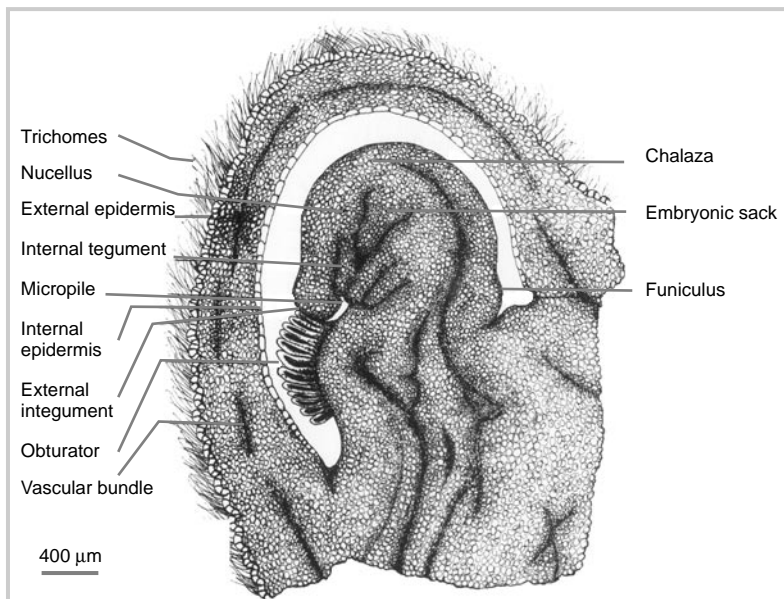
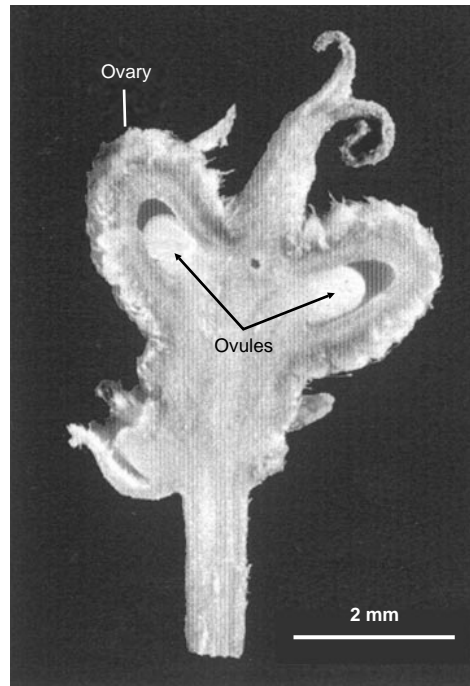


Figure 5.
Litchi chinensis Sonn.:
longitudinal section
of the ovary.

An external epidermis rich in trichomes and an internal epidermis can be distinguished in the ovary. Vascular bundles of the parenchyma run between the external and internal layers (*figure 5*).

The ovary usually contains two ovules (*figures 4, 6*) and, occasionally, three

(*figure 7*) with one fruit usually ripening (*figure 8*), rarely two (*figure 6*). Three ovules may be fertilized, but only one fruit reaches maturity (*figures 6, 7*).

The fruit is a drupe, elongated when young (*figure 8*), turning cordiform as it develops (*figure 9*). It measures approximately (38.62 ± 0.75) mm in length by (36.10 ± 0.88) mm in diameter, and weighs on average (23.90 ± 1.60) g. It is fleshy and indehiscent (*figure 9*), the pericarp is thin, hard, rough, warty and brittle with a reddish color when ripe (*figures 9, 10*). It contains one seed. The aril (pulp), which is the edible portion of the fruit, is white, juicy, translucent and soft (*figures 10, 11*) and covers the entire seed (*figures 11, 12*).

3.2. Pericarp anatomy and development

Litchi differentiation starts with formation of the pericarp, consisting of small, isodiametric cells. These characteristics, typical of dividing cells, were observed in the pericarps of all fruits 7 d after anthesis (daa) (*figure 13*). Later, growth was due to cellular expansion, which started 21 daa (*figure 14*). During this period the cells increased in size, approximately equally in all directions, starting primarily in the peripheral region of the pericarp (*figure 14*).

As the fruit develops, the pericarp differentiates into epicarp, mesocarp, and endocarp (*figure 15*).

