

Running title: Fruit aril development in longan

Fruiting pattern in longan (*Dimocarpus longan*). From pollination to aril development

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Abstract

In most fleshy fruits the edible part of the fruit is formed either by the pericarp derived from the ovary wall or by an engrossed receptacle. However, in a number of fruit tree species the edible part is the aril, an outgrowth of the ovule that envelops the seed, and there is a paucity of information on fruit development and fruiting pattern in these crops. In order to fill this gap, in this work we characterize the progamic phase, fruit development, and fruit/fruitlet drop in longan (*Dimocarpus longan*). The progamic phase from pollination to fertilization took just three days; the pollen tubes arrived to the base of the style in one day, reached the locule one day later and penetrated the ovules three days after pollination. Only one of the two ovules present in each longan flower got fertilized. However, this did not result in rapid fruitlet growth, since a very slow fruit development occurred up to six weeks after flowering. Pollination was required for fruiting and unpollinated flowers dropped 9 days after anthesis. Only 7% of the female flowers produced became fruits. There was a first flower/fruitlet drop 2-4 weeks after flowering that appeared to be related to lack of fertilization followed by a second fruitlet drop 5-6 weeks after flowering, concomitantly with the rapid growth of the embryo and the aril, that appears to be related to competition for nutrients. These results set a frameline to understand fruiting in tree species in which the edible part of the fruit is a fleshy aril.

Keywords: aril; fruit drop; fruit set; longan; pollen tube growth; progamic phase

Introduction

Longan (*Dimocarpus longan* Lour.) is a subtropical fruit tree crop native from Southern Asia, extending from Myanmar to southern China, Southwestern India and Sri Lanka (Tindall, 1994). It was first described in 1790 by the Jesuit missionary and botanist Joao de Loureiro (Loureiro, 1790), although it has a long tradition of cultivation in China and Southeastern Asia (Wong, 2000). Longan is also cultivated in other regions of the world with subtropical climates such as Queensland in Australia or Florida in the United States (Wong, 2000), and has potential as a new crop in other countries with subtropical climates. World longan production reached more than 2,500 million tons in 2010; the main world longan producing countries are China, with about 1,300 million tons, Vietnam with more than 600 million tons, and Thailand with over 500 million tons in 2010 (FAO, 2011). In China, Vietnam and Thailand, fresh longan fruits have been appreciated for centuries due to their excellent nutritive and pharmaceutical properties (Wong, 2000). Longan fruits can also be marketed in various processed ways such as frozen, canned or dried. Dried longan arils, leaves and flowers are also ingredients in traditional medicine, with properties against stomachache, insomnia and as an antidote for different poisons (Wong, 2000).

Longan belongs to the Sapindaceae, or soapberry family, with about 150 genera and 2000 species that includes species of interest for their valuable wood, such as maple (*Acer* spp) and buckeye (*Aesculus* spp), or as ornamental plants as the Taiwanese rain tree [*Koelreuteria elegans* (Seem) A.C.Sm.]. Most importantly, the Sapindaceae is a rich family in edible fruit species such as lychee (*Litchi chinensis* Sonn.), rambutan (*Nephelium lappaceum* L.), guarana (*Paullinia cupana* Kunth), korlan (*Nephelium hypoleucum* Kurz), pitomba (*Talisia esculenta* Radlk.), Spanish lime (*Melicoccus bijugatus* Jacq.), pulasan (*Nephelium mutabile* Blume) or ackee (*Blighia sapida* K.D.Koenig). Many of these species share the fact that the fruit is not a developed pericarp or receptacle, as it occurs in most fleshy fruit tree species (Esau, 1977), but the edible fruit (in fact a fruit-like structure) is a fleshy aril, which is an outgrowth of the ovule that envelops the seed (Endress, 1973). Fruit tree species with fleshy arils are also found in other families as pomegranate (*Punica granatum* L.) in the Lythraceae (Morton, 1987), nutmeg (*Myristica fragrans* Houtt.) in the Myristicaceae (Endress, 1973), passion fruit (*Passiflora* spp.) in the Passifloraceae or mangosteen (*Garcinia mangostana* L.) in the Clusiaceae. It is also present in some gymnosperms such as the yews (*Taxus* spp.) in the Taxaceae or *Dacrycarpus* spp. in the Podocarpaceae.

