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A Scanning Electron Microscope Study of Flowers of Carambola, Durian and Rambutan

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RINGKASAN

Spesimen-spesimen segar bunga belimbing manis (Averrhoa carambola *L.*), *durian* (Durio zibethinus *Murr.*) *dan rambutan* (Nephelium lappaceum *L.*) *telah diperiksa dengan mikroskop elektron pengimbasan (SEM). Bunga menyeluroh dan bahagian-bahagian bunga telah dihuraikan dan digambarkan dalam mikrograf. Struktur-struktur bunga dan perbezaan-p. rbezaannya dalam spesis telah diberi penekanan, pertalian dan fungsi mereka dibincangkan dengan rujukan khusus kepada mekanisma pendebungaan dan morfologi debunga.*

SUMMARY

Fresh specimens of flowers of carambola (Averrhoa carambola *L.*), *durian* (Durio zibethinus *Murr.*) *and rambutan* (Nephelium lappaceum *L.*) *were examined using the scanning electron microscope (SEM). Whole flowers and floral parts were described and illustrated in the micrographs. Floral structures and their differences within species were highlighted, their relationship and functions are discussed with special reference to pollination mechanisms and pollen morphology.*

INTRODUCTION

The carambola or star fruit *(Averrhoa carambola* L.*)*, Durian *(Durio zibethinus* Murr.*)* and rambutan *(Nephelium lappaceum* L.*)* are some of the more important and popular fruits of the tropics particularly in South East Asian countries. These economically important crops have been cultivated and described over half a century ago by Popenoe (1920), Ochse and Barkhuizen (1931) and Burkill (1935). Clonal or varietal selection, identification and propagation of the rambutan were carried out later as reported by Whitehead (1959) and Milsum (1960). Despite the economic value and importance of these fruits, for example the durian in Malaysia, there is no large scale plantation or estate in this region (Lai, 1974) nor is there a well documented programme on the selection and breeding of these fruits. Studies are lacking on the autecology, flowering biology, cytology and breeding systems, but these are the pre-requisites in any breeding project. Lately selection of individuals followed by vegetative propagation is commonly used, instead of breeding by various crosses. The reproductive biology of durian has been studied by Valmayor *et al.* (1965); Soepadmo and Eow (1976) and the floral biology of carambola by Knight (1965); and Nand (1967, 1971).

The study of floral structures using a scanning electron microscope (SEM) gave greater details and depth of focus than the ordinary light microscope (Troughton and Donaldson, 1972). Scholefield (1982) used this technique to illustrate and described four tropical and sub-tropical species, the avocado *(Persea americana* Mill.*)*, litchi *(Litchi chinensis* Sonn.*)*, macadamia *(Macadamia integrifolia* Maiden and Betche*)* and mango *(Mangifera indica* L.*)*.

There is still a lack of interest and information on the floral biology of the tropical fruit species. This paper contains scanning electron micrographs of the structure of our most important fruits, the durian, rambutan and carambola. The aim of this paper is to provide further information and appreciation of the floral biology of these three

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popular fruit species to breeders and horticulturists. Emphasis is also given to the relationship of floral parts and the pollen grains in the process of pollination, fertilization and subsequently fruit and seed set.

MATERIALS AND METHODS

Plant Materials

Flowers were picked and collected both in the morning and evening from grafted trees of carambola (5 years old), durian (10 years old) and rambutan (8 years old) in the university farm. Flowers of carambola are available all year round but those of durian and rambutan are seasonal and varieties can overlap in their flowering dates. In Malaysia, the durian flowers in March-April and September-October; the rambutan between March-May and August-September. Flowers for this study were picked in mid August, and were placed in clear plastic bags with moist cotton wool.

Method of Scanning

The durian flowers are very large compared with those of the rambutan and carambola. Hence the floral parts of durian flowers were scanned separately. Fresh flowers were dissected with the removal of the epicalyx, calyx and corolla. The stigma and stamens (uncoated with gold) were mounted on a SEM brass stub and examined.

With carambola flowers three sepals and petals were first removed before mounting on the stub; in case of the rambutan flowers the whole flower was mounted on the brass stub. These mounted specimens of floral parts or whole flowers were examined in a JEOL JSM 35C scanning electron microscope using an accelerating voltage of 6kV. During the examination of specimens over a period of 15 to 30 minutes desiccation effects were noted when specimens were under high power of x 3000. Pollen morphology was studied by dusting pollen grains on to a double sided cello-tape on a stub and examined in the same manner.

