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Short Communication

Evaluation on Durian var. *Musang King* Pollination Compatibility Regarding High Fruit Set

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ABSTRACT

Durian or *Durio zibethinus* of variety *Musang King* is growing in popularity and with high international demands. With the ever-increasing demands for fruits, growers are exploring ways to maximize production by looking at the feasibility of planting single or mono varieties in a planting area. Previous investigations revealed that many durian varieties are self-incompatible, and the condition varies from one variety to another. Against this background, the present study evaluated *Musang King*'s compatibility status in fruit sets. The study was conducted in Raub, Pahang, from 2017 through 2018 with five different pollination treatments. Crossing *Musang King* with D24 showed the highest fruit set rate of 16.28% at harvest and suggested this variety is self-incompatible. Observations on the flowering process revealed that *Musang King* possessed herkogamy condition, which posed a morphological barrier to self-pollination. The study proposes that *Musang King* is best planted in a multi-variety planting system instead of mono-variety to achieve a higher rate of fruit sets.

Keywords: Autogamy, herkogamy, *Musang King* variety, self-incompatibility, xenogamy

INTRODUCTION

In recent years, durian or *Durio zibethinus*, whose tree looks regal and majestic befitting its royal title as 'King of Fruits,' has become one of the most popular fruits for export by many Southeast Asian countries like Malaysia. In 2020 alone, Malaysia exported about 30,000 tonnes of the fruits valued at about RM74.1 million and has been expected to increase in the coming years leading to the

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establishment of more durian orchards with *Musang King* as the leading variety (Ahmad & Pfordten, 2021). Conventional planting of durian is by having a few varieties in a planting area with the main objective of getting high production capacity (Abidin et al.,1991). However, questions have arisen among growers about its viability in a monoculture cropping system. Thus, planting several varieties in a planting area has been recommended. It is being supported based on the occurrence of self-incompatibility among durian varieties. The ability to set fruits is associated with pollination, defined as the transfer of pollen (male gametophytes) to stigmas of female parts, which occurs in the same flower as a complete flower or another flower (Abrol, 2015). However, pollination is not always successful. It is due to the stigmas' ability to detect the genetic compatibility of the pollens, which dictates the eventual fertilization. Successful pollination leading to fertilization is indicated by fruit set. For that to happen, Sanzol and Herrero (2001) cited that an adequate quantity of pollen must be transferred to stigmas and consequent growth of pollen tubes takes place.

Variations in self-incompatibility (SI) within plants' families occur typically due to only one or few genes which control SI, which segregates self-incompatibility within the families for alleles at the gene(s) level. Lipow and Wyatt (1999) put forward that the pattern of inter-compatibility depends on the particular genetic system involved, which differs from inbreeding depression which is generally caused by many loci with no segregation. Assessing the plant's ability in terms of SI is important to understand the significant changes that occurred in the self-pollination avoidance system in angiosperms (Navarro et al., 2012). Lim and Luders (1997) published variabilities in the magnitude of SI among durian varieties studied, and in a separate report (Lim & Luders, 1998) stated that SI is cultivar dependent. Studies on Thai durian by Honsho et al. (2009) noted the existence of SI, although in earlier pollination studies. Honsho et al. (2004) stated that, in self-pollination tests, all self-pollinated durian showed low percentages of fruit sets except for variety *Kradum Thong* in which self-pollination exceeded the success rate recorded in cross-pollinated variety, *Phaung Manee*. Against this background, the variety *Musang King* held the potential of producing higher yield if self-pollination is enhanced, which could assist growers in deciding the planting system to be employed to maximize production.

The present study examined flower morphology in its contribution to the pollination habits of *Musang King*. The study aimed at investigating pollination compatibility of *Musang King* variety with respect to higher fruit settings by utilizing different pollen transfer procedures as treatments.

MATERIALS AND METHODS

Location

This study was conducted at Lembah Temir Resort, Lembah Klau, Raub Pahang (3.7182° N, 102.0347° E) from January until December 2017 until 2018. The study location was at Raub, a popular durian town with its extensive cultivation of durian, especially the variety, *Musang King*. An orchard with two major varieties, *Musang King* and D24, bearing an age exceeding 20 years, was selected. The orchard had a history of being well-maintained with good farm management practices in fertilization, irrigation, and pest control carried throughout the cultivation of the crop.