The fruiting pattern in fruit tree species in which the edible part is a developed pericarp or receptacle, is well characterized (Westwood, 1978). The progamic phase, which goes from pollination to fertilization

(Linskens, 1986; De Graaf *et al.*, 2001), has proven to play a major role on the establishment of fruit set in most fruit trees such as apricots (Rodrigo & Herrero, 2002), cherries (Hedhly *et al.*, 2007), apples (Losada & Herrero, 2013), or cherimoyas (Lora *et al.*, 2010). Along the progamic phase important successive events, as pollen germination, pollen tube growth in the style, and penetration into ovule, take place (Mauseth, 2014; Williams, 2009; De Graaf *et al.*, 2001; Herrero, 1992; Linskens, 1986; *et al.*, 1984). Following this phase, in angiosperms, double fertilization occurs, which is followed by growth of the ovary or receptacle, and fruit development (Mauseth, 2014; Rost *et al.*, 1984). Lack of pollination or fertilization may cause fruit abortion and reduce crop production (Stephenson, 1981). In some cases, seedless or parthenocarpic fruits can be produced (Lee, 1988; Voraquaux *et al.*, 2000), due to an uncoupling of ovary development from the fertilization process (Distefano *et al.*, 2011). However, information on the relevance of the progamic phase on fruit development is scarce when the edible part of the fruit is an aril. This may be based in the fact that information is also scarce on the fruiting pattern in these species.

In longan, information is available on flowering (Davenport & Stern, 2005; Pham *et al.*, 2015), and fruit development appears to be similar to lychee (Stern, 2005). The longan inflorescences are compound dichasia (Fig. 1A) with a large number of unisexual flowers that appear generally in three waves with different degrees of overlap among functionally female (Fig. 1B) and male (Fig. 1C) flowers depending on the cultivar and environmental conditions (Subhadrabandhu & Stern, 2005; Davenport & Stern, 2005; Pham *et al.*, 2015). The female flowers have a bicarpellate ovary (Fig. 1D) although usually only one locule will develop into a fruit. The reproductive process including pollination biology (Liu *et al.*, 1996), the fertilization process (Li, 1984), early embryology (Xu *et al.*, 2012), aril initiation (Ke *et al.*, 1992), fruit growth (Chen *et al.*, 1995), and fruit contents (Xu *et al.*, 1997) have been studied in this species. However, information is lacking on the fruiting pattern and on how fruiting relates to the reproductive process. In order to fill this gap, in this work we characterize the fruiting pattern in longan and evaluate the influence of the progamic phase on this process. For this purpose, we follow pollen tube behavior and the onset of fruiting and relate these events with fruiting in the field, characterizing the pattern of fruit drop. These results provide a line basis for the understanding of fruiting in species in which the edible fruit is the aril.

Materials and Methods

Plant material

Data were collected from 15 year old adult trees of *Dimocarpus longan* cultivars ‘Choompoo’, ‘Fuk How’, ‘Biew Kiew’, and ‘Duan Yu’, propagated by air layering, located at IHSM la Mayora in Malaga (Spain), at latitude 36°45N, longitude 4°4W and elevation 35m above sea level. The climate data of the last 50 years show a mean annual temperature for this location of 18.5°C, average maximum temperatures of 28.9°C in the hottest month (August) and average minimum temperatures of 9.8°C in the coolest month (January).

Field experiments

The fruiting pattern was characterized in the field. Observations were made weekly from flowering to fruit ripening, and photographed with a Canon Rebel XT 8.0 MP digital camera. To characterize the fruit drop pattern, flower/fruit abscission was monitored weekly from flowering to harvest. For this purpose, eighteen labeled inflorescences were left to open pollination, and a plastic sheet was placed underneath to collect all dropped flowers/fruits, without preventing natural pollination.

