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Characterization of Male Reproductive Organs in Durian; Anther Dehiscence and Pollen Longevity

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We characterized the pollen germination and longevity in durian (*Durio zibethinus* Murr.) under *in vitro* conditions and observed the morphological changes during anther dehiscence. Approximately 10 anthers aggregate on a filament; these merge with others at the base to form five phalanges in the flower. Anther dehiscence occurred at 19:00, at the same time as floral anthesis. Observation with a low-vacuum scanning electron microscope revealed pollen grains clinging to the anther even after the anther had dehisced. *In vitro* pollen germination was highest at a sucrose concentration of 10%. In the test of pollen germination on Brewbaker and Kwack (BK) medium, the germination rate was highest (59.1%). The germination rate was decreased (5.0% to 46.0%) when any of the minerals of B, Ca, Mg, and K was removed. The pollen germination rate at anthesis was 40.4 to 50.6% in four cultivars ('Mon Thong', 'Chanee', 'Kradum Thong', and 'Phaung Manee'), and the rate was maintained at more than 40% until 24 h after anthesis and then decreased gradually. When pollen was stored under desiccating conditions with silica gel, pollen following 120-h storage could germinate after rehydration. Rehydration for 12 h achieved the best results, with 47.0% germination occurring after this rehydration period.

Key Words: anther dehiscence, durian, pollen germination, pollen longevity.

Introduction

The reproductive biology of durian (*Durio zibethinus* Murr.) is relatively unique. Durian trees are large, bearing numerous cauliflorous-flowers that form clusters. They set fruits even on branches at a higher position. The durian flower begins to open in the early evening and is fully open at night. Subsequently, all floral organs except the gynoecium abscise by the following morning (Honsho et al., 2004b). Because the flowers open at night, herbivorous giant bats are thought to be the responsible pollinators. If only a single cultivar is planted in the orchard, most of the young fruit drop by self-incompatibility two to four weeks after setting (Honsho et al., 2004a), giving a poor yield. The yield of durian in most Southeast Asian countries appears to be low and erratic, mainly due to inadequate fruit set (Subhadrabandhu et al., 1991). Somsri (1987) reported that cross-pollination improved both fruit shape and size. Thus, artificial cross-pollination is recommended for commercial production. However, not all durian growers conduct artificial pollination because dangerous and laborious work for pollination has to be performed during the night with a ladder to pollinate the flowers on higher branches.

The success of artificial pollination is dependent on the viability of the pollen. However, information on the longevity of pollen and optimal condition for *in vitro* germination are not readily available for durian. Although flowers are hermaphroditic, self-pollination is not usually possible; this is ensured by the spatial separation between the anther and stigma owing to the difference in the lengths of the style and filament. Durian is not anemophilous (Valmayor et al., 1965); pollen should be transferred by some pollinator for successful pollination. However, if pollen longevity is short, it

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would reduce the opportunity for a pollinator to visit the nocturnal flower, even though bats are a possible pollinator. Thus, pollen longevity would be a limiting factor for durian productivity as well as flower receptivity. Further, the morphological structure of the anther and the time of anther dehiscence may be associated with the characteristics of durian pollination. Thus, information on the morphological features of anther dehiscence and pollen longevity is required for successful artificial pollination. In addition, a method to extend pollen longevity is required for pollination at an appropriate time as well as the transportation of pollen between orchards.

In four commercially important Thai durian cultivars, we first observed anther dehiscence using stereo-microscopy and low-vacuum scanning electron microscopy (SEM), then determined the optimal concentrations of sugar and requirement of minerals for germination *in vitro*. In addition, we investigated the pollen longevity, and the effects of desiccation on increasing the longevity.

Materials and Methods

Durian trees, planted in a durian collection orchard at the Chantaburi Horticultural Research Center in Thailand, were used in this study.

