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Short communication

Effective pollination period in durian (*Durio zibethinus* Murr.) and the factors regulating it

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Abstract

The effective pollination period (EPP) of durian was determined by both delayed and bud pollination, during which reproductive factors affecting the EPP, e.g., stigma receptivity, pollen tube growth in the style, and ovule longevity were studied histologically. This study was conducted in three distinct locations in Thailand, namely, the Chantaburi Horticultural Research Center and two private orchards in Chantaburi and Trat provinces. Results from artificial pollination revealed that at anthesis, the durian flower is receptive and has a high fruit set ratio. A mean fruit set of 50% was obtained at anthesis in the private orchard in Chantaburi province. However, the EPP of durian was found to be very short, lasting for only one night; the fruit set from pollination on the morning after anthesis ranged from 0% to 3.4%. No fruit set occurred following pollination 24 or more hours after anthesis. When compared with the flowers of other fruit species, the durian flower has a unique feature in that it blooms overnight; the following morning, there is abscission of all parts of the flower, except the gynoecium. Thus, EPP appears to be synchronized with flower longevity. On the other hand, the durian flower was receptive several hours before anthesis. The results of chemical tests, including the hydrogen peroxide test and Perex-Test®, for the evaluation of stigma receptivity appeared to be in agreement with the EPP. However, fluorescent microscopy revealed that pollen could germinate even in the stigmas pollinated 48 h after anthesis, but the number of pollen tubes at the top of the style rapidly decreased from 34.6 (at anthesis) to 0.5 (48 h after anthesis). A correlation test demonstrated a higher correlation coefficient between the fruit set and the number of pollen than that between the fruit set and the result of Perex-Test®. This indicated that pollen tube penetration or elongation in the style was inhibited, probably due to the deterioration of nutritional support from the pistil to the pollen tubes; this can be a limiting factor of the EPP in durian.

Keywords: Durian; Effective pollination period (EPP); Stigma receptivity; Ovule longevity; Pollen tube growth

1. Introduction

Fruit set is an essential event among many events that must occur successfully for fruit production. In this process, adequate pollen needs to be transferred to the stigma and pollen tube growth has to occur successfully for the pollen tube to fertilize the embryo sac. The fruit set is an important factor limiting fruit crop yields. In the case of the durian, which is one of the most important fruits in the tropics, the fruit set is often dependent on successful pollination (Brown, 1997; Honsho et al., 2004b). In an agricultural context, the durian yield in most Southeast Asian countries appears to be low and erratic, mainly due to inadequate fruit set (Subhadrabandhu et al., 1991). Our previous research showed that compared to open- and self-pollination, cross-pollination resulted in a higher fruit set (Honsho et al., 2004b). Thus, we recommended artificial cross-pollination to achieve a high and stable fruit set. In addition, durian fruits do not develop an aril surrounding an unfertilized ovule; this results in an abnormal shape that lowers the commercial value of the fruits (Brown, 1997). Therefore, pollination and fertilization are key events in durian production. In such situations, flower receptivity can be a limiting factor of the fruit set.

Regarding the ability of a flower to set, the term ‘‘effective pollination period’’ (EPP) was a concept introduced by Williams (1965) to assess flower receptivity. A short EPP

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often limits the potential productivity of fruit tree species. The EPP has proved to be a good tool for the detection of factors limiting the fruit set. Although several experiments have been conducted over the last 40 years to determine the EPP of fruit trees worldwide, little attention has been paid to the EPP of tropical fruits. No information on the EPP of durian is available thus far. Although we recommended artificial cross-pollination for better productivity, performing the procedure at night is laborious and dangerous for farmers. If the durian flower is receptive to pollen at either the beginning or the end of anthesis during the daylight hours, a new approach to artificial pollination could be developed. Thus, it is important to elucidate the EPP of durian for improving its production and to obtain a better understanding of its reproductive physiology.

In this study, we determined the duration of the EPP in durian at different flower developmental stages from −12 to 48 h from anthesis. We also confirmed the stigma receptivity, pollen germination, pollen tube growth, and ovule longevity, which are the reproductive parameters that determine flower receptivity. The stigma receptivity was verified using hydrogen peroxide and the Perex-Test®. Pollen germination and pollen tube growth in the pistil were then monitored by fluorescent microscopy, and the development of the ovule and embryo sac was observed by light microscopy.