Plant Materials

At the commencement of the study, flowers were tagged *in situ* from the bud initiation stage. Flower blooming timelines were recorded (Figure 1) to establish when the flowers were fully open and to identify the gaps in time between the full-bloom state and the beginning of anthers' dehiscence. The observation could assist in understanding pollination ecology and determining the most suitable time to initiate pollination treatments. Prior to treatments, pollens were sourced from freshly dehisced flowers from the varieties grown within the experimental location. *Musang King* was the maternal flower, while the paternal or pollen donors were from *Musang King* for self-pollination and variety D24 for cross-pollination treatments depending on the fresh availability of pollens in the orchard. Only D24 was used in this study as it is the only other variety accessible and reported available with flowers bearing in the Lembah Temir Resort besides *Musang King*. The timing of flowering was simultaneous with *Musang King* flowering period. Therefore, only available varieties in the same location were selected to preserve the freshness and viability of the pollen used in this study. Flower clusters were thinned out to make 10–12 cm gaps between clusters to reduce flower density and competition.

Pollination Compatibility Test

For the compatibility test, each flower cluster was treated as one replication. According to the pollen sources, five pollination treatments were used to pollinate the maternal flowers (*Musang King*). Treatments consisted of the following:

i) Self-pollination treatment with pollens of *Musang King* from the same tree (PST)
ii) Cross-pollination treatment within variety where pollens of *Musang King* sourced from different trees were used (PDT)
iii) Autonomous autogamy pollination treatment where *Musang King* flowers were left untouched, no thinning and no emasculation but covered with plastic bags (autogamy)
iv) Pollination treatment with D24 pollens (xenogamy), and
v) Open-pollination treatment where flower clusters were tagged without alteration or modification (control).

Flower clusters in all treatments, except open pollination, were wrapped in plastic bags for seven days before and after anthesis (DAA) to eliminate contamination and visitation by other visitors. All anthers of flowers on treatment plants were emasculated at noon before the flowers were fully open, and all flower clusters of treatment plants were thinned out, leaving only seven to 12 flowers per cluster, except for autonomous autogamy and open-pollination treatments. All flowers for PST, PDT, and xenogamy treatments were pollinated by hand pollination or assisted pollination. Flowers were pollinated with freshly dehisced pollens collected late evening and re-wrapped with plastic bags after treatments. All parts of the stigmas were fully covered with fresh pollens to ensure sufficient pollens were applied to stigmas. Each flower cluster used in this experiment was considered a replicate. Pollination treatments were performed on 12 flower clusters of *Musang King* for each pollination treatment (n = 60).

Pollen Tube Observation

In the procedure, ovaries of treated flowers were cut-off from pistil samples, and the outer layers of the ovaries were excised to expose the ovules. Samples were collected three days after anthesis and stored in a formaldehyde alcohol acetic acid (FAA) fixative. Subsequently, the samples were softened using 8M sodium hydroxide (NaOH)for 14 days in a 100 ml glass bottle. Next, the samples were clean-off from NaOH solution with distilled water before staining with aniline blue in 0.1M potassium phosphate (K_3PO_4) adjusted to an acidic pH 5. Overnight staining was allowed in the dark before placing the samples on microscope slides with drops of glycerol on the slides before covering the samples for observation. The samples were observed under fluorescence microscope Leica DFC310 FX (Germany) with excitation of 360 nm Filter 1. Procedures were modified from Kozai et al. (2014) and Bumrungsri et al. (2009) to suit this experiment.

Data Collection

Honsho et al. (2004) stated that many young fruits dropped two to eight weeks after pollination, and their data showed stability in fruit set (%) at eight days after pollination treatments, and before that showed the same pattern of decreased number of fruit set for all their pollination treatments. On the other hand, Kozai et al. (2014) study stated that the frequencies of deformed ovules among the treatments between three days and seven days do not significantly differ. In addition, according to Bumrungsri et al. (2009), the majority of the fruit set abortions happen within 20 days after pollination experiments, and it decreased after that period. Thus, the data collections began on the seventh day after the pollination date and continued at the 14th, 21st, 28th, and at harvest was suitable to portray the fruit set (%) pattern during the overall period from pollination to harvest.

Statistical Analysis

Pollination treatments on the fruit set were calculated as a percentage per cluster for each replication. Fruit sets were recorded on the 7th, 14th, 21st, and 28th days after anthesis (DAA) and harvest day. The collected data were subjected for normality test using diagnostic regression plot in SAS (version 9.4), and from a fit diagnostic graph, residual of data collected is normally distributed. In addition, data of fruit set (%) recorded were subjected to analysis of variance (ANOVA), and comparison of means was subjected to Tukey's range test.