1. Observation of anther dehiscence by stereo-microscopy and low-vacuum SEM

Anthers of 'Chanee', one of the most important durian cultivars in Thailand, were collected every six hours from 7:00 on the day before anthesis (36 h before anthesis) to 1:00 on the day after anthesis (6 h after anthesis). After collection, the anthers were immediately fixed in formalin-acetic acid-alcohol (FAA). Their entire structure was observed with a stereo-microscope. Additionally, a low-vacuum scanning electron microscope (S-3000N, Hitachi Co., Ltd., Japan) was used to observe the morphological features of the anther.

2. Pollen germination in vitro

Dehisced anthers containing pollen were collected at 19:00 from open flowers. Due to the difficulty in isolating pollen from the anthers, the pollen and anthers were kept together on weighing paper. The pollen was separated from the anthers with a brush, and the grains that adhered to the brush were placed on the germination media. The media were placed in a petri dish, in which a few drops of water were loaded to prevent them from drying, and they were incubated at room temperature (≈27°C) for at least 12 h. After incubation, the germinated pollen grains were counted with a hand-held tally counter under a light microscope, and the germination rate was calculated. When the length of the pollen tube was greater than the diameter of the pollen, the pollen was regarded as germinated. Three replications were performed for experiments 2.2, and four replications for experiment 2.1 and 2.3. More than 300 pollen grains were recorded in each replication. When ANOVA was performed, the percentages of pollen germination were subjected to arcsine transformation before analysis. Mean separation was performed by Tukey's test.

2.1. The optimal concentrations of sucrose and minerals for germination

'Mon Thong' pollen was used in this experiment. For the germination test, BK medium consisting of 100 mg·L^{-1} H_3BO_4, 200 mg·L^{-1} $MgSO_4 \cdot 7H_2O$, 300 mg·L^{-1} $Ca(NO_3)_2 \cdot 4H_2O$, and 100 mg·L^{-1} KNO_3 (Brewbaker and Kwack, 1963) was used. The medium was solidified with 1% (w/v) agar. Four sucrose concentrations of 0, 10, 20, and 30% (w/v) were examined to find out the optimal sucrose concentration for pollen germination.

To determine the inorganic elements required for pollen germination, six kinds of medium were designed. In addition to the BK medium described above, BKLB, BKLMg, BKLCa, BKLK, and BKLA media were prepared. The first four media lacked H_3BO_4, $MgSO_4 \cdot 7H_2O$, $Ca(NO_3)_2 \cdot 4H_2O$, and KNO_3, respectively; BKLA eliminated all of these elements. All germination media contained 10% sucrose and were solidified with 1% agar.

2.2. Pollen longevity

Anthers were collected from 'Mon Thong', 'Chanee', 'Kradum Thong', and 'Phaung Manee' and kept on paper at room temperature. Pollen grains of each cultivar were sown on the BK media with 10% sucrose, which was the optimal condition determined in experiment 2.1, at 0, 6, 12, 24, 48, 72, or 120 h after collection. Two-way ANOVA was performed for each cultivar and time after collection in this experiment.

2.3. Desiccation of pollen in storage

'Chanee' anthers were dried with approximately 100 g of silica gel in 1 L closed columnar-containers for 120 h. Each container had anthers from 3–5 flowers. After a 120-h incubation, the anthers and pollen grains were rehydrated under normal room conditions (relative humidity above 70% and temperature of 27°C) for 0, 3, 6, 12, and 24 h. Subsequently, the germinating ability of pollens was evaluated on the BK medium containing 10% sucrose.