To evaluate flower/fruitlet growth in pollinated flowers, female flowers were marked with colored threads, and hand pollinated on the day of anthesis. For this purpose anthers were collected from flowers just opened, but prior to anther dehiscence, and left in the shade in a Petri dish for one to two hours, until they dehisced. Flowers were hand pollinated by touching the stigma of a receptive flower with a dehisced anther. Ten female flowers were marked per inflorescence, in five inflorescences per tree, over 13 trees. Ten flowers were collected 6 hours after pollination and, in the following days, ten flowers were collected daily from 1 to 25 days after pollination, making a total of 260 collected flowers. To evaluate flower/fruitlet growth in unpollinated flowers, and to avoid unwanted insect pollination, a whole inflorescence was covered with fleece mesh before flower opening. Every day, all opened female flowers were labeled with colored threads; concurrently, all opened male flowers were

removed to prevent self-pollination in the same tree. Those flowers were left without pollination, and ten labeled flowers were collected daily, from the day they opened at anthesis (day 0) until they dropped, 9 days after anthesis (DAA), making a total of 100 flowers. In each unpollinated and pollinated flower, the peduncle, petals, sepals, anthers and filaments were removed and the remaining gynoecium, with the nectar disc, was individually weighed, before fixing.

Additionally, fruitlet, seed and aril development was sequentially observed under a binocular Leica S6DA stereomicroscope in longitudinally opened fruitlets. The fruits were observed either directly or following staining with Toluidine Blue 0.2% in water. Photographs were taken with a Canon PowerShot S50 5.0MP camera attached to the binocular microscope.

Microscope preparations

The collected gynoecia were fixed in 2.5% glutaraldehyde in 0.03M phosphate buffer saline (PBS) (Sabatini *et al.*, 1963). They were left in 0.03M PBS overnight in the fridge and washed for one hour in each 0.03M PBS, 35% alcohol, 50% alcohol, 70% alcohol and 95% alcohol. Then the gynoecia were boiled for 15 minutes in 5% Na₂SO₃. Each gynoecium is composed of two or three carpels, each one with one ovule. Pollen tube growth was observed in squash preparations of stigma-style, and also in dissected obturators and ovules. For this purpose the stigma-style and ovary were separated. The two or three stigma lobes were dissected before staining under a binocular Leica S6DA stereomicroscope. Both the stigma-style and the dissected obturators and ovules were stained with 0.1% aniline blue in PO4K3 (Linskens & Esser, 1957) and squashed under the coverslip. Preparations were examined under a Leica DM LB2 fluorescence microscope with epifluorescent UV with a 515-560 excitation filter and a LP 590 barrier filter. Five pollinated flowers were examined for each fixing time: six hours, one, two and three days after pollination, when the pollen tubes penetrated the ovules. In each pistil, the number of pollen grains in the stigma and the number of pollen tubes growing in the style, reaching the obturator and the ovule were recorded. Photographs were taken with a LEICA DFC310 FX camera, attached to the fluorescence microscope.

Results

Pollen tube kinetics in longan pistils

Pollen grains were captured by long papilla at the surface of the stigma and rapidly germinated. Pollen germination was already observed 6h after pollination (Fig. 2A), although some pollen tubes appeared arrested in the stigma (Fig 2B) and many of them with very short pollen tubes were washed away during the fixing procedure. The number of adhered and germinated pollen grains increased from day one to day two after pollination from 16 to 64 (Table 1). The first pollen tubes were already present in the style six hours after pollination reaching 40% of the style length. Pollen tubes grew in the style (Fig. 2C), showing clear callose plugs (Fig. 2D) and the first pollen tubes reached the base of the style one day after pollination. Two days after pollination, the first pollen tubes could be seen in the ovary growing on the surface of the obturator (Fig. 2E), which is a placental protuberance located at the suture line and facing the ovule. At that time, the first pollen tubes started penetrating the ovule and continued up to three days after pollination (Fig. 2F). Upon penetration in the style, there was a reduction in the number of pollen tubes growing in the stigma, and some 20-27 pollen tubes were observed in the style. This number was further reduced upon penetrating the ovary and three days after pollination one or two pollen tubes entered each locule, but only one out of the two ovules was penetrated by a pollen tube.