RESULTS AND DISCUSSION

The Carambola or Star Fruit (Fig. 1a and 1b)

The carambola flower is heterodistylous borne in small cymose inflorescences at the axils of leaves. Individual trees may have the 'long-style' or 'pin-eye' flowers or 'short-style', 'thrum-eye' flowers *(Fig. 1a and 1b)* but never both. This condition was noted by Ochse *et al.* (1961) and Knight (1965). Nand (1966, 1967)

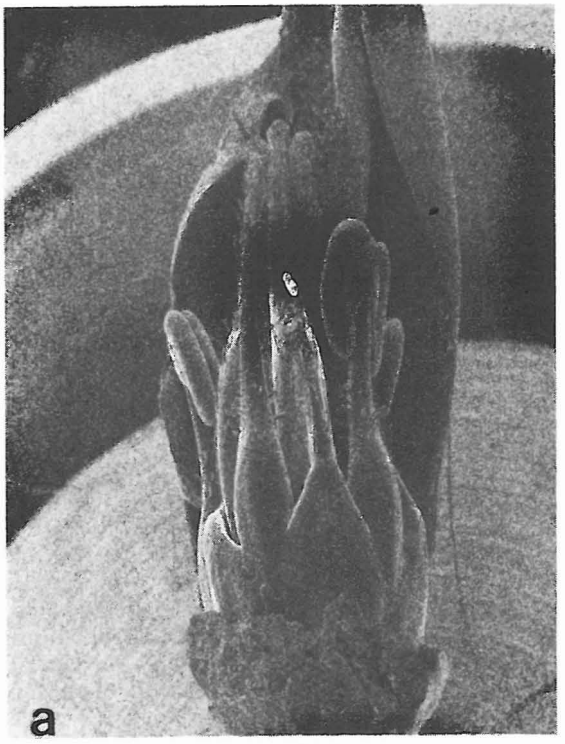

Fig. 1(a)

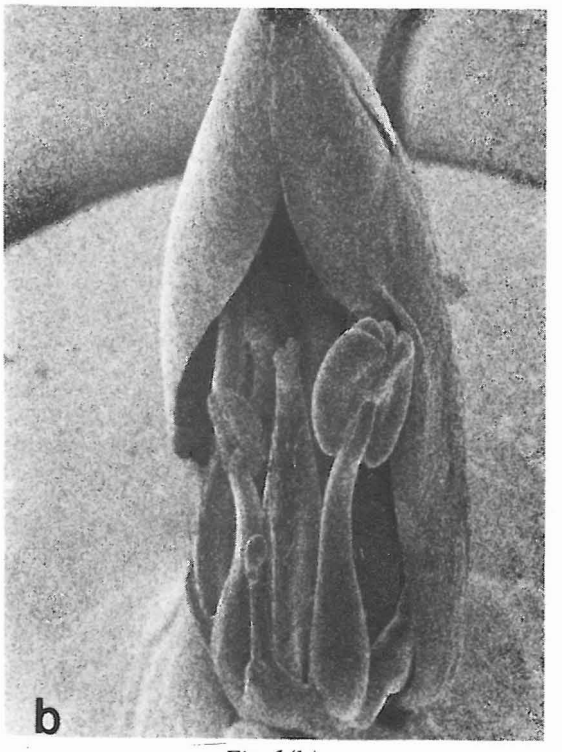

Fig. 1(b)

studied the morphology and abnormalities of the 'pin-eye' flower and its fruit respectively.

Each flower is about 6–7 mm tall by 9–10 mm wide at the mouth of the corolla. The calyx has 5 imbricate rose-pink sepals with whitish borders and almost half as tall as the corolla tube. The corolla is gamopetalous with five recurved lobes. Each petal is rose-pink with the basal portion and the borders whitish. The inner surface is covered by tiny hairs which give it a velvety appearance *(Fig. 1g)*.

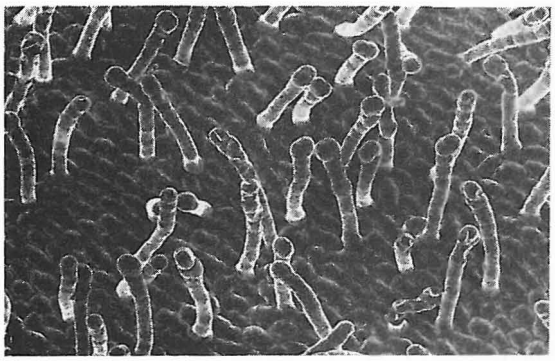

Fig. 1(g)