Results and Discussion

The anthers and filaments of durian are shown in Figure 1. Each filament has multiple reniform anthers (10.3 ± 1.0; mean ± SE, n = 10). Each stamen is polyadelphous with several filaments are joined at the base forming a phalanx (Fig. 1A). Each flower has five phalanges, which originate from five staminal primordia (Honsho et al., 2004b). The periphery of an anther, which looks like a labrum, contains the pollen sac (Fig. 1B). The sample collected at 7:00 on the day before anthesis had pollen grains in the pollen sac (data not shown). The anthers had not dehisced at 13:00 (Fig. 1C, D) but

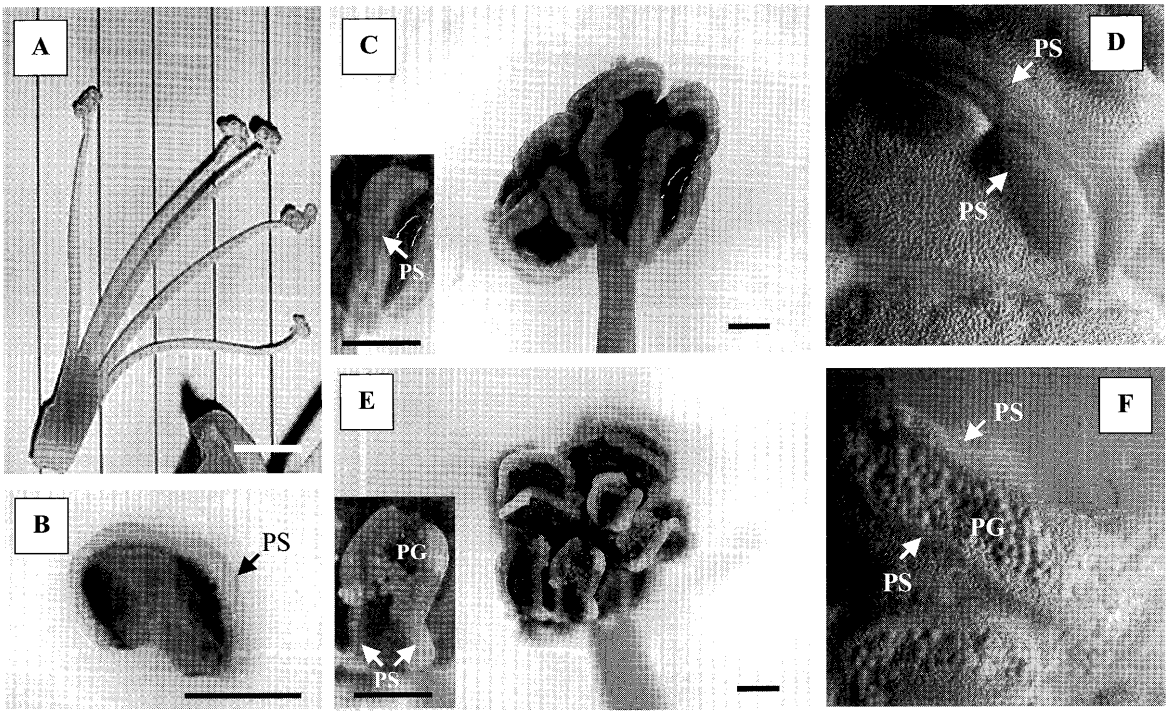

Fig. 1. Photographs of the phalanx (A) and anther (B–F). Photograph B shows a section of the anther, the periphery of which is the pollen sac (PS). Photographs C and D and photographs E and F show the anthers at 13:00 (6 h before anthesis) and 19:00 (at anthesis), respectively. The anthers shown in C and D did not dehisce, while those shown in E and F dehisced longitudinally. The lower left corners in photographs C and E show enlarged images of sections of the undehisced and dehisced anthers. In photograph E, the pollen grains (PG) appear at the longitudinal slip. Photographs D and F are low-vacuum SEM images of the anther. The pollen sac (PS) and pollen grains (PG) are indicated. The bar in photograph A represents 1 cm; those in the rest, 1 mm.