The immature epicarp is composed of the external epiderm and two hypodermic layers (*figure 15*). The external epiderm consists of a series of small, cube-like cells with radially thickened walls, covered by a thick cuticle which is impregnated with phenolic compounds and numerous trichomes that persist until 14 daa (*figure 13*).

Formation of sclerenchymatous tissue starts just below the epiderm at 7 daa (*figure 16*) and becomes completely differentiated at 62 daa. Sclerenchymatous tissue is distributed in two or three discontinuous layers separated by very noticeable protuberances present in the pericarp at 56 daa (*figure 17*).

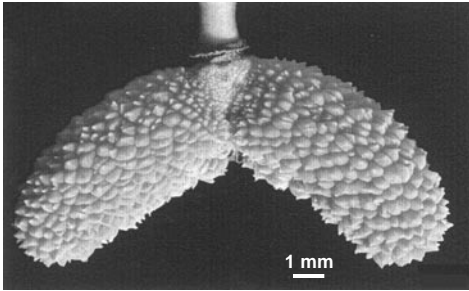


Figure 6.
Two unripe normal fruits of *Litchi chinensis* Sonn.

The mesocarp is composed of parenchymatous cells (figure 18) and the endocarp is formed from very small, thin-walled, non-lignified cells (figures 19, 20).

Vascular tissue is distributed throughout the pericarpial region and is found in the greatest quantity in the external mesocarp, near the protuberances (figures 14, 15).

4. Discussion

4.1. Fruit morphology and development

As seen above, the ovary contains two ovules. Frequently, however, only one fruit ripens although well developed double fruits are found on a single peduncle at times. This phenomenon is quite rare. In general only ovarian lobules contain an ovule that develops into a fruit [16].

Mustard [17] demonstrated that most litchi ovules are abnormal. Embryo sacs are missing in many ovules and the embryo sac synergides in 'Brewster' litchi flowers degenerate before anthesis.

According to Stern *et al.* [5], in 'Mauritus' and 'Floridan' litchi flowers, many ovules lack embryo sacs, and many embryo sacs are abnormal or degenerate, due to the absence of essential components (egg cells, polar nucleus and synergides).

In many species, genetic, nutritional and environmental factors are considered

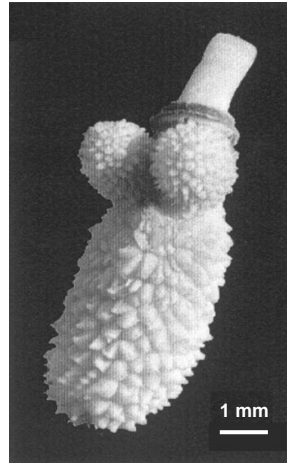


Figure 7.
Unripe fruit with two atrophied fruits of *Litchi chinensis* Sonn.

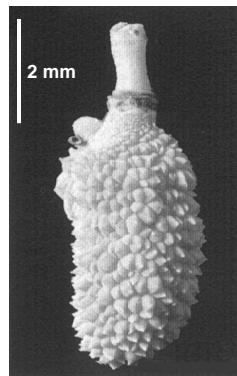


Figure 8.
Unripe fruit of *Litchi chinensis* Sonn., 38 d after anthesis.

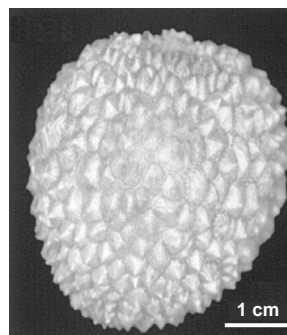


Figure 9.
External view of a *L. chinensis* ripe fruit.

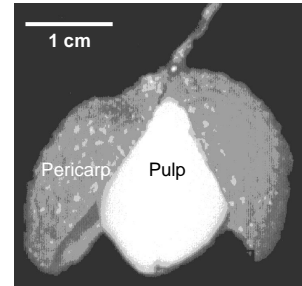


Figure 10.
Ripe fruit of *L. chinensis* showing the thin and fragile pericarp.

