Effect of Temperature before and after Pollination on Pollen Function in ‘Chance’ Durian

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Abstract: Ambient temperature at anthesis is important for pollen germination and pollen tube elongation. In this experiment, the effect of ambient temperature (15, 20, 25, and 30°C) at flowering (1600 to 1900 hours) on subsequent pollen germination was analyzed in ‘Chanee’ and ‘Monthong’ durian. The effect of ambient temperature (15, 20, 25, and 30°C) after pollination on pollen tube elongation was also examined in ‘Chanee’ at 12, 24 and 48 hours after pollination. Low temperatures during anther dehiscence decreased pollen germination in both cultivars; however, the rate was higher in ‘Chanee’ (27–63%) than ‘Monthong’ (4–37%) under all temperature conditions. Pollen tube length was significantly shorter in ‘Chanee’ at 15°C 12 hours after pollination (12% of the length of the pistil); however, elongation did not differ from that under other temperature conditions at 20°C (57–63%). These findings suggest the effectiveness of ‘Chanee’ pollen under low temperatures from flowering through fertilization.

Keywords: anther dehiscence, Durio zibethinus, pollen germination, pollen tube elongation

INTRODUCTION

Hand pollination is commonly practiced in commercial durian orchards to guarantee fruit set and production of evenly shaped fruit. Durian flowers usually contain five locules in the ovary, with each holding 5–7 ovules (Kozai et al., 2014a), which develop into seeds. The seeds are concealed by the edible part of durian, called the aril (Enoch, 1980). The presence of seeds affects durian fruit shape, with fruits having a good shape graded as high quality when traded in the market (Tantrakoonnsab and Tantrakoonsab, 2018). Artificial hand pollination is therefore important for production and yield.

Pollen function is one of the most important factors determining successful fertilization (Dag et al., 2000; Alcaraz et al., 2011; Matsuda et al., 2011; Kozai et al., 2014b; Pham et al., 2015). Fresh pollen germination has been shown to be optimal at 20–25°C in durian (Kozai et al., 2014b). The same study reported that pollen germination decrease differed between two consecutive years under different temperature regimes, with 15°C having no effect observed compared with 20–25°C in the first year, but a decrease observed in the second year. The difference might have resulted from different temperature conditions during flower opening. The pollen material used in their study was collected at 1900 hours from the orchard, when anther dehiscence had completed. Durian flowers begin to open at around 1600 hours followed by anther dehiscence at approximately 1700 hours, with both ending at around 1900 hours. Growers therefore tend to commence hand pollination using fresh pollen at around 1900 hours; hence collection of pollen at 1900 hours in the experiment by Kozai et al. (2014b) is inappropriate. The effect of temperature conditions on pollen function during flowering is not yet fully understood. Air temperatures start to decrease at around 1600 hours and can drop below 20°C even during the evening time. Thus, to achieve successful fertilization, it is important to understand the effect of ambient temperatures before and during anther dehiscence (1600 to 1900 hours) on subsequent pollen germination.

Pollen tube elongation is also an important factor affecting fruit set and quality (Dag et al., 2000; Matcha et al., 2006; Matsuda et al., 2011; Kozai et al., 2014b; Pham et al., 2015). Kozai et al. (2014b) reported a decrease in pollen tube elongation at both low (<15°C) and high temperatures (>30°C) in ‘Monthong’ durian. They further revealed that reproductive development in ‘Monthong’ is also sensitive to low temperatures; however, the information on other cultivars is lacking. Most research on durian is conducted using ‘Monthong’ because it is the dominant cultivar in Thailand. Furthermore, although comparisons of fruit setting (Honsho et al., 2004), ovule development (Kozai et al., 2014a), and pollen germination have been carried out between cultivars (Salakpetch et al., 1992), little is known about how pollen tube elongation is affected by temperature.

‘Chance’ is another commercial cultivar in Thailand...
humidity. The preparations were completed by 1930 hours, with pollen grains were placed in plastic Petri dishes containing 100 mg L⁻¹ KNO₃, 15 mg L⁻¹ MgSO₄·7H₂O, 300 mg L⁻¹ Ca(NO₃)₂·4H₂O, 100 mg L⁻¹ KN0₃, 15% (w/v) sucrose, and 1% (w/v) agar for pollen germination. Three replicates were prepared on 24 slides of agar medium, 1 per flower. The agar medium with pollen grains were placed in plastic Petri dishes containing wet tissue paper, with lids closed to maintain humidity. The preparations were completed by 1930 hours, and the slides were incubated at 25°C for 12 hours beginning at 1930 hours. Pollen germination was observed for more than 50 pollen grains per replicate from each flower. Observations were repeated the following day.

