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Unravelling self-incompatibility and polliniser requirements in 'Musang King' durian for enhanced cultivation practices

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Abstract

The objective of this study was to study the 'Musang King' durian (*Durio zibethinus*) variety's self-incompatibility status. The study was conducted in Parit Sulong, Batu Pahat, Johor. The first experimental phase used four pollination treatments (open pollination, assisted self-pollination, cross-pollination with 'D24' pollen and autonomous autogamy). The second phase introduced supplementary pollens 'D190' and 'D200'. The fruit set was closely monitored for 12 weeks after pollination. Data were analysed using SAS 9.4 software. The study found that cross-pollination significantly improved the fruit set rate. The average fruit set for cross-pollination with 'D24' was 36.24% at 12 weeks after pollination in the first experiment compared to no fruit setting for assisted self-pollination. A lower fruit set was produced during the second phase of the study using 'D24', 'D190' and 'D200' pollens, while no fruit set was observed for assisted self-pollination. The study highlighted the importance of selecting compatible varieties as pollen sources for successful pollination and affirmed the need for multi-variety planting systems in 'Musang King' cultivation.

Keywords: *Musang King durian, cross-pollination, self-incompatibility, pollen source varieties, fruit setting, pollination compatibility*

Introduction

Durian, known as the 'King of Fruits', holds a regal position among Malaysia's fruit crops. Among its 215 recognised varieties listed in the National Crop List by the Department of Agriculture, the 'Musang King', or 'D197', enjoys a special reputation (Abdul Rashid 2021). Renowned for its exceptional taste, fine texture, and attractive yellow aril colour, 'Musang King' has captured local and international markets, including China, where it is sold at a premium price. These fruits were exported from Malaysia using the cryopreservation method (Nur Azlin et al. 2020).

While durian enjoys considerable popularity, the planting systems and mechanisms of its pollination raise several questions. Typically, conventional planting systems plant a few durian varieties in one area to maximise production. Responding to the escalating fruit demands, agricultural producers are investigating strategies to augment yield. These strategies include assessing the viability of cultivating homogenous or monovarietal durian plantations within a designated agricultural zone (Ei & Ismail 2022). However, the concern lies in the viability and sustainability of such a system, given the self-incompatibility observed in wide durian varieties.

The phenomenon of self-incompatibility in durian was initially documented by Valmayor et al. in 1965. Subsequent research delved deeper into this aspect, exploring its implications across various durian varieties, as seen in studies by Shaari et al. (1985), Soepadma and Eow (1976), Chin and Poon (1982), Zainal Abidin (1990), and Honsho et al. (2004). Honsho et al. (2004) proposed late-acting self-incompatibility in durians, drawing this conclusion from a significant reduction in fruit settings observed four weeks after artificial self-pollination. Thus, these collective findings increase the understanding of the intricate reproductive mechanisms in durian plants.

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The durian's anthers shed pollen around 8:00 p.m. and fall before sunrise (Ei & Ismail 2022). Their nocturnal pollinators include the nectar bat (*Eonycteris spelaea* Dobson), lesser dog-faced fruit bat (*Cynopterus brachyotis* Muller), and the giant honeybee (*Apis dorsata* Fabricius), and they play a vital role in ensuring successful pollination (Bumrungsri et al. 2009). Durian pollen grains clump together and cannot be dispersed by the wind.

These complexities and the prevailing evidence of self-incompatibility in durians will force most farmers to focus on 'Musang King' cultivation and their self-incompatibility. However, their lack of awareness is due to insufficient concrete evidence for self-incompatibility in 'Musang King'. To address this gap, this study aims to determine whether 'Musang King' is self-incompatible and to identify potential polliniser varieties for this variety, thus offering insights into its pollination compatibility and the possibility of enhancing self-pollination for increased yield.

Materials and method

Location

The research design encompassed pilot and large-scale studies conducted within a durian orchard in Parit Sulong, Batu Pahat, Johor. The orchard has over 20 durian varieties, such as 'Musang King' (D197), 'D24', 'Kim Hong' ('D198'), 'MDUR 88' ('D190') and 'Black Thorn' ('D200'). The trees were over 20 years old and have been cared for and cultivated meticulously. The orchard possesses a Malaysian Good Agricultural Practices (MyGAP) certificate that encompasses quality agriculture, aquaculture, and livestock certification programmes. A good agricultural approach (GAP) is an agricultural practice that emphasises environmental, economic, and social considerations to ensure agricultural products' safety and quality.

Research design

The pilot studies were conducted in 2019, specifically during the primary durian flowering and the fruiting period from February to May 2019. Extensive research was undertaken in 2020, specifically during the flowering and fruiting period, lasting from March through May. This study was performed on the 'Musang King' durian variety to evaluate the effects of various pollination techniques on fruit sets.

Pilot Study 1: Six fully developed 'Musang King' trees were subjected to four distinct pollination treatments: open pollination, assisted self-pollination, cross-pollination with 'D24' pollen, and autonomous autogamy. Four flower clusters per tree were treated with each treatment. The fruit-setting observation was conducted bi-weekly for each treatment from week 2 to week 12 (7th March 2019 – 16th May 2019) following the method by Honsho et al. (2004).

Pilot Study 2: The second pilot study aimed to assess the effects of various pollen sources ('D24', 'D168', 'D190', and 'D200') on the growth and development of a solitary 'Musang King' tree. Seven flower clusters were subjected to each treatment, and the subsequent fruit setting was observed concurrently with the initial pilot study.

Large-scale study: After the pilot studies, a comprehensive study was conducted in 2020, by replicating the design of the Pilot Study 1 but incorporating two additional pollen sources, namely 'D190' and 'D200'. The present investigation was conducted on twelve trees belonging to the 'Musang King' variety. The data collection procedure was modified to accommodate the Movement Restricted Orders implemented during the COVID-19 pandemic.