2. Materials and methods

This study was conducted at three different locations in Thailand, i.e., the Chantaburi Horticultural Research Center (CHRC) and two private orchards in Trat and Chantaburi provinces. These locations in eastern Thailand are the main durian production areas of the country (Subhadrabandhu and Ketsa, 2001). The experiment was conducted in CHRC and the private orchard in Trat in the 2002–2003 season and in the private orchard in Chantaburi in the 2004–2005 season.

2.1. Determination of EPP by delayed and bud pollination

In CHRC and the private orchard in Chantaburi, 'Chanee' and 'Kradum Thong' were used as the maternal and paternal parents, respectively, to prevent fruit drop due to a self-incompatibility reaction. However, in Trat province, 'Mon Thong' was self-pollinated because no other cultivars were available as pollen donors. The flower buds that developed a prominent corolla, indicating the onset of nocturnal anthesis (Honsho et al., 2004a), were emasculated in the late afternoon. Pollination procedures were performed according to Honsho et al. (2004b). Since the durian flowers bloom at approximately 19:00, it was considered as the time of anthesis in this study. Pollen was obtained from anthers collected at anthesis because it was difficult to isolate pollen from the anthers due to their stickiness. Pollen was applied to the emasculated flowers at −12, 0, 6, 12, 24, and 48 h from anthesis (HFA) in CHRC; at −6, 0, 24, and 48 HFA in Trat; and at −6, −3, 0, 6, 12, 24, and 48 HFA in Chantaburi. Pollen used for pollination at 0, 24, and 48 HFA was collected from blooming flowers just prior to the procedure, while that used at other times was collected at anthesis and kept on a paper at room temperature. Since pollen remains viable for at least 24 h after anthesis (unpublished data), the pollen that was collected within 24 h after anthesis was applied to the stigmas at each pollination treatment. More than 20 flowers were pollinated at each pollination treatment. The fruit set in all locations was recorded 2 weeks after pollination, because it was almost stable thereafter (Honsho et al., 2004b).

2.2. Histochemical tests for stigma receptivity, observation of pollen germination and pollen tube growth in the style, and anatomical study of time-course alteration in the ovule

Histochemical tests to determine the duration of stigmatic receptivity were performed in the 'Chanee' cultivar in the private orchard in Chantaburi by using the hydrogen peroxide (H_2O_2) test and Perex-Test® (Merck, Germany) according to Dafni and Maués (1998). The flower buds were emasculated and covered to avoid a false-positive reaction due to any foreign pollen and to prevent damage to the stigma. The stigmas were collected at −6, −3, 0, 6, 12, 24, and 48 HFA for each test. Five samples were immediately immersed in each test solution in glass vials. In the H_2O_2 test, the stigma receptivity was evaluated based on the intensity of effervescence observed after the samples were immersed in 10% H_2O_2. The results recorded were as follows: −, none; +, little; ++, moderate; and +++, intense. In the Perex-Test® treatment for determining the H_2O_2 concentration in the stigma, the color of the test solution was compared with the color chart supplied with the kit, according to the instructions provided by the company and those of Dafni and Maués (1998).

Pollination between 'Chanee' (female) and 'Kradum Thong' (male) was carried out at −6, −3, 0, 6, 12, 24, and 48 HFA at the private orchard in Chantaburi. At 24 h after pollination, at least 10 pistils from each pollination treatment were fixed in FAA. For fluorescent microscopic observation, the fixed pistils were washed with distilled water, softened with 8N NaOH overnight under room temperature, and then split longitudinally. They were stained with 0.1% (w/v) decolorized aniline blue in 0.1M K_3PO_4 (Martin, 1959) and squashed by cover slips. Pollen germination on the stigma and elongation within the styles were observed by using a fluorescent microscope (BH-2; Olympus Co. Ltd., Tokyo, Japan) that was equipped with UV excitation filters with a main wavelength of 356 nm. Transverse sections from the top (near the stigma) and the base (near the ovary) of styles were also prepared manually using razor blade. The same procedure was used for preparing longitudinal sections for fluorescent microscopy, with the exception that the samples were cut before softening and were not squashed by cover slips. The number of pollen tubes was counted in both transverse sections.