**Experiment 2. Effect of temperature after hand pollination on pollen tube elongation**

This experiment was conducted at CHRC in January 2017. To obtain fresh pollen, flowers were collected before anther dehiscence from fully grown ‘Chanee’ trees by 1700 hours. Anthers were detached from the flowers and placed at room temperature (RT; 25–27°C) for 2 hours; after anther dehiscence was complete by 1900 hours. Pistils of ‘Kradumthong’ durian were used to evaluate pollen tube elongation in ‘Chanee’ because ‘Chanee’ is self-incompatible. More than 40 fresh ‘Kradumthong’ flowers were collected from 1500 hours until 1600 hours and were immediately emasculated at the laboratory. The ‘Kradumthong’ pistils were put into vials of tap water and divided into four groups. Each group was then placed into one of four temperature-controlled incubators (15, 20, 25, or 30°C). At 1930 hours, ‘Chanee’ pollen grains were pollinated onto ‘Kradumthong’ stigmas. The rate of germination of the ‘Chanee’ pollen used for this experiment was approximately 40% at 25°C. At 12, 24, and 48 hours after pollination (HAP), 3–5 pistils were collected from each treatment, then fixed with Copenhagen mixture [10% ethanol: 1 glicerol: 8 distilled water (by volume)] to preserve them until analysis. The pistils were then washed with distilled water for 3 hours and soaked in 2.5 N NaOH for 3 hours at RT. The pistils were washed again with distilled water and stained with 0.1% (w/v) aniline blue in 0.1 M K₃PO₄ overnight. The styles were removed from the ovaries on a glass slide and squashed under coverslips. Pollen tubes were observed under a microscope (Labophot, Nikon Corporation). Lengths of the style and the longest pollen tube were measured in each sample. Pollen tube elongation was evaluated as a ratio of the length of the pollen tube to the style length.

**Statistical analysis**

To clarify the influences of temperature and cultivar on pollen germination, the ratio of germinated pollen to all tested pollen for eight treatment combinations of temperature and cultivar (four temperature conditions and ‘Chanee’ compared with ‘Monthong’) were analyzed using a generalized linear mixed model with binomial error. The day of the experiment was considered as a random effect because each experiment was repeated a total of two times on different days. Then the significance of each fixed effect (i.e., temperature, cultivar, and their interaction) was determined using analysis of deviance. The ratio of germinated pollen to all tested pollen between each pair of the eight treatments were also compared using a general linear hypothesis (GLHT) function with Tukey’s method. Furthermore, to understand the influence of temperature on the elongation rate of ‘Chanee’ pollen tubes, we analyzed the ratio of the length of each pollen tube to that of the style for 12 combined temperature and time treatments after pollination using a generalized linear model with gamma error. The length of style was set as an offset.
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Effect of temperature during anther dehiscence on pollen germination

Pollen germination was significantly affected by temperature \((P < 0.001)\), cultivar \((P < 0.001)\), and their interaction \((P = 0.026, \text{Table 1})\). Temperature conditions at the initial flowering stages for 3 hours from 1600 hours significantly affected pollen germination in ‘Chanee’ (Fig. 2). The pollen germination rates were significantly higher in ‘Chanee’ at 25 and 30°C than for the other treatments. However, similarly in ‘Monthong’, the pollen germination percentage was significantly lower at 15°C than at 20°C.

Effect of temperature after hand pollination on pollen tube elongation

Pollen tube elongation was significantly influenced by temperature \((P < 0.001)\), hours after pollination \((P < 0.001)\), and their interaction \((P < 0.001, \text{Table 2})\). As for the interaction between cultivar and temperature, we observed that cultivars reacted to low temperature differently. There was no significant difference in pollen germination in ‘Chanee’ between 15°C and 20°C; however, in ‘Monthong’, the pollen germination percentage was significantly lower at 15°C than at 20°C.

Results

Table 1 Analysis results for deviance in pollen germination (type II Wald chi-square tests)

| Source          | Df | Chisq  | P     |
|-----------------|----|--------|-------|
| Temperature (T) | 3  | 191.718| <0.001|
| Cultivar (C)    | 1  | 172.329| <0.001|
| T×C             | 3  | 9.224  | 0.026 |
| Df: Degrees of freedom, Chisq: Chi-squared value, P: P-value.

Fig. 2 Effect of temperature during anther dehiscence on subsequent pollen germination in ‘Chanee’ and ‘Monthong’. Bars indicate standard error. Different letters indicate significant difference according to Tukey’s method \((P<0.0001)\).

Table 2 Analysis results for deviance in pollen tube elongation (type II tests)

| Source          | SS  | Df | F     | P     |
|-----------------|-----|----|-------|-------|
| Temperature (T) | 5.3231 | 3  | 30.057| <0.001|
| Hours after pollination (H) | 5.7208 | 2  | 48.455| <0.001|
| T×H             | 2.6364 | 6  | 7.443 | <0.001|
| SS: Sum of square, Df: Degrees of freedom, F: F-value, P: P-value.

