THE REPRODUCTIVE BIOLOGY OF THE LONGAN

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The longan or "Nhãn" (*Dimocarpus longan* Lour., Sapindaceae), a subtropical fruit tree species native from Southern Asia is an important crop in Vietnam. There are several popular types of longan in Vietnam such as "Nhãn lồng hưng yên" in Hung Yen province which produces one crop a year, "Nhãn tiêu da bò" in Tien Giang province which produces three crops in 2 years or "Nhãn xuồng cơm vàng" in Vung Tau province which produces one crop a year. According to Vietnam Ministry of Agricultural and Rural Development (MARD), in 2011 the total of longan cultivation area is close to 100,000 hectares and the production of longan is higher than 600,000 tons (Ministry of Industry and Trade of Vietnam, 2012). Most of the longan cultivation area is concentrated in Cuu Long River Delta (Mekong River Delta) with more than 35,000 hectares (MARD, 2011), mainly in Vinh Long, Ben Tre, Tien Giang or Dong Thap (MARD, 2011). In the north, provinces with large longan cultivation areas include Hung Yen, Bac Giang, Ha Noi, Tuyen Quang, Yen Bai and Hai Duong.

Longan is the fifth fruit crop in importance in Vietnam after cashew, coconut, banana, lychee and rambutan (MARD, 2011). While the reproductive process plays a clear part on the subsequent fruit set, very little is known on longan reproductive biology. In this work we describe the basics of longan reproductive biology.

I. MATERIALS AND METHODS

1. Materials

Longan flowers were collected in orchards of Hai Duong provinces in Vietnam and in the Instituto de Hortofruticultura Subtropical "La Mayora" in Spain during 2012 and 2013. Four longan cultivars were studied in Vietnam ("Nhãn lồng hưng yên", "Nhãn cùi", "Nhãn nước" and "Nhãn muộn hà tây") and four in Spain ("Biew Khiew2, "Fuk How", "Chom Poo2 and "Duan Yu").

2. Methods

Time of flower opening was observed every two hours. Hand pollination was performed by cross pollination. Female flowers were collected daily from one day after anthesis up to eleven days after anthesis. The flowers were fixed by FAA (90% Alcohol 70^0 + 5% Formaldehyde + 5% Glacial acetic acid) and 2.5% Glutaraldehyde in 0.03M phosphate buffer (Sabatini *et al.*, 1963). Pistils conserved in FAA samples were stained with 0.1% aniline blue in PO4K3 (Currier, 1957; Linskens & Esser, 1957) and observed under a fluorescence microscope. Pistils

fixed in Glutaraldehyde were infiltrated with plastic and were cut longitudinally by a Ultramicrotome, into 2µm sections and then were stained with 0.07% Calcofluor in water (Hughes & McCully, 1975), 0.1% aniline blue in 0.1N PO4K3, 0.2% toluidine blue in water and periodic acid-Schiff (PAS) (Feder & O'Brien, 1968).

II. RESULTS AND DISCUSSION

1. Biology

Normally in the North of Vietnam, flowering of longan takes place from the end of March to the beginning of April, but this can be variable depending on the weather. Harvesting is usually performed from July to August. In the South of Vietnam, production is all year round. In Instituto de Hortofruticultura Subtropical "La Mayora" of Spain, flowering takes place from the end of May to the beginning of June, whereas harvesting is usually performed from November to January.

2. Flowering

The inflorescence and flowers: M1, M2, F: The longan inflorescences are compound dichasia, terminal, usually leafless, erect, with widely branched panicles 10-40cm long. Flowering in each panicle progresses as staminate flowers (M1), functionally female hermaphrodite flowers (F), and finally, functionally male hermaphrodite flowers (M2) (Davenport and Stern, 2005) (Fig. 1). Flowers are about 7µm size with five couple of hairy petals and sepals. The M1 and M2 are yellow-light-brown and have eight long stamens in a single circle on a disc. The stamens are dorsifixed anther with two-lobes. The F flowers are yellow-brown and have a bicarpellate or tricarpellary ovary on the disc with a two or three lobed stigma. Usually, one carpel grows and develops into a fruit. The eight stamens on the F flowers are undeveloped, with a short filament, about 1mm tall ((Fig.1. F).). The M2 flowers have undeveloped ovary and stigma. The ovary is smaller and pistil is shorter than those of the F flowers. Stamens are more or less erecter and longer than those of the M1 flowers (Fig.1. M2).

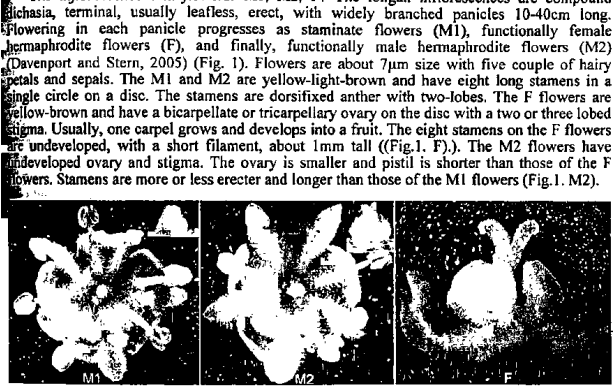

Fig. 1. Three types of Longan flowers: Staminate flowers (M1), functionally male hermaphrodite flowers (M2), and the functionally female hermaphrodite flowers (F).