RESULTS AND DISCUSSION

Flower Blooming and Anther Dehiscence

Figure 1 presents flowering timelines in the durian variety *Musang King*. The study observed that the epicalyx of a flower bud started to break a day before the flowers bloomed. The blooming of *Musang King* flowers could be seen as protrusions of flower buds in the morning and proceeded by an elongation of the corolla before the flowers started to open in late the afternoon. Blooming progressed until the petals were fully retracted, touching the calyx in the evening at around 6.30 p.m. and exposing the stigmas and stamens.

In anther dehiscence, pollens were observed to consistently release pollens only around 7.30 p.m. when the sun had already set. The release of pollens started with the break of stomium. At the beginning of the release, pollens were observed to be dry and subsequently seen to become wet after an hour. Salakpetch et al. (1991) recorded that the round-shape durian pollen grains appeared sticky and released in clumps. Sanchez et al. (2004) reported that this sticky condition of the pollen combined with stigma exudate, which contained both proteins and sugars, helped in the adhesion of pollens. Due to this stickiness of the pollens, pollen transfers were possible, without which, and without the help of a pollinator, were reported to be impossible (Bumrungsri et al., 2009). Shivanna and Tandon (2014), in their studies, reported that there were time gaps of about three hours and 30 minutes between the time when the stigmas started to be exposed (which was the time when the flower buds started to open at 4 p.m.) and time when anthers released the pollens (7.30 p.m.) making a condition known as protogyny (where stigmas became receptive before the pollens started to function).

Pollination Compatibility

The percentages of fruit sets after anthesis and pollination treatments and after harvest are presented in Table 1. Treatment with pollens from different *Musang King* trees (PDT) recorded a higher rate of fruit set

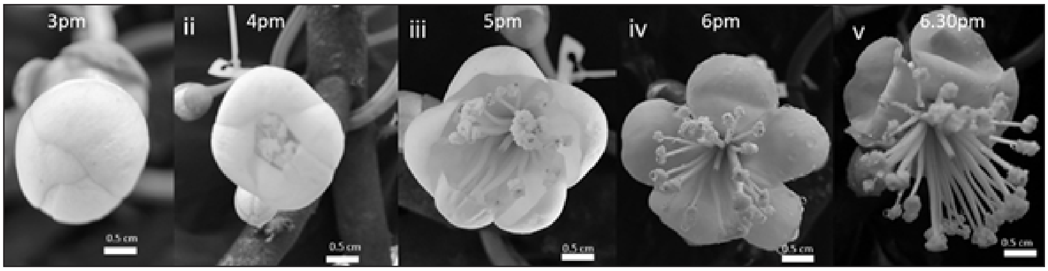

Figure 1. Flowering Timeline in *Musang King* durian

Table 1
Percentages of fruit sets at days after anthesis

Treatment	Days after anthesis (DAA)				
	7th	14th	21st	28th	Harvest
Control	22.36[ab*]	3.49[b]	3.49[b]	2.05[b]	0.87[b]
PDT	37.15[a]	0[b]	0[b]	0[b]	0[b]
PST	9.27[b]	0.85[b]	0[b]	0[b]	0[b]
Xenogamy	20.79[ab]	16.28[a]	16.28[a]	16.28[a]	16.28[a]
Autogamy	5.2[b]	0[b]	0[b]	0[b]	0[b]

Note. *Means with the same letter vertically are not significantly different at $P \leq 0.05$ using the Tukey test.
DAA: Days after anthesis; PDT: Pollination from different trees; PST: Pollination from the same tree

(31.15%) compared to pollination with pollen from the same tree (PST) recorded at 9.27%. Autonomous autogamy pollination showed a significantly low fruit set at 5.2%, whereas control or open pollination yielded 22.36%, and xenogamy resulted in a 20.79% fruit set. Fruit set for all treatments continuously dropped except for xenogamy in which fruit set stable started from 14 days after pollination or day after anthesis (DAA) and consistently maintained at 16.28% until harvest time. Open pollination (control) recorded 0.87% fruit set at harvest. The data suggest that on day 14th after anthesis, the fruit sets were stable and could be used as an indicator in predicting fruit production if the appropriate pollination procedure was carried out. The significant difference in fruit sets for control (open pollination) and xenogamy (*Musang King* crossed with D24) gave an insight into the importance of not only pollen load and availability and pollens' compatibility to yield high fruit sets. In similar studies on durian, Bumrungsri et al. (2009), Honsho et al. (2004, 2007) reported that the percentages of fruit sets were generally the lowest for open pollination, followed by self-pollination, while assisted pollination was recorded higher fruit sets.