Fruit set and fruit drop pattern

At the day of anthesis, the petals opened and the stigmas spread (Fig. 3A). One week after anthesis, the petals had felt down and the stigmas dried (Fig. 3B), as the ovary color changed to light green and was slightly bigger than the day the flower opened. Two weeks after anthesis, the two carpels of the ovary were equally developed (Fig. 3C). However, one week later usually only one carpel of the ovary develops into a young

fruitlet, as the other carpel remains underdeveloped (Fig. 3D). At this point, while some carpels remained small, the set fruits continued growing (Fig. 3E), and developed attaining full size 17 weeks after flowering (Fig 3F). Fruit maturation and harvest took place 4-5 months after flowering in the conditions of Southern Spain. At this time, 60-80 fruits could be harvested per inflorescence. Along this process average maximum and minimum temperatures were 26.7°C and 12.8°C.

Flowers/fruits dropped from 1 to 9 weeks after flowering (Fig. 4A), in an exponential trendline ($R^2 = 0.9331$), following the equation $y = 144.69e^{-0.285x}$, where 'x' means weeks after anthesis. Two main waves of flower/fruit drop were observed (Fig. 4B). The first drop wave occurred 2-4 weeks after flowering, when 33% of the fruitlets dropped. Most of those dropped fruitlets had both carpels equally developed. This was followed by a second wave 5-6 weeks after flowering, when an additional 48% of the initial flowers/fruitlets dropped. These dropped fruits had only one carpel developed. Thereafter, there was a continuous slight fruit drop resulting in 7% final fruit set at harvest, which took place 17-20 weeks after flowering.

Initial fruit development

Pollinated flowers did not show receptacle enlargement for the first 9 days after pollination. But two weeks after pollination, while some pistils did not show further growth, others continued growing (Fig. 5A). Gynoecia grew very slowly from day 10 to day 22 after pollination, and a significant growth was observed from day 23 after pollination. Thus, at anthesis, the weight of the gynoecium plus the nectar disc averaged 17.7 mg (ranging from 14.8 to 19.4 mg), and 25 days after pollination the weight of the remaining fruitlets in the tree averaged 120.4 mg (ranging from 82.8 to 158.7 mg). Gynoecium weight increased following the equation $y = 12.762e^{0.0724x}$ ($x = \text{DAA}$) and showing an exponential trendline ($R^2 = 0.9098$). Unpollinated flowers dropped 9 days after anthesis. In this period, there was no much change in the weight of flowers (Fig. 5B) since the average weight of ten unpollinated flowers in the day of anthesis was 16.5 mg (ranging from 14.5 to 20.8 mg) and increased up to 18.9 mg (ranging from 15.9 to 27.9 mg) 9 days after anthesis. By this time, the flowers had dried and lost weight. This is reflected in an exponential trendline ($R^2 = 0.2574$) and an equation $y = 17.672e^{-0.018x}$ where x is number of days. Fruit weight at harvest averaged 11 g (ranging from 8.66 g to 12.61 g).

Two weeks after anthesis, both carpels showed equally developed ovules (Fig. 6A). However, one week later differences were clear between both carpels and ovules, and as one grew the other degenerated (Fig. 6B). In the degenerated carpel, the pericarp still had a fleshy appearance, but the ovule turned brown (Fig 6C). Six weeks after anthesis, in the young developing fruits, a small embryo and an incipient aril were apparent (Fig. 6D). The aril continued development to surround the seed and could be observed to the naked eye 10 weeks after anthesis (Fig. 6E). At harvest, some 17 weeks after anthesis, the aril had its characteristic fleshy appearance (Fig. 6F).

Discussion

Results herein in longan show that the fact that the edible fruit is an aril results in some common points and some peculiarities for fruit development as compared with other fruits where the edible fruit is derived from different structures of the ovary, such as the pericarp. It shares with other fruits the need for pollination, but fruit development instead of starting following fertilization is further delayed to the time of aril development. As in other plant species, only a small proportion of the flowers develop into fruits and two main waves of drop occur relating to particular critical steps of fruit development.