Floral development: Flowering phenology of longan is very different from lychee. In lychee, there are three distinct waves of flowering inflorescences. The first wave consists of M1 flowers, the second of F flowers, and the third of M2 flowers (Stern and Gazit, 2003). But observations in this work show that in longan, there are three indistinct waves of flowering inflorescences. The first wave consists of F flowers, the second of M2 flowers, and the third of M1 flowers. Usually, in the first wave few M2 flowers are also present, whereas in the second wave a lot of M1 flowers are present and in the third wave few F flowers are present. In Vietnam, female flowers start opening from afternoon (around 18: 00h), fully open at midnight, mostly from 23: 00h to 1: 00h, nectar appears and flower is ready for pollination around 2: 00h in the middle of the night. Also, male flowers start opening at the same time and fully open at midnight. Nectar appears and anther

dehiscence starts at around 2: 00h. The eight anthers will dehisce sequentiality up to 12: 00h. After that time, when the dehiscence has finished, the anthers become dry and the male flowers start dropping at about 16: 00h up to next few days. In Spain, the female flowers start opening at night, fully open at mid-day (around 13: 00h) then nectar appears and flower is ready for pollination at this time. Also, male flowers start and fully open at the same time. The nectar appears and the anther dehiscence starts at around 13: 00h. The anthers will dehisce sequentiality up to evening. The longan flowering season in the same individual tree and orchard can last from 30 days to 40 days. These differences in phenology time between Vietnam and Spain could be related to temperature or to differences in the duration of the day/night time. In Vietnam, the time on day/night is 12h/12h, while in Spain it is 14h/10h.

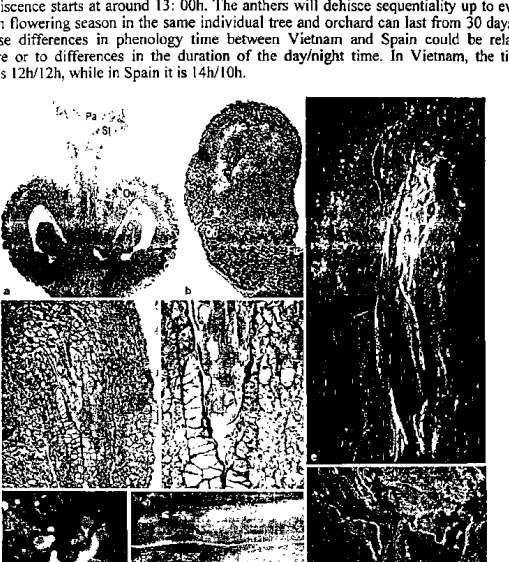

Fig. 2. The reproductive structures

a. Pistil with two united carpels, each one with an ovule, and a papillated bilobed stigma; b. Anatropous ovule showing the funiculus and facing the obturator; c. Integuments closing the micropyle; d. Detail of an ovule showing the embryo sac; e. Pollen tubes growing in the style eleven days after anthesis; f. Pollen grains growing in surface of papillated tigma one day after anthesis;g. Detail of a pollen tube growing in the style ten days after anthesis showing callose plugs; h. some pollen tube growing in the style one days after anthesis (a, c, d-Steaned with PAS; b-Steaned with PAS + toluidine blue; e, f, g, h-Steaned with aniline blue; Pa-Papila, St-Stigma, Ow-Ovary wall, Ov-Ovulus, Fu-Funiculus, Ob-Obturator)

The female reproductive organ: The pistil has a stigma with two or three lobes. When the stigma lobes starts to separate the opening of the female flowers starts. The stigma is receptivte at this stage and retains full receptivity for few days. The surface of the receptive stigma is

covered with long papillae (Fig. 2a). With age, the stigma becomes dry and the color turns from white-yellow to green-brown. The pistil is composed by two or three united carpels. Each carpel has an anatropous ovule that hosts the embryo sac, and the exostome of the micropyle faces an obturator, close to the funiculus (Fig. 2b). The female flower persists on inflorescence up to ten days, then drop in case of unsuccessful pollination.

The male reproductive organs and pollen tube growth: All three flower types (Fig. 1) have stamens and anthers. The stamens of the two male flower types (M1, M2) are more or less erect and have a long filament with a yellow anther. Dehiscence of the anther takes place longitudinally. In the female type flower (F), the stamen is curved and close covering the ovary (Fig. 1). It has a short filament with an indehiscent anther. The pollen grain is orbicular-ovate (Fig. 2f). When the pollen grains are carried by a pollinator from an M1 or M2 flower to an F flower, it is captured by the long papillae of the stigma. In this situation, the pollen grain will grow within one days after anthesis (Fig. 2f,h). In our observations, very few pollen grains germinated and grew. Pollen tubes were clearly observed two days after anthesis at the microscope and long pollen tubes with callose plugs can be observed in the style eleven days after anthesis (Fig. 2e,g).