The control (open pollination) recorded a significantly higher percentage of fruit sets. Similar responses were recorded with xenogamy on the 7th day after anthesis, but the percentage was significantly lower on the 14th day as it dropped to 3.49%. In autogamy, pollination had resulted in a significantly low percentage of fruit set on the seventh day after anthesis. No fruit set was recorded on the 14th day after anthesis. The significantly low fruit set rate in treatment by autogamy could be due to absence or very low pollen load. Wilcock and Neiland (2002) reported that the number of pollens transferred during assisted pollination had significant effects on pollination success as insufficient pollens quantity caused a low number of ovules being fertilized and resulted in low fruit sets. In the present study, assisted cross-pollination of *Musang King* and D24 yielded confirmed high fruit sets starting on the 14th day after anthesis compared to other pollination treatments suggesting that assisted cross-pollination had a higher rate for fruit set in comparison with assisted self-pollination treatment.

Wilcock and Neiland (2002) cited that one of the reasons for pollination failure in plants was insufficient pollens, which resulted in a low number of ovules compared to the total number of ovules being fertilized, thus negatively impacting stimulation for fruits to set. Data on treatments by PDT and PST, which yielded 0% of fruit set on the 21st day after anthesis, proved no difference in reaction on compatibility when *Musang King* was pollinated within the variety. Kozai et al. (2014) studied ovule development in cross-pollinated and self-pollinated Thai durian cultivars and recorded that all non-pollinated flowers under the study had all ovules degenerated. About 82% degenerated ten days after anthesis (DAA), and on 14 days after anthesis (DAA), there were still 5% fruit sets suggesting that although there was no pollination that took place, the ovaries could set fruiting and remain on the tree for a period after anthesis. In the present study on *Musang King*, fruit setting in autogamy treatment on the 7th day after anthesis could be caused by the apomixis development but later by abortion significantly on the 14th day after anthesis. A similar phenomenon occurred in self-pollinated (PST) and cross-pollinated same variety (PDT) pollination treatments. The pistil from these treatments remained on the branches and dropped 14th day after anthesis or hand-pollination. The pollination compatibility test on *Musang King* confirmed self-incompatibility syndrome on the 21st day after pollination. There was 0% fruit set in self-pollinated (PST and PDT) treatments.

Results of cross-pollination between *Musang King* and D24 in xenogamy treatments agreed with previous studies of Bumrungsri et al. (2009), Honsho et al. (2004, 2007), who reported high fruit sets from cross-pollination of different varieties of durian. The ability to yield higher fruit sets in cross-pollination instead of self-pollination was caused by self-incompatibility (Honsho et al., 2004). Self-incompatibility in the Bombacaceae family in which *Durio zibethinus* belongs, have been discussed in several species such as *Eriotheca gracilipes*, *Ceiba petandra*, and *Theobroma cocoa*, many of which have self-incompatibility issues and have high fruit sets when cross-pollinated (Ford & Wilkinson, 2012; Gribel et al., 1999; Oliveira et al., 1992). The possibility of self-incompatibility to cluster within family and close families was discussed by Gibbs and Bianchi (1999), where the heredity of a single locus established by the SI mechanism could have been passed down within the family. From flower blooming stages as presented in Figure 1, the *Musang King*'s flowers at full bloom have their stigmas and anthers in spatial separation. It was observed that the flowers have protogyny conditions as the stigmas were exposed earlier than the anthers. The spatial separation between the anthers and stigmas showed that *Musang King*'s flowers have herkogamy conditions. Previous studies by Lim and Luders (1997) cited that at anthesis, the stamens and stigmas had the same height but did not elaborate the conditions to the effect on self-pollination ability. Webb and Llyod (1986) reported that many self-incompatible plants possessed herkogamy conditions which could be the reason for failed self-pollination. Luijten et al. (1999) discussed herkogamy conditions and suggested reducing risk using pollen from anthers of the same flower.

Reduction of self-fertilization had been reported for species *Gentianella germanica* and *Narcissus cyclamineus* (Luijten et al., 1999; Navarro et al., 2012) caused by herkogamy. In a study on *Habranthus gracilifolius*, Streher et al. (2018) reported that herkogamy was a barrier to self-pollination and self-incompatibility. They concluded that both herkogamy and self-incompatibility were a pre-and post-barrier of self-pollination and self-incompatibility. In a study on durian variety *Mon Thong*, Honsho et al. (2004) mentioned heterostyly, a reciprocal herkogamy where distyly or tristyly exist in a population (Jesson, 2017); however, approached herkogamy condition was consistently observed on all flowers of durian variety *Musang King* with a height of stigma exceeds the height of the anthers with spatial separation. Distyly or tristyly conditions were not observed from samples of *Musang King* flowers. Webb & Llyod (1986) had classified different types of herkogamy with different families classified under it, which means the herkogamy condition could be fixed as a morphological trait within the family. Despite the failure to retain fruit set after 14th-day anthesis (DAA) as seen in Table 1, pollens were successfully grown into the micropyles as seen in Figure 2 for PDT pollination treatment. It indicates that *Musang King* could grow the pollen tube, and the termination happens in the ovule as in late acting self-incompatibility.