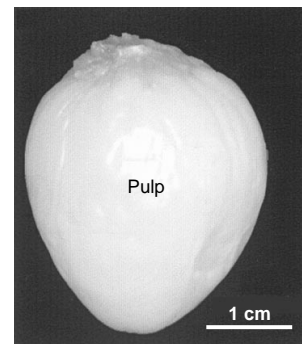


Figure 11.
External view of the ripe fruit pulp of *L. chinensis*.

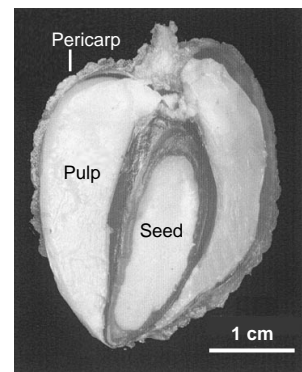


Figure 12.
Ripe fruit of *L. chinensis* in longitudinal section, displaying the seed covering pulp.

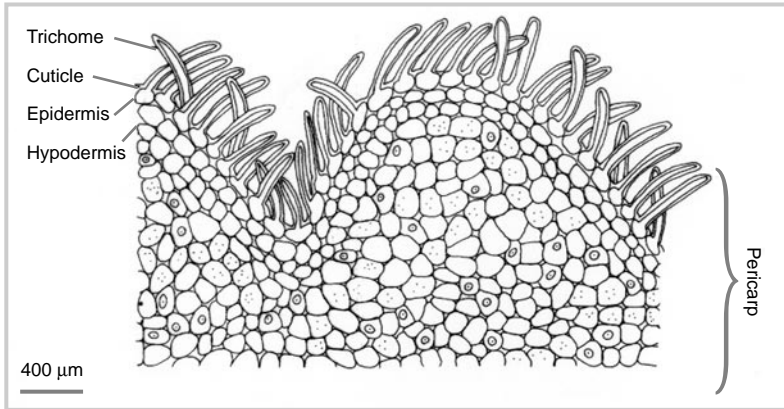


Figure 13. Cross section of an unripe fruit pericarp of *L. chinensis*.

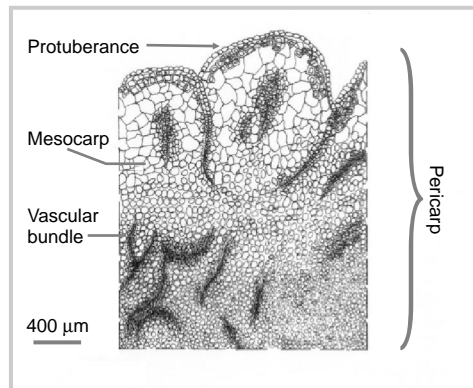


Figure 14. Cross section of a ripe fruit pericarp of *L. chinensis*.

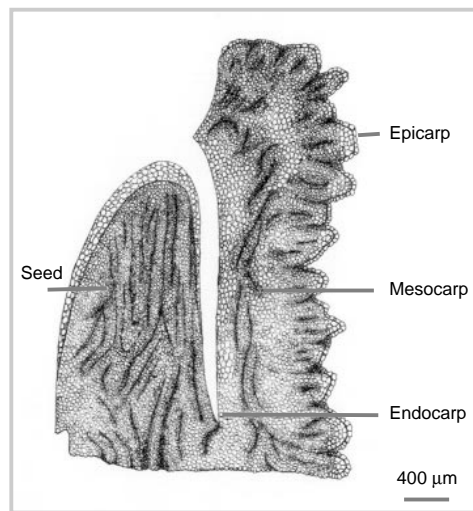


Figure 15. Longitudinal section of an unripe fruit pericarp of *L. chinensis*.

responsible for the abnormal or degenerate development of reproductive organs [18, 19].

The 'Brewster' litchi flower presents an ovule with funicular obturator. According to Corner [12] and Steyn and Roberts [20], other litchi cultivars also contain an obturator. Examples include the HLH Mauritius in which the obturator is placental with a half-moon-shaped protuberance around the sessile ovule, and the species *Dodonea vicosa* which possesses a well developed placental obturator.

The 'Brewster' flower also possesses an ovary with a large, thick septum. Studies carried out by Barroso [2] showed that this structure is very important and serves as the basis for establishing genera sections. Barroso [2] separated the species *Serjania* into two groups, with one containing the majority of species, belonging to sections whose fruits have thinner septa than locules, while the other smaller group contained sections whose fruits had septa larger than the locules.