A correlation test was performed between the percentages of fruit set in Chantaburi and the results of Perex-Test® or the number of pollen tubes since these experiments were conducted at the same location. The percentages of fruit set were subjected to angular transformation before analysis.

The selected 'Chanee' flowers were emasculated in the late afternoon prior to their blooming, and pollination and fertilization were prevented by covering them with paper bags until sampling. Ovaries from emasculated and non-pollinated flowers were collected at −12, 0, 6, 12, 24, and 48 HFA. Five flowers were sampled from the same tree each time. They were immediately fixed in FAA, dehydrated through an alcohol and butanol series, and embedded in paraffin wax. Sections that were 10–20-μm thick were prepared using a microtome and counterstained with safranine and fastgreen (Johansen, 1940). The developing ovules and embryo sacs were photographed under a light microscope. Their alteration was monitored and the length and width were estimated from the photographs by using Scion Image software (Scion Corporation).

3. Results and discussion

Our data showed that the EPP of durian lasted until the morning (12 HFA) after nocturnal anthesis, and this duration was very short (Table 1). The highest percentages of fruit set were obtained from 6 HFA and at anthesis in CHRC (37.5%) and Chantaburi (50.0%), whereas that in Trat (12.0%) was obtained at −6 HFA. In the 12 HFA tests, the fruit set was 3.4% and 0% in CHRC and in Chantaburi, respectively. At all locations, no fruit set occurred after 24 HFA, indicating a complete loss of pollination capacity. This evanescent EPP of approximately 12 h at night is a unique feature of the durian since flowers of most temperate fruits have EPPs that last more than 1 day (Sanzol and Herrero, 2001). We confirmed the results of our previous study in which durian flowering started in the evening and ended the following morning with the abscission of floral organs, except the pistil (Honsho et al., 2004a). Regarding the EPP prior to anthesis, our results showed that the durian flower was receptive approximately 6 h before anthesis (12.0% in Trat; 45.8% in Chantaburi). However, this was not consistent with the finding of a previous research in which a flower was found to be receptive as early as 24 h before anthesis and yet have a fruit set of 80% (Valmayor et al., 1965). In this study, the fruit set varied among the three experimental locations; there was no definitive explanation for this phenomenon. The variation might be attributed to the difference in cultivars and/or the local microclimate because pollination and EPP are generally affected by temperature and relative humidity (Westwood, 1993; Sanzol and Herrero, 2001). This might also be due to the self-incompatiblity (Honsho et al., 2004b) in the durian in Trat where a single cultivar was self-pollinated.

The EPP may be limited by three reproductive parameters; stigmatic receptivity, pollen tube kinetics, and ovule longevity (Sanzol and Herrero, 2001). The results of this study indicated that the ovule did not show any alterations such as collapse and degeneration at 48 HFA. Its size was almost constant from anthesis until 48 HFA (Table 2). Our previous research demonstrated that the pollen tube approached the base of the style within 24 h after pollination (Honsho et al., 2004b), indicating that the ovule remained viable at the time when the stigma was no longer receptive. On the contrary, two chemical tests measuring the stigmatic receptivity showed that the stigma remained receptive for a short period; this was in agreement with the fruit set records obtained following delayed pollinations (Table 2). Thus, the ovule longevity in the durian is not a critical factor limiting the EPP. However, the results of the Perex-Test® were not significantly correlated with the fruit set, while the correlation between the fruit set and the number of pollen tubes was significant. The number of pollen tubes reached a peak at anthesis (top (34.6) and base (32.4) of the style); their number significantly decreased as pollination was delayed (top (0.5) and base (1.6) of the style at 48 HFA). Correlation coefficients for the number of pollen tubes were greater than 0.85 (Table 1).