had dehisced longitudinally at 19:00 (Fig. 1E, F). Anthesis of the durian flower occurs at 19:00 (Honsho et al., 2004b); therefore, anthesis and anther dehiscence occur simultaneously. Low-vacuum SEM revealed that the pollen grains (approximately 50 μm in diameter) were still held in the pollen sac from anthesis (Fig. 1F) to 1:00 (6 h after anthesis; data not shown), indicating that the pollen was not released at dehiscence. The size of pollen was nearly equal to that of avocado (30–50 μm, Inoue et al., 1992), mango (25–45 μm, Davenport and Núñez-Elisea, 1997), and cherimoya (50 μm, Yonemoto et al., 1999). In addition, the grains adhered to the pollen sac due to their stickiness and clump formation. Valmayor et al. (1965) stated that durian flowers are not anemophilous and require pollinators under natural conditions. Our results support this report since sticky pollen grains are characteristic of zoophily, which includes entomophily and chiropterophily.

The optimal sucrose concentration for germination in 'Mon Thong' was 10%, at which a pollen germination rate of 59.1% was observed (Table 1). At the sucrose concentrations of 0%, 20%, and 30%, the pollen germination rate was 7.1%, 19.5%, and 0.3%, respectively; each ratio was significantly lower than that of 10%. An extremely high sugar concentration has been reported to impair pollen germination in many kinds of plants. In durian, the D2 clone in Malaysia germinated optimally with 20% sucrose (Bala, 1996); this variation is probably caused by the difference of the clone and minerals used. In other tropical fruit crops, a concentration of 10% was best in cherimoya as well (Rosell et al., 1999), while 30% was optimal for dragon fruit (Macha et al., 2006). The reason for the 10% sucrose medium being optimal might be that sucrose plays an osmoregulatory as well as nutritive role (Iwanami, 1980; Rosell et al., 1999).

The pollen germination rate was nearly 60% in the BK medium, but removing some or all elements from the medium decreased the rate (Table 2). Pollen germination was the lowest (5.0%) in the BKLK medium. The germination rate was moderately decreased to 46.0% in the BKLMg medium and 34.8% in the BKLCa medium. In the BKLB medium, this rate was

Table 1. Pollen germination on BK media at different sucrose concentrations.

Sucrose concentration (%)	Pollen germination (%)
0	7.1±1.5 b[z]
10	59.1±4.9 a
20	19.5±5.5 b
30	0.3±0.2 c

[z] Means with different letters within a column are significantly different by Tukey's test at $P<0.05$ (n=4).

Table 2. Pollen germination on BK media without one or all minerals.

Medium	Pollen germination (%)
BK[z]	59.1±4.9 a[y]
BKLB	16.6±1.0 c
BKLCa	34.8±1.8 b
BKLMg	46.0±3.2 b
BKLK	5.0±0.5 d
BKLA	7.5±1.3 d

[z] Components of each medium are described in the Materials and Methods.
[y] Means with different letters within a column are significantly different by Tukey's test at $P<0.05$ (n=4).

relatively low (16.6%) but was higher than that in the BKLA and BKLK media. Thus, potassium was the most important nutrient for pollen germination among elements. Potassium is necessary for germination and pollen tube growth (Brewbaker and Kwack, 1963; Feijó et al., 1995; Weisenseel and Jaffe, 1976).