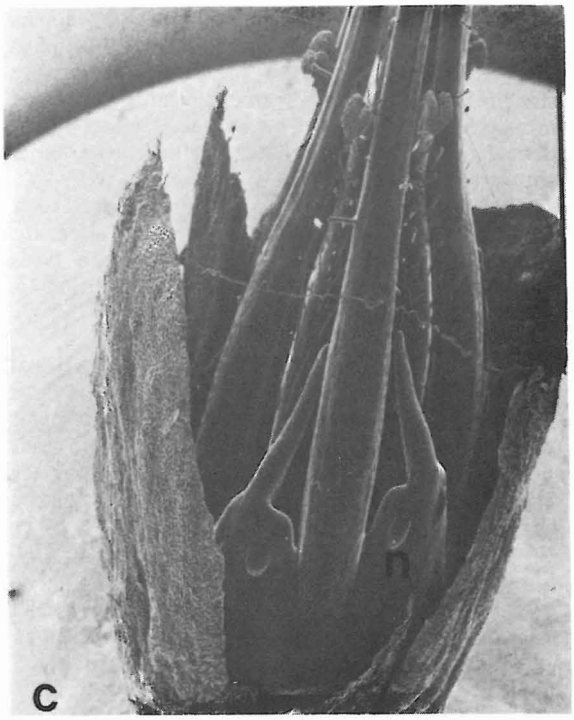

Fig. 1(c)

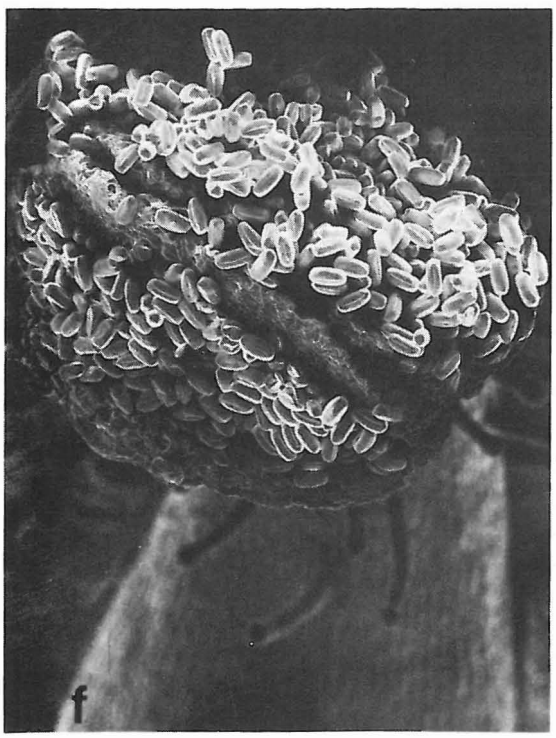

Fig. 1(f)

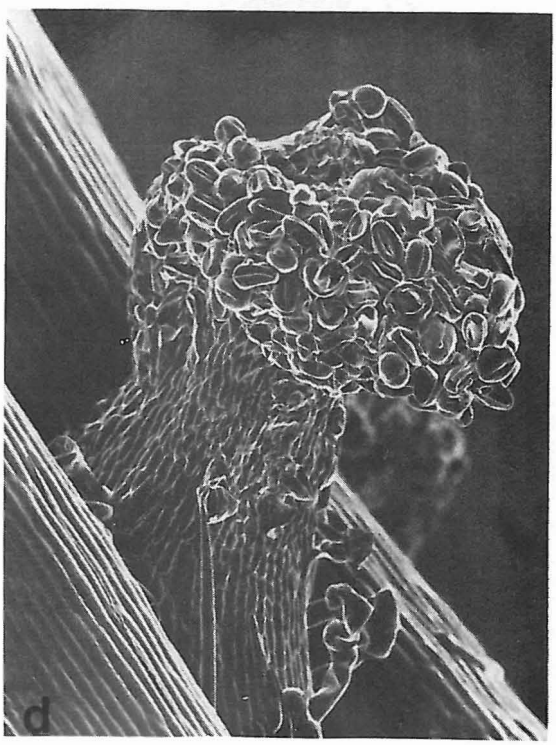

Fig. 1(d)

floral structure has been described by Ochse *et al.* (1931, 1961). The development of flowers and fruits has been described by Valmayor *et al.* (1965) while Soepadmo and Eow (1976) have described the floral differentiation and floral anatomy. The durian flower is perfect and regular. It is enclosed by an epicalyx, inside which are five to six bundles, each bundle consisting of six to ten stamens *(Fig. 2).* The durian exhibits the heterostylous condition among the clones. The ovary is superior, ovoid with a long slender style terminated by a yellow orangy stigma.

The flowers open late in the afternoon with the splitting of epicalyx into two or three lobes followed by the calyx. Soon after dark petals become recurved outwards exposing both stamens and stigma *(Fig. 3a).* Around 18:00-20:00 hours the anthers dehisce *(Fig. 3b).* The stigmatic surface is receptive at anthesis, a sticky mucilaginous secretion is produced on the surface and pollen grains can become attached to it *(Fig. 3c).* The stigma remains receptive till 01:00 hours (Soepadmo and Eow, 1976) while Valmayor *et al.* (1965) reported receptivity lasted until 06:00 hours the following morning.