The fruit drop pattern

In Southern Spain, two waves of longan fruit abscission were recorded, a first one from 2 to 4 weeks after flowering, and a second one 5 to 6 weeks after flowering. A similar fruit abscission pattern has also been reported in Vietnam (Tran & Huynh, 2008; Tran & Do, 2011), and in China, with a similar first wave, but where

the second wave occurred a bit later, 5-8 weeks before harvest (Zee *et al.*, 1998). Besides these two main abscission waves, scattered fruit drop was also observed along the fruiting season resulting in 7% final fruit set at harvest. This proportion is similar to that reported for lychee, where three or four drop waves have been shown (Yuan & Huang, 1988; Stern *et al.*, 1995; Stern and Gazit, 2003), although a higher fruit set has also been reported for longan in other environmental conditions (Stern, 2005).

Flower and fruitlet/fruit abscission is a common phenomenon in most plants (Stephenson, 1981; Charlesworth, 1989) resulting in a reduction in the final number of fruits harvested (Alcaraz *et al.*, 2010, 2013). Different non-exclusive hypotheses, such as pollen limitation, pollinator attraction, bet hedging, selective abortion or pollen donation, have been proposed to explain the reasons why plants produce significantly more flowers than fruits. In biochemical terms, longan fruit abscission has been related to a reduction in auxin and gibberellin concentrations (Zhou *et al.*, 1999). In fact, application of exogenous gibberellins has been used in China to reduce fruit drop (Liu & Ma, 2001). And yet, even under the apparently most favorable conditions, flower/fruit drop also occurs consistently in a good number of species (Stephenson, 1981). The fact that drop follows a conserved pattern suggests that particular critical development stages should be behind these drop waves. In order to understand this process in longan, we investigated the progamic phase and fruit development.

The progamic phase

In spite to the fact that the edible part of the longan fruit is an aril, the development of the fruit requires fertilization, as it occurs in species where the edible part of the fruit is the pericarp that results from the enlargement of the ovary wall (Esau, 1977). Thus, in longan, an appropriate progamic phase that results in fertilization success is required for fruiting, as it is put forward by the drop of all the unpollinated flowers. The process from pollination to fertilization lasted three days, a time length similar to the two days reported for lychee (Stern & Gazit, 1998). Previous work in longan reported fertilization at different times after pollination, ranging from three hours to three days (Li, 1984; Liu *et al.*, 1996; Xu *et al.*, 2012). In other unrelated species adapted to subtropical climates, such as avocado and cherimoya, the pollen tube reached the ovary in one day (Alcaraz *et al.*, 2010; Lora *et al.*, 2010). Conversely, longer periods have been observed in temperate fruit tree crops such as apple, where the pollen tube reached the ovule one week after pollination (Losada & Herrero, 2013), or even longer in peach (Herrero & Arbeloa, 1989), plum (Thompson & Liu, 1973), or cork oak trees (Boavida *et al.*, 1999). In any case, compared to gymnosperms, a fast pollen tube growth rate resulting in a short progamic phase has been considered a key innovation of angiosperms (Williams, 2008) although environmental factors, mainly temperature, play a major part in regulating pollen tube kinetics (Sanzol & Herrero, 2001; Hedhly *et al.*, 2004; Hedhly *et al.*, 2009).

Once pollen grains were captured on the stigmatic surface, pollen germination was conspicuous six hours after pollination, a similar situation to that observed in lychee (Stern & Gazit, 2003). Differences were observed in the speed of pollen tube growth along the different regions of the pistil. Thus, while pollen tubes reached the base of the style one day after pollination, it took them two days to reach to the obturator and three days to penetrate the ovules. A similar situation was recorded in the peach pistil where the pollen tubes took twice more from the base of the style to ovule penetration than to cover the length of the style (Herrero & Arbeloa, 1989). Changes in the speed of pollen tube growth rate along the pistil have been reported for different species (Mascarenhas, 1975, 1993; De Graaf *et al.*, 2001). These are related to the different territories the pollen tube has to traverse (Losada & Herrero, 2014), and to a differential maturation of the different pistil structures (Herrero & Arbeloa, 1989; Arbeloa & Herrero, 1987; Herrero, 1992, 2000, 2001; Herrero & Hormaza, 1996; Sogo & Tobe, 2005) since for a successful mating male-female synchrony is required (Herrero, 2003). In lychee, fertilization also takes place two or three days after pollination in spite of a fast pollen germination process (Stern & Gazit, 2003).