Pollen germination at anthesis was 40.2% to 52.9% in the four cultivars (Table 3). At 24 h from anthesis, the germination ability decreased but was still maintained at over 40%. Although two-way ANOVA demonstrated significant differences in cultivar and time, there were no significant differences by Tukey's test among the four cultivars at each time except at 72 h from anthesis. At 72 h from anthesis, pollen germination in 'Kradum Thong' was significantly lower than that in the others due to a rapid loss of the germination ability. Pollen germination in all cultivars approached approximately zero at 120 h from anthesis. As a result of two-way ANOVA, there was a significant difference between 24 and 36 h from anthesis, while no significant difference was found between 0, 12, and 24 h from anthesis (data not shown). Thus, the pollen could be effective for pollination at 24 h after anthesis in terms of pollen germination. However, practical pollination tests are required to obtain the precise answer for how long the pollen is effective for pollination. The pollen grains retained their germination ability until at least 24 h from anthesis. Since the next anthesis occurs and new fresh pollen is released 24 h later, a continuous supply of germinable pollen can be possible. The androecium of durian generally abscises and drops within 12 h of the flower opening (Honsho et al., 2004b). However, our result showed that the pollen grains in the anthers are viable for at least another 12 h, indicating that they can still contribute to pollination in the daylight hours if they are successfully transferred to the stigma by natural pollinators. Cross-pollination gave a higher fruit set and better fruit quality in durian compared with self-pollination (Honsho et al., 2004a; Somsri, 1987). However, because the durian flower opens at night, artificial pollination is so far performed after sunset. The result of this study indicated that pollen is viable for at least 24 h. The stigma of durian becomes receptive before nocturnal flower opening while this receptiveness is rapidly lost by the next morning of flowering (Honsho et al., unpublished data). Thus, it is possible to perform artificial pollination before dark to avoid danger in conducting cross-pollination at night.

Desiccation is effective for the extension of pollen viability (Table 4). However, the pollen grains required rehydration to recover their germination ability. The direct transfer of dried pollen on the media resulted in no germination (0%), while rehydration for 12 h resulted in 47% pollen germination. The necessity of rehydration for the restoration of the germinability of the dry-stored pollen grains in durian is consistent with the results obtained by Akihama and Omura (1986); for the dried pollen of both peach and pear stored at room temperature (20°C), the effective period for restoration was found to be 6 to 12 h under an relative humidity of 100%, which corresponds to that observed in the present study. The desiccation method makes it possible to carry out cross-pollination in an orchard where only a single cultivar is grown by transporting the pollen of different cultivars from another orchard.

In conclusion, we have elucidated the characteristics of the male organ in durian in terms of pollen release and pollen longevity. Although anther dehiscence and floral anthesis occurred simultaneously, pollen was held

Table 3. Pollen germination (%) of four durian cultivars on BK medium at different times after collection.

Cultivar	Time after collection (h)						
	0	12	24	36	48	72	120
Mon Thong	52.9±5.1 a[z]	52.3±4.7 a	42.8±1.9 a	37.7±3.4 a	35.8± 8.0 a	13.1±0.6 a	2.3±1.8 a
Chanee	56.0±2.0 a	55.5±0.4 a	44.4±3.4 a	33.7±2.8 a	32.4±10.1 a	14.7±1.7 a	1.8±1.4 a
Kradum Thong	48.2±2.7 a	44.1±6.2 a	40.3±4.7 a	25.4±7.4 a	21.6± 6.2 a	5.6±0.8 b	0.9±0.3 a
Phaung Manee	40.2±4.4 a	49.3±1.7 a	40.1±4.8 a	32.0±2.0 a	32.4± 1.2 a	15.7±0.3 a	0.9±0.6 a
Significance							
Cultivar				**			
Time				**			
Cultivar × Time				NS			

[z] Means with different letters within a column are significantly different among cultivars at each time by Tukey's test at $P<0.05$ (n=3).
** and NS indicate significance at $P<0.01$ and nonsignificance, respectively.

Table 4. Effect of the time for rehydration on the recovery of pollen germination after 120 h of storage with silica gel.

Time for rehydration after 120-h storage (h)	Pollen germination (%)
0	0 d[z]
3	35.6±2.9 b
6	37.4±3.2 b
12	47.0±1.7 a
24	23.6±0.5 c

[z] Means with different letters within a column are significantly different by Tukey's test at $P<0.05$ (n=4).

in the anther sac after dehiscence. The *in vitro* study on pollen germination in 'Mon Thong' revealed that the optimal concentration of sucrose was 10% and potassium was the most important element for germination. The pollen grains retained their germination ability for 24 h or more, and for at least 5 days under desiccation. These results suggest a new practical method for artificial cross-pollination, such as the transportation of pollen from remote orchards and pollination in daylight hours.