The flowers of the durian can be considered to be very large; the pedicels are violet to 7 cm long, the calyx measures 2 cm and 1.5 cm wide and petals are 3 cm long by 1.5 cm wide; the stigma and the stamens are 5-7 cm and 2-3 cm long respectively. Pollination is attributed to bats which feed on the nectar or the large pollen grains or both. The nectary, made up of large cells, is located at the base of calyx tube *(Fig. 3g).* Mature pollen grains are more or less spherical, three to four porate measuring 80-150 microns in diameter (Soepadmo and Eow, 1976). At anthesis they are released singly or in clumps *(Fig. 3d).* The pollen grain is large and covered with mucilage *(Fig. d)* which can be removed with ether revealing the three porate pollen grain *(Fig. 3f).*

The receptive stigma is papillate and sticky *(Fig. 3c).* The stigma located at the tip of the long style is placed in such a position that pollinators will come into contact with it while feeding on the nectar or pollen. Self-incompatibility has been reported by Arasu, (1976); Valmayor *et al.* (1965), but compatibility by Soepadmo and Eow (1976). The pollen grains germinate within three or four hours through the pore *(Fig. 3e);* the pollen tubes are sent through the stigmatic papillae into the style; and the successful one finally enters the embryo sac in the process of fertilization. Subsequently the calyx, petals and stamens begin to drop off leaving only the ovary and stigma attached to the branch. The ovary is initially covered with scales, a cross section of which shows the thorny ovary surface *(Fig. 3h)* and scales over it. Within a week all unsuccessfully pollinated ovaries drop off leaving one or two per inflorescence. The fertilized spiny ovary will develop and enlarge changing from light brown to the light green fruit. The spiny fruit is made up usually of five carpels containing 10 to 20 very large seeds which mature in about three months.

The Rambutan (Fig. 4a)

The rambutan is androdioecious with separate male and hermaphrodite trees. The male tree is seldom found since nowadays the rambutan is usually planted from budded material. The hermaphrodite tree is often referred to as the female because it bears abundant fruits. Brief descriptions of flowers have been given by Popenoe (1920), Corner (1952), Ochse *et al.* (1961) and Pursglove (1974).

The flowers are borne on terminal or axillary cymose inflorescences. The flowers are apetalous, greenish white in colour about 2 mm in diameter. The calyx has four to six pubescent lobes.

In the male flower there are five to seven stamens which arise from the disc between the lobes of the nectaries *(Fig. 4a).* Each stamen has a whitish tomentose filament with a yellowish bilobed anther. The anther lobes split along a longitudinal line to release large amounts of pollen *(Fig. 4e).* At the centre of the flower is the abortive ovary which is highly pubescent *(Fig. 4c).*

The hermaphrodite flower has six to seven stamens but the anthers do not dehisce to release the well developed pollen grains inside. Functionally it serves as a female flower. The ovary is two or three lobed and bears a bifid or trifid stigma. The outer surfaces of the ovary and stigma are pubescent while the stigmatic surface is highly papillose *(Fig. 4d).* Only the nectaries are not pubescent.

The male pollen grain is sticky and is barrel-shaped *(Fig. 4g).* When wetted it swells up to a size of about 20 microns and reveals three narrow colpi. The exine is finely patterned with minute fusiform depressions *(Fig. 4h).*

The rambutan flowers open at all times of the day but the majority do so at about 06:30 hours. The first sign of anthesis is the parting of the calyx in the male flower whereas in the hermaphrodite flower this is indicated by the recurving of the bifid stigma. The greenish white stigma is receptive at anthesis and remains so for a day after which it turns brown. Nectar is secreted at anthesis whereas anther dehiscence in the male flower begins at about 08:30 hours onwards.

drate oxidation (Simpson and Boyd, 1971), leading to enhanced cholesterol side-chain cleavage activity.

The above effect of glucose was further compounded by a marked dependence of the cells on BSA: increaing concentrations of the albumin appeared to enhance progesterone synthesis in both the control and LH-treated cells. A similar dependence on BSA for corticosterone production by adrenal cells has been reported previously (Sayers *et al.*, 1971), but corticosteroidogenesis in this case was maximal at 0.5% BSA. The mechanism of this albumin effect is not clear, although it is probably due to a general protective action of the albumin on the integrity and functional viability of the cells.

These studies with isolated luteal cells provide a basis for a convenient and extremely sensitive *in vitro* system for investigations of LH action, as well as a possible bioassay for the hormone.

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