The success of pollen tubes of self-pollinated to grow in incompatible ovules suggests gametophytic self-incompatibility (Golz et al.,1995; Takayama & Isogai, 2005). Another plant species that exhibited incompatibility through tests of cross and self pollinations was *Lycium cestroides*. In pollination treatments of self-cross, geitonomous, autogamous, autonomous, and control treatment, Aguilar and Bernadello (2001) recorded that only cross-and open-pollination yielded fruits. On the other hand, self-and geitonomous hand-pollination and autonomous self-pollination were observed to have successful growth of pollen tubes in the ovules. Therefore, the authors concluded that the plant species had ovarian self-incompatibility or late-acting self-incompatibility conditions (Aguilar & Bernadello, 2001).

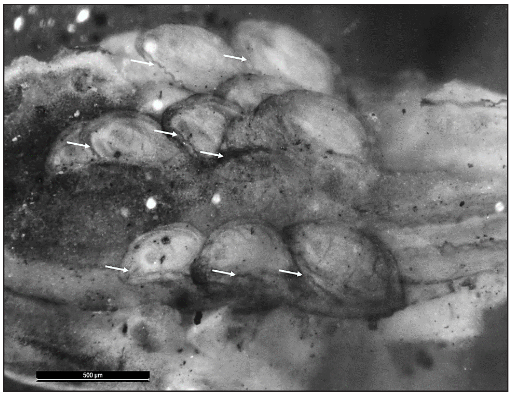

Figure 2. The pollen tubes grow in micropyles in PDT treatment with arrows pointing to the pollen tubes. ×100 scale bar = 500µm

Literature has it that the self-incompatibility system is divided into three types: Solanaceae, Papaveraceae, and Brassicaceae systems. The Solanaceae system acts by blocking growth incompatible pollen tubes growth in the pistil by the reaction of multi-allelic RNase. In contrast, the Papaveracea system acts by building calcium fluxes, actin rearrangements, and occurrence of cell death once the incompatible pollens were detected as a reaction from complex multicellular responses. The activation of the receptor kinase signaling pathway in the pistil to reject pollen is how the Brassicaceae system works (Silva & Goring, 2001). In the case of *Musang King*, it was not feasible to differentiate if the self-incompatibility system was one of the categories of self-incompatibility as the present study observed the ability of pollen tubes to grow in the micropyle of PDT and PST treatments. Furthermore, Kozai et al. (2014) recorded the occurrence of abortion after fruit set. Further investigation on the type of self-incompatibility system in *Musang King* would be useful for breeding purposes in the future.

CONCLUSION

Failure of autogamy in the present study suggests that *Musang King* was unable to set fruits by apomixis without the help of a pollinator agent. Failures in PST and PDT treatments suggest that *Musang King* could not produce yield by its pollens. Ruling out of autogamy and geitonogamy, the only option left in the breeding system for high fruit set and high fruit production at harvest in *Musang King* was xenogamy compared to open pollination. Herkogamy, which exists in the flower morphology of *Musang King,* explains the reduced potential for self-pollination, as well as an important morphological marker to analyze the plant's ability to self-pollinate. An extensive study on flower morphology of other durian varieties should be carried out to enlighten us further on the pollination pattern and relation to self-incompatibility in durian species. Examination of the pollen tubes and their ability to set fruit compared to different pollens used either originated from *Musang King* or other durian variety reflects the capability of self-fertilized or *vice versa*. Low fruit-set percentages after self-pollination confirmed the self-incompatibility status of *Musang King*, and it should be planted with other varieties in a planting area. Results from the present study could guide growers of *Musang King* to decide on the implementation of a multi-varieties planting system instead of mono-variety. Although the multi-variety system could raise the number of trees to produce more fruits, it solely could not ensure pollination success. Compatible pollens, the existence of pollinator agents, and quality pollens should co-exist or simultaneously improve to increase durian fruit production. Further evaluation on different potential pollen donors could be done to examine crossing capabilities with *Musang King* as maternal to produce the highest number of fruits at harvest.

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