Fluorescent microscopic observation of the longitudinal sections revealed that many pollen grains germinated on the stigma in the 24 HFA or earlier tests, while fewer pollen tubes were present in the style in the 12 HFA or later tests (data not

Table 1
Percentages of fruit set following early or delayed pollination in three different locations, results of the chemical tests, and number of pollen tubes at two positions in the style

Time of pollination or collection	Fruit set (%)			Chemical tests		Number of pollen tubes	
	Trat	CHRC	Chantaburi	H_2O_2	Perex-Test (mg L^{-1})	Top of style	Base of style
−12 HFA[a]	–[b]	4.2	–	–	–	–	
−6 HFA	12.0	–	45.8	+++	4.0 bc[c]	18.7 b	13.3 b
−3 HFA	–	–	16.7	+++	14.0 b	18.0 b	17.1 b
At anthesis	8.7	22.6	50.0	+++	39.0 a	34.6 a	32.4 a
6 HFA	–	37.5	30.0	+++	9.0 bc	15.8 b	16.7 b
12 HFA	–	3.4	0	++	2.4 c	6.8 c	5.5 c
24 HFA	0	0	0	+	3.2 c	4.7 d	1.0 cd
48 HFA	0	0	0	+	0.6 c	0.5 cd	1.6 d
Correlation test							
Significance					NS	**	*
Correlation coefficient (r)					0.64	0.89	0.86

NS, **, * indicate non-significant, significant at $P < 0.01$ and $P < 0.05$, respectively.
[a] Hours from anthesis.
[b] Not examined.
[c] Means with different letters within columns are significantly different; Tukey's test ($P < 0.05$).

Table 2
Size of ovule and embryo sac at different times

Time of collection	No. of ovules examined	Ovule		Embryo sac	
		Length (μm)	Width (μm)	Length (μm)	Width (μm)
−12 HFA[a]	79	711.0 a[b]	302.1 c	277.5 c	87.3 c
At anthesis	49	740.8 a	333.9 ab	304.8 bc	104.0 b
6 HFA	73	765.9 a	342.4 a	317.5 ab	113.8 ab
12 HFA	75	722.9 a	324.9 ab	297.3 bc	110.8 ab
24 HFA	69	726.5 a	328.4 ab	311.3 bc	116.1 ab
48 HFA	66	734.0 a	310.9 b	325.5 ab	119.1 a

[a] Hours from anthesis.
[b] Means with different letters within the columns are significantly different; Tukey's test ($P < 0.05$).

shown). Durian pollen apparently can germinate on the stigma, but most pollen tubes did not penetrate and/or elongate in the style at 12 HFA. The pollen germination and pollen tube growth in the stigma are autotrophic, i.e., the processes rely on reserves within the pollen (Herrero, 1992; Herrero and Dickinson, 1981), whereas the pollen tube growth in styles is heterotrophic, i.e., the tube absorbs stylar reserves for elongation (Herrero and Hormaza, 1996; Herrero and Dickinson, 1979, 1981). The heterotrophy of the pollen tube in the style suggests that any reduction in the availability of these reserves may affect the number of pollen tubes growing within the styles and/or their growth rate (Herrero and Hormaza, 1996). Changes in the values obtained in the H_2O_2 test and Perex-Test® could indicate a decreased nutritional supply. Although pollen can germinate within 48 HFA, their growth in the style may stop due to the lack of nutritional reserves if their own reserves were depleted. Thus, the inhibition of pollen tube penetration or elongation in the style may account for the short EPP in the durian, which is supported by the significant correlation the between fruit set and the number of pollen tubes.

Insufficient pollination on the day after anthesis led to many inferior fruits, which resulted in an early drop durian fruit development; on the other hand, a high and stable fruit set could be achieved by pollination conducted a few hours before anthesis because the flowers were already receptive. Artificial cross-pollination is recommended to achieve a high and stable fruit set; however, performing it in the dark is time-consuming and hazardous to the workers. Our results demonstrate that artificial cross-pollination during the day is feasible and reduces the risk to farmers. Furthermore, since the stigma protrudes from the unopened bud, laborers can carry out pollination without cutting any flower parts to expose the stigma. It also minimizes the chances of self-pollination, which induces fruit drop due to self-incompatibility.

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