The litchi is a drupe; however it has a membranous endocarp, in contrast to the majority of typical drupes. According to Roth [21], typical drupes frequently possess sclerified endocarps constituting the pit and are considered highly specialized fruits.

In litchi cv. Brewster, the fruit has one seed that is found attached to the internal angles of the marginal seam. Due to this characteristic, the fruit is denominated chamber, following Spjut's classification [22].

4.2. Pericarp anatomy and development

The fruit originates from terminal panicles primarily by formation of the pericarp. As the fruit develops, the pericarp differentiates morphologically into three zones: epicarp, mesocarp and endocarp.

At times during development, it is only possible to distinguish the epicarp and mesocarp since the endocarp remains slightly differentiated, becoming distinct when the fruit matures. The mature fruit pericarp possesses a thin epicarp, fleshy mesocarp and membranous endocarp.

During early development, the epicarp consists of a continuous and thick cuticle, which thins towards the end of fruit development. Studies carried out by Underhill and Critchley [10] with litchi cv. Kway May Pink demonstrated that, during pericarpial development, the cuticle undergoes an accentuated reduction in thickness, and this reduction can in part result from the rapid expansion of the pericarp, with cuticle synthesis being involved in the process. Underhill and Critchley [10] also observed that small cracks appear in the pericarp surface due to a reduction in cuticle thickness and, according to Joubert [23], the litchi fruit is susceptible to cracking during development principally because of rapid growth of the aril.

The mesocarp is fleshy and constitutes the major part of the pericarp. When immature, it contains photosynthetic organelles. When mature, it presents a large quantity of anthocyanins.

The endocarp is membranous and very delicate, with a pinkish color. It is often separated from the aril when the fruit ripens. In studies carried out with 'Mauritus', Joubert and Van Lelyveld [11] also demonstrated that the pinkish color of the endocarp appears when the fruit is completely mature.

The pericarp presents intense protuberances during the initial stages of development. After seed and aril development, these protuberances become progressively smaller, and the pericarp thins out gaining a warty aspect due to the presence of brachysclereides just under the epiderm. The function of these structures could be to protect against mechanical, physiological and herbivorous stresses.

During litchi development, most vascular tissues increase in quantity through differentiation of additional vascular lines within the fundamental parenchyma. According to Fahn [18], vascular tissue is found in the pericarp and other fruit parts in different fruit types and its localization is related to the way the carpels fuse or unite.

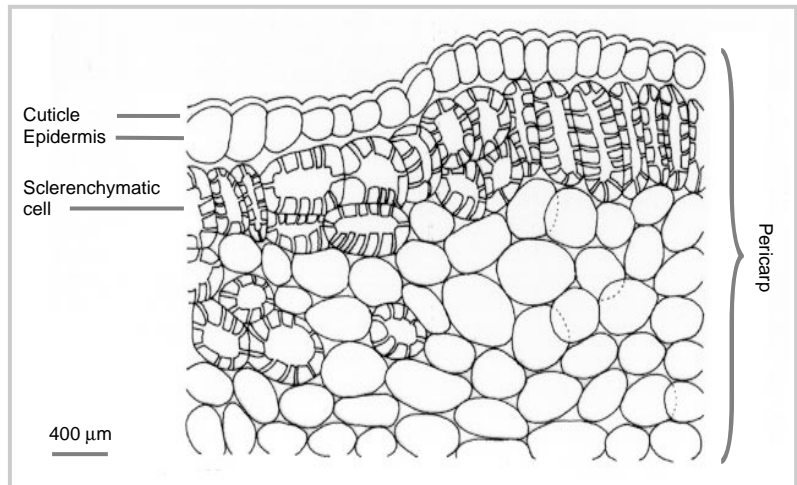


Figure 16. Detailed cross section of a ripe fruit pericarp of *L. chinensis*.