Although many pollen grains were observed on the surface of the longan stigmas and many of them germinated, a clear reduction in the number of pollen tubes occurred when they penetrated the style. A reduction occurred also along the style and, finally, only one pollen tube penetrated the ovule three days after pollination. A reduction in the number of pollen tubes growing along the pistil has been recorded in other angiosperms such as petunia (Herrero & Dickinson, 1980, 1981), cherries (Hormaza & Herrero, 1996), cork oak trees (Boavida *et al.*, 1999), *Amborella trichopoda* (Williams, 2009) or citrus (Distefano *et al.*, 2011). In longan this reduction

was most notorious in the ovary, where out of the two ovules in the two locules only one got fertilized, and this resulted in the development of a single carpel. The causes behind this behavior require further elucidation, but it is not exclusive of longan or lychee (Stern & Gazit, 2003). Fertilization of just one ovule, out of the two present in a flower, has also been reported in *Prunus* species (Arbeloa & Herrero, 1991) where differences in starch content prior to fertilization appear to be related with ovule fate (Rodrigo & Herrero, 1998).

Fruit drop in relation to fruit development

Two big waves of flower/fruitlet drop could be observed from anthesis to fruit maturation in longan, a similar situation to that reported for lychee cultivars that produce seeded fruits (Huang, 2005). These two waves of drop appear to be related to critical developmental stages. The first drop wave just followed the time when one of the two carpels of the gynoecium degenerated and the other developed into a fruit. The fact that only one of the two ovules of a gynoecium got fertilized suggests that lack of fertilization is the cause of this degeneration. This is further supported by the fact that the first sign of carpel degeneration was browning of the ovule and that most of the fruitlets that naturally dropped during this first wave had still the two carpels equally developed.

In spite to the fact that fertilization was required for carpel development, the gynoecium showed a very small weight increase for the first nine days after anthesis, either in pollinated or unpollinated flowers. All unpollinated flowers dropped at this time whereas, while some pollinated flowers arrested development, others continued growing. A similar situation has been observed in apple (Losada & Herrero, 2013), pear (Herrero & Gascon, 1987), cherimoya (Lora *et al.*, 2010), or avocado (Alcaraz *et al.*, 2010). But, in longan, fruiting does not occur as an immediate response to pollen tube penetration to the ovule, as it may occur in apple (Losada & Herrero, 2013), but is delayed. This may be related to the fact that, while in apple the fruit is the developing receptacle, in longan the fruit is formed by the aril. Indeed, for the first 25 days after pollination, longan fruits grew very slowly to only 1.1% of their final fruit weight, and similar observations have been made under different environmental conditions (Ke *et al.*, 1992; Xu *et al.*, 2012) and also in lychee (Stern *et al.*, 1995). This initial slow fruit growth may be related to the fact that, in this period, the development of the fruit concentrates in developing a pericarp and seed, while the fleshy aril that will constitute most of the mature fruit has still to develop (Ke *et al.*, 1992; Zheng *et al.*, 1994; Chen *et al.*, 1995; Xu *et al.*, 1997). A similar situation has also been reported for lychee (Stern *et al.*, 1995; Huang, 2005) in which pericarp development contributes to most of the fruit weight during 50 days after anthesis. A second wave of fruitlet drop occurred when the embryo started to grow, as an incipient aril was already visible to the naked eye. The fact that, following this time point, a rapid embryo and aril development took place suggests that this second wave could be related to competition for nutrients among developing fruitlets. Thus, two critical steps appear to mark fruit set in longan: fertilization with the development of a single carpel out of the two carpels present in a flower, and aril development in fertilized ovules. In these two critical points, a massive abscission of those flowers/fruits that do not achieve these developmental stages occur.