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Literature Cited

Akihama, T. and M. Omura. 1986. Preservation of fruit tree pollen. p. 101–112. In: Y. P. S. Bajaj (ed.). Biotechnology in agriculture and forestry. vol. 1: Trees I. Springer-Verlag, Berlin.

Bala, J. I. 1996. Practical and efficient techniques of pollen delivery for pollination in selected fruit tree crops. p. 59–64. In: S. Vijaysegaran, M. Pauziah, M. S. Mohamed and S. Ahmad Tarmizi (eds.). Proceedings of the international conference on tropical fruits Volume II.

Brewbaker, J. L. and B. H. Kwack. 1963. The essential role of calcium ion in pollen germination and pollen tube growth. Amer. J. Bot. 50: 859–865.

Davenport, T. L. and R. Núñez-Elisea. 1997. Reproductive physiology. p. 69–146. In: R. E. Litz (ed.). The mango: botany, production, and uses. CABI publishing, NewYork.

Feijó, J. A., R. Malhó and G. Obermeyer. 1995. Ion dynamics and its possible role during in vitro pollen germination and tube growth. Protoplasma 187: 155–167.

Honsho, C., K. Yonemori, S. Somsri, S. Subadrabandhu and A. Sugiura. 2004a. Marked improvement of fruit set in Thai durian by artificial cross-pollination. Sci. Hort. 101: 399–406.

Honsho, C., K. Yonemori, A. Sugiura, S. Somsri and S. Subhadrabandhu. 2004b. Durian floral differentiation and flowering habit. J. Amer. Soc. Hort. Sci. 129: 42–45.

Inoue, H., B. Takahashi and K. Shirato. 1992. Morphology and fertility of pollen of avocado (*Persea americana* Mill.). Japan. J. Trop. Agr. 36: 45–50 (In Japanese with English summary).

Iwanami, Y. 1980. Kafungaku (In Japanese). Kodansha, Tokyo.

Macha, M. M., A. K. Chowdhury, T. Murata and Y. Yonemoto. 2006. Effect of artificial media, temperature conditions and storage methods on in vitro germination of dragon fruit (*Hylocereus undatus* Britt & Rose) pollen. Japan. J. Trop. Agr. 50: 51–56.

Rosell, P., M. Herrero and V. G. Saúco. 1999. Pollen germination of cherimoya (*Annona cherimola* Mill.). In vivo characterization and optimization of in vitro germination. Sci. Hort. 81: 251–265.

Somsri, S. 1987. Studies on hand pollination of durian (*Durio zibethinus* L.) cvs. Cha-nee and Kanyao by certain pollinizers. (in Thai with English summary). MS Thesis. Kasetsart Univ., Bangkok.

Subhadrabandhu, S., J. M. P. Schneemann and E. W. M. Verheij. 1991. *Durio zibethinus* Murray. p. 157–161. In: E. W. M. Verheij and R. E. Coronel (eds.). PROSEA: Plant resources of south-east Asia No. 2. Edible fruits and nuts. Pudoc, Wageningen.

Valmayor, R. V., R. E. Coronel and D. A. Ramirez. 1965. Studies on the floral biology, fruit set and fruit development in durian. Philippine Agriculturist 48: 355–366.

Weisenseel, M. H. and L. F. Jaffe. 1976. The major growth current through lily pollen tubes enters as K^+ and leaves as H^+. Planta 133: 1–7.

Yonemoto, Y., H. Higuchi, T. Nakanishi and E. Tomita. 1999. Conditions of artificial media for pollen germination and tube growth of cherimoya (*Annona cherimola* Mill.). Japan. J. Trop. Agr. 43: 260–264 (In Japanese with English summary).