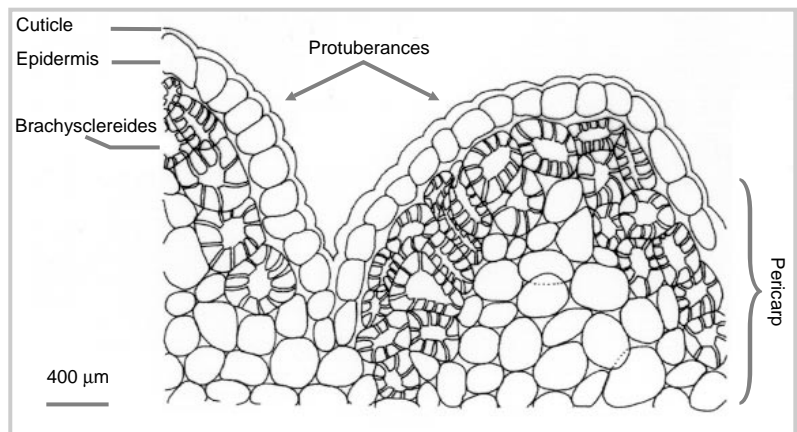


Figure 17. Cross section of a ripe fruit pericarp of *L. chinensis* showing the protuberances.

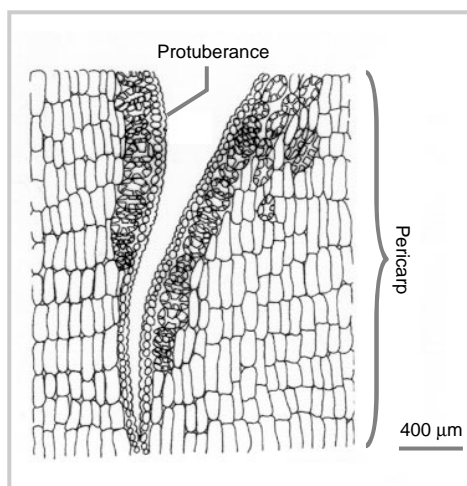


Figure 18. Cross section of an unripe fruit pericarp of *L. chinensis* showing accentuated protuberance.

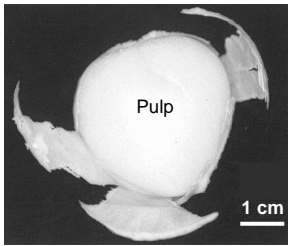


Figure 19.
Ripe fruit of *L. chinensis* indicating the detachable endocarp.

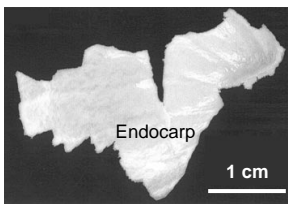


Figure 20.
Ripe fruit endocarp showing their membranaceous appearance.

Acknowledgements

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Desarrollo morfológico y anatómico del litchi (*Litchi chinensis* Sonn. cv. Brewster).

Resumen — Introducción. En el litchi, los frutos están agrupados en racimos terminales tras un ciclo de desarrollo de aproximadamente 4 meses. A pesar de la importancia fundamental del fruto en la sistemática de las sapindáceas, la información sobre la morfología y anatomía del litchi es limitada. El presente estudio tiene como objetivo estudiar la morfología del fruto y la anatomía del pericarpo durante el desarrollo del fruto del cv. Brewster. **Material y métodos.** Se recogieron frutos con diferentes fases de desarrollo en plantas de 12 años. Se estudiaron morfológica y anatómicamente. Una vez fijadas, se seccionaron las muestras en diferentes niveles anatómicos y se montaron en láminas para su observación con microscopio. **Resultados.** Las flores son unisexuales, con un ovario supero bicarpelar, con dos óvulos, raramente tres; la mayor parte de las veces, sólo uno se desarrolla en fruto. El fruto es una drupa cordiforme, carnosa e indehiscente tras la maduración. El pericarpo es delgado, coriáceo, rugoso y rojo. La carne recubre todo el hueso. El desarrollo del fruto comienza con la formación del pericarpo, constituido de epicarpo, mesocarpo y endocarpo. **Discusión.** En el inicio del crecimiento, el fruto es alargado para volverse cordiforme una vez maduro. Cada uno de los huesos, uno por fruto, está sujeto al ángulo interno del punto de sutura marginal. Maduro, el litchi se presenta con un pericarpo coriáceo y quebradizo debido a la presencia de braquisclereidas bajo la epidermis. Éstas garantizan una protección contra el estrés mecánico y fisiológico pero también contra los animales herbívoros.

Brasil / *Litchi chinensis* / órganos reproductores vegetales / fruto / anatomía de la planta / pericarpo