In some fruit tree species the edible part of the fruit is the aril, an outgrowth of the ovule that envelops the seed, and there is a paucity of information on fruit development and fruiting pattern in these species. In this work we have described the progamic phase and the initial steps in fruit development in longan, a subtropical fruit crop in which the edible part of the fruit is a fleshy aril. The results show two critical steps that mark fruit set in longan (fertilization and aril development) providing a line basis for the understanding of fruiting in species where the edible fruit is an aril. It will be of interest to complement this work with additional studies involving other fruit crops that produce fleshy edible arils in order to understand the implications of the progamic phase for fruit production in those species.

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Tables

Table 1: Number of pollen grains and germinated pollen grains in the stigma, number of pollen tubes in the style, and penetrated locules and ovules by a pollen tube in the first three days following pollination.

Days after anthesis	N° of pollen grains	N° of germinated pollen grains	N° of pollen tubes in the style	N° of penetrated locules by a pollen tube	N° of penetrated ovule/ ovules in a flower
+1	65	16	6	0	0
+2	233	64	20	0/2; 0/2; 1/2; 1/2; 2/2	0/2; 0/2; 0/2; 0/2; 1/2
+3	204	38	27	2/3; 2/2; 2/2; 2/2; 2/2	1/3; 1/2; 1/2; 1/2; 2/2

Figure legends

Figure 1 Diagrams showing the details of the longan inflorescence and flowers. (A) Inflorescence. (B) Staminate flower. (C) Functionally female hermaphrodite flower. (D) Detail of the gynoecium of a functionally female hermaphrodite flower showing two carpels.

Figure 2 Pollen tube growth in the longan pistil. (A) Stigma with germinated pollen grains 6 h after pollination. (B) Some pollen grains showed pollen tube arrest on the stigmatic surface. (C) Pollen tubes growing close to the base of style 24 h after pollination. (D) Detail of pollen tube in the style showing a callose plug. (E) Pollen tube growing in the ovary on the obturator surface and approaching an ovule. (F) Pollen tube penetrating the ovule 72 h after pollination. Squash preparations of glutaraldehyde fixed pistils, stained with aniline blue, *bars* 20µm. *ob* obturator, *ov* ovule

Figure 3 From flower to fruit in longan. (A) Female flower at anthesis, week 0 (w0). (B) Flowers showed petal fall and a dry stigma, one week after anthesis (w1). (C) Initial fruit set with two conspicuous carpels, 2 weeks after anthesis (w2). (D) Fruit set with only one developing carpel, as the other remains undeveloped, 3 weeks after anthesis (w3). (E) While some carpels remained undeveloped, set fruits increased in size and weight, 6 weeks after anthesis (w6). (F) Fruits continued to grow until harvest, some 17 weeks after anthesis (w17)

Figure 4 Fruit drop in ‘Chompoo’ longan in open pollination conditions. (A) Fruit drop pattern followed an exponential trendline. Only 13% of the fruits remained in the tree, 9 weeks after anthesis. (B) Relative fruit drop showed one first peak of flower/fruit drop 2-4 weeks after flowering and a second drop 5-6 weeks after flowering

Figure 5 Gynoecium weight of initial fruits. (A) Pollinated flowers. (B) Unpollinated flowers

Figure 6 Fruit development in longan. (A) Initial fruit development with two conspicuous carpels, 2 weeks after anthesis (w2). (B) Fruit set with only one growing carpel whereas the other is aborting, 3 weeks after anthesis (w3). (C) Ovule with a brown coloration in the aborting carpel, 3 weeks after anthesis (w3). (D) Developing fruits showed a small embryo (*white arrowhead*) and an incipient aril (*black arrow*), 6 weeks after anthesis (w6). (E) As the embryo (*white arrowhead*) developed, the aril (*black arrow*) grew surrounding the seed, 10 weeks after anthesis. (F) Fruits continued to enlarge until harvest, some 17 weeks after anthesis, showing a fleshy aril (*black arrow*) (w17). Cross sections. Stained with Toluidine Blue (a, d, e), *bars* 2mm