3. Pollination

Longan flowers are very fragrant so they are visited by many insects. Most pollinators are honeybees, flies, and different species of mosquytoes. At night, many insect species have been found in Hai Duong orchards during flowering time and some of them can easily be mistaken for pollinators: Popillia flavosellata, Adoretus simicus, A. cambodjensis, Petrophora chlorosata, Spodoptera litura, Blattella germanica.

Pollination is considered to be carried out mostly by honeybees such as *Apis dorsata,* which was observed in Hai Duong, and the European honeybee, which was observed in Hung Yen and La Mayora orchards. In addition, *Apis cerana* and *A. mellifera* were found in other orchards with enumeration more than 90% (Pham, 2012). In Thailand, the species *Apis mellifera* is the most important pollinator (Davenport and Stern 2005). The European honeybee is the most important pollinator in lychee in Florida, South Africa, Israel, and Australia (Stern and Gazit, 2003) and it was imported to Vietnam by bee keepers. Although results of this work showing the flower behaviour opening at night, both in male and female flowers, suggests that nocturnal insects surely do play a clear part in pollination.

In Vietnam, pollination occurs from early morning (6: 00h) to mid-afternoon (16: 00h), concentrate at 10: 00h. In overcast days, pollination can be extended to the end of afternoon although this weather is not the most appropriate for honeybee activity. In addition, western winds are very dry and hot, resulting in rapid drying of the stigma and, consequently, affecting pollination success.

III. CONCLUSION

There are three types of flower in longan: Staminate flowers (M1), functionally female hermaphrodite flowers (F), and functionally male hermaphrodite flowers (M2). In Vietnam, flowers start opening in the afternoon, fully open at midnight and they are ready for pollination around 2: 00h at night. The eight anthers will dehisce sequentially up to 12: 00h; Male flowers start dropping at 16: 00h up to next few days. In Spain, flowers start opening at night, fully open at mid-day (around 13: 00h) and flower is ready for pollination at this time. The anthers will dehisce sequentiality up to evening. Longan flowers do not open at the same time in individual trees and orchard.

The F flowers have a bicarpellate or tricarpellary ovary with a two or three lobed stigma. The eight stamens on the F flowers are undeveloped, with a short filament and an indehiscent anther. The surface of the receptive stigma is covered with long papillae. The ovule is composed of two or three united carpels; each carpel includes an anatropous ovule. The ovule hosts the embryo sac, is bound to the placenta by the funiculus, and the micropylar exostome faces an obturator. The M1

and M2 flowers have eight long developed stamens. The M2 flowers have a short and small undeveloped ovary and stigma. Pollen grains will grow within one day after anthesis.

Pollination occurs from early morning to mid-afternoon in Vietnam. It was carried out mostly by honeybee *Apis cerana* and European honeybees *Apis melifera*, although seeing the flower behaviour the relevance of other insects in pollination is worth to investigate.

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SINH HỌC SINH SẢN Ở NHÃN

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TÓM TẮT

Nhãn (*Dimocarpus longan* Lour., Sapindaceae) là một trong năm loại cây ăn quả quan trọng của Việt Nam. Cơ quan sinh sản của nhãn gồm hoa đực (M1), hoa lưỡng tính có chức năng cái (F) và hoa lưỡng tính có chức năng đực (M2). Ở Việt Nam, hoa bắt đầu nở vào buổi chiều, nở hoàn toàn vào nửa đêm và sẵn sàng cho thụ phấn từ khoảng 2 giờ sáng. Các bao phấn sẽ nứt liên tục cho đến 12 giờ trưa. Hoa đực bắt đầu rụng từ 4 giờ chiều cùng ngày cho đến một vài ngày tiếp theo. Ở Tây Ban Nha, hoa bắt đầu nở vào ban tối, nở hoàn toàn và sẵn sàng cho việc thụ phấn vào khoảng 13 giờ. Các bao phấn nứt liên tục từ khoảng thời gian đó cho đến tối. Hoa không nở cùng một lúc giữa các cây trong vườn cũng như giữa các cụm hoa trong từng cá thể.

Hoa F có một bầu với hai hoặc ba lá noãn và một nhụy xẻ hai hoặc ba thuỳ. Tám nhị của hoa F không phát triển, các chỉ nhị ngắn và bao phấn không bao giờ nứt. Trên bề mặt của núm nhụy được bao phủ bởi các nhú dài. Noãn gồm hai hoặc ba lá noãn hợp lại, mỗi lá noãn gồm có một noãn đảo. Noãn chứa túi phôi, được giới hạn với giá noãn bởi cán noãn và lỗ noãn mặt ngoài được che bởi một nắp bịt. Hoa M1 và M2 gồm có 8 nhị hữu thụ dài. Hoa M2 có một bầu và nhụy không phát triển. Hạt phấn nảy mầm trong khoảng thời gian một ngày sau khi thụ phấn.

Ở Việt Nam, sự thụ phấn diễn ra vào buổi sáng sớm cho đến giữa buổi chiều. Chúng được thụ phấn chủ yếu bởi Ong mật nội địa *Apis serana* và Ong ý nhập nội *Apis melifera*.