Fig. 3 Relative growth rate of ‘Chanee’ pollen tube within pistil of ‘Kradingthong’ durian at 12, 24, and 48 hours after pollination (HAP) at each temperature. Bars indicate standard error. Different letters indicate significant differences according to Tukey’s method \((P<0.001)\).
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DISCUSSION

Effect of temperature during anther dehiscence on pollen germination

In Experiment 1, durian pollen germination decreased upon exposure to relatively low temperatures and decreased with decreasing temperatures; however, there were no significant differences between 25 and 30°C in either ‘Chanee’ or ‘Monthong’ (Fig. 2). Some studies have compared germination under different temperature conditions using fresh normal pollen. For example, Kozai et al. (2014b) suggested that the optimal temperature for durian pollen germination was 20-25°C in ‘Monthong’ durian. Similarly, the effect of temperature on pollen germination in vitro has also been reported in fruit crops such as mango (Dag et al., 2000), avocado (Acaraz et al., 2011), longan (Pham et al., 2015), and cherimoya (Matsuda et al., 2011). Findings also suggest that pollen germination is dependent on temperature conditions; however, these previous experiments were conducted using normal pollen dehisced under optimal conditions. In the present study, temperature treatment was applied to flowers before anthers dehisced. The results showed that low temperature conditions during the 3 hours of anther dehiscence only (1600 to 1900 hours) strongly affected pollen germination, suggesting that low temperature exposure during anthesis has an adverse effect on pollen germination in durian. In cherimoya flowers, pollen germination was tested using temperature treatment from the day before anthesis (Matsuda et al., 2016). As a result, pollen germination was 70% when the flowers were exposed to temperatures of ~20-22°C during the night; however, germination decreased significantly when the flowers were exposed to 14°C. They found that cherimoya pollen was filled with starch grains just 10 minutes before flower opening, but that the starch grains had disappeared in half of the flowers at anthesis. Starch is one of the most important nutrient reserves for developing pollen tubes and low temperatures can inhibit starch metabolism (Thakur et al., 2010). Although we didn’t observe starch grains of pollen in the current study, starch metabolism might be inhibited by relatively low temperatures such as 15°C.

Effect of temperature after pollination on pollen tube elongation

In the Experiment 2, pollen tube elongation decreased at 15°C even though we used normal pollen, which resulted in a germination rate of approximately 40% at 25°C following hand pollination. This result indicates that the decrease in pollen tube elongation was not the result of a decrease in pollen germination. Furthermore, pollen tube elongation did not significantly differ at 20, 25, and 30°C. Similar results were also reported in ‘Monthong’, whereby pollen tube elongation decreased at low temperatures of 10 and 15°C and a high temperature of 35°C (Kozai et al., 2014b). Taken together, these findings suggest that 15°C is the critical low temperature for durian pollen tube elongation. The critical low temperature for pollen tube elongation is 15°C for mango (Dag et al., 2000) and cherimoya (Matsuda et al., 2011), and 25°C for pitaya (Matcha et al., 2006).

Effectiveness of ‘Chanee’ pollen under low temperature regimes

The comparison of ‘Chanee’ and ‘Monthong’ pollen germination in Experiment 1 (Fig. 2) suggests that ‘Chanee’ pollen has a higher low temperature tolerance than ‘Monthong’. Furthermore, in Experiment 2, compared with our previous study using ‘Monthong’ pollen (Kozai et al., 2014b), pollen tube elongation appeared to ‘Chanee’ under low temperatures. The longest pollen tube in ‘Chanee’ reached about 84% of the pistil length by 48 HAP at 15°C (Fig. 3); however, in ‘Monthong’ the length was about 50% of the pistil length (Kozai et al., 2014b). Because 27-56% of ovules were mature-stage at 3 days after anthesis in ‘Monthong’ (Kozai et al., 2014a), it was considered that ‘Chanee’ pollen tubes have chance to meet normal ovule within the 3 days. Although the combination of pollen-pistil was different from that of the previous experiment, it is assumed that ‘Chanee’ pollen is hardly affected by lower temperature compared with ‘Monthong’ pollen.

‘Monthong’ is a predominant cultivar in Thailand and is planted most often in monoculture because ‘Monthong’ is known to have a high capacity for self-pollinating (Subhadurabandhu and Ketsa, 2001). However, the night temperature in durian production areas can sometimes drop to around 15°C. Even in the evening (1600 to 1900 hours), temperatures can drop below 20°C. Overall, our findings indicate that pollen germination at 15°C was not significantly different from that at 20°C in ‘Chanee’ in contrast to ‘Monthong’. Similarity, pollen tube elongation appeared to be less affected in ‘Chanee’ than in ‘Monthong’. Other researchers have observed improved fruit set in ‘Monthong’ under cross-pollination by ‘Chanee’ (Lo et al., 2007). They indicated that self-pollinated ‘Monthong’ set fruit only 15%, while cross-pollinated with ‘Chanee’ set about 50%. Thus, ‘Chanee’ could be a good pollinator to support fruit set in ‘Monthong’ and other durian cultivars, especially when night temperatures start to drop.

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