'D200' was chosen based on its influence and status as Malaysia's second highest quality durian variety, after the 'Musang King'. 'D24' was selected based on its enduring popularity and widespread prevalence in Malaysia and 'D190' was selected as the preferred option due to its prominent status and endorsement by MARDI.

The Randomised Complete Block Design (RCBD) was chosen as the statistical approach, and it considers factors such as distinct pollen sources and specific time intervals post-pollination.

Pollination treatments

The pollination treatments followed by Honsho et al. (2004) with modifications on the method of transferring pollen and improvement in the flower bagging method. The pollens were gathered from a single plant per variety to facilitate cross-pollination. The assisted self-pollination and cross-pollination process selects and reduces the number of flowers in clusters at the 'white stage' (Kozai and Higuchi, 2011) to an average of five flowers per cluster. During the white stage phase, the flowers will expose half of their petals which will be in white colour. Later in the day, at about 1600 H, the flowers will open and expose all part of the petals. Emasculation took place from 0900 H to 1500 H, involving the removal of petals and stamen from each flower bud. As a precaution, the treated buds were covered with custom-made muslin bag to ensure that no pollinators, such as bats or insects, pollinate the stigma in the studies. Subsequently, pollination was performed in the evening, beginning at 2000 H and concluding at 2300 H, and the flower clusters were re-covered with the muslin bag. During open pollination, clusters of flowers were carefully selected in the pre-anthesis stage, and any flowers that had already reached the anthesis stage were removed. A plastic tag was affixed near each flower cluster, and the number of remaining flowers was subsequently enumerated.

The process of autogamy was autonomously implemented as follows: Flower clusters at the pre-anthesis phase were identified selectively. In contrast, the ones that had transitioned to the anthesis phase were eliminated. Following this selection, a custom-

made muslin bag covered these flower clusters. This encapsulation persisted until all enclosed flowers either transitioned to the fruiting stage or experienced abscission.

Data analysis

Fruit settings were recorded every two weeks from the second to the twelfth week after pollination. Subsequently, the recorded fruit set data was inserted into a Microsoft Excel file and converted into a percentage format. Then, the data was analysed using SAS 9.4 software. The General Linear Model (GLM) procedure identified statistically significant variations in means across treatments and periods, accounting for the unequal sample sizes. Post-hoc pairwise comparisons were conducted using Duncan's Multiple Range Test at a significance level of $\alpha = 0.05$ to assess differences between treatments.

Results and discussion

The research endeavour was initiated with a pilot study in 2019 to comprehend the pollination behaviour of the 'Musang King' durian variety. Next, it was followed by a comprehensive investigation in 2020. This investigation validated the preliminary findings and explored the impact of various pollination methods on the 'Musang King' fruit settings.

The pilot study showed a consistent lack of development of fruit sets in cases of assisted self-pollination. As shown in *Table 1*, no fruit settings occurred in week 12 after pollination because in week 2, it was found that all pollinated flowers underwent abscission. Statistical analyses highlighted the significant effects of different pollination treatments on the fruit setting of 'Musang King' durian trees over a 12 week observation period. Based on the result, when 'Musang King' was cross-pollinated with the 'D24' flowers, it showed promising results, outperforming other methods, where at twelfth weeks after pollination, 36.83 % of fruits were setting and ready for fruit harvest following natural fruit abscission. The pollination compatibility study of 'Musang King' durian revealed several crucial discoveries, aligning with previous studies by Ei and Honsho et al. (2004), Lo et al. (2007) and Ismail (2022).

The persistent failure of fruit set in 'Musang King' flowers caused by assisted self-pollination underscores its self-incompatibility trait. The 2020 research further corroborated this variety's inability to self-fertilise across various developmental stages. The enduring self-incompatibility highlights the importance of cross-pollination strategies for successful fruit production in 'Musang King' durian cultivation, paralleling patterns in varieties such as 'D2', 'D24', 'D66' and 'D84', as documented by Wong (1999).

The results in the pilot study showed limited fruit set without fertilisation, with a minimal occurrence of 0.40 %, suggesting possible apomixis, though further investigation is necessary to substantiate this finding. Apomixis is a form of asexual reproduction where seeds are formed without fertilisation, leading to offspring genetically identical to the parent. Ei and Ismail (2022) observed fruit set in their study due to autonomous autogamy during the 7th day after pollination, which then all the flowers that failed to set fruit underwent abscission by the 14th day after pollination. This might be explained by the 'Musang King' variety's late-self incompatibility characters, similar to observation on other durian varieties by Honsho et al. (2204). The low fruit setting percentage observed during the 4th to 12th week after anthesis in our investigation suggests the importance of cross-pollination in 'Musang King' durian trees.

The second pilot study in 2019 provided insights into the impact of different pollen sources on the 'Musang King' fruit setting. Pollens from varieties like 'D24', 'D168', 'D190' and 'D200' were used, confirming the initial observations of self-incompatibility in the 'Musang King' durian variety.

Our research documented the occurrence of self-incompatibility in 'Musang King' durians, supporting Ei and Ismail's (2022) findings. The significant increase in fruit sets when subjected to cross-pollination with 'D24' durians is consistent with observations by Honsho et al. (2004) and Lo et al. (2007), confirming the widespread occurrence of self-incompatibility in durian varieties.

Table 1. Fruit set percentages at weeks after anthesis (Pilot Study 1, 2019)

Treatment (Sample size)	Weeks after anthesis					
	2nd	4th	6th	8th	10th	12th
Open pollination (n=114)	18.05 ± 25.77^{b}	1.00 ± 2.21^{b}	1.00 ± 2.21^{b}	0.79 ± 1.62^{b}	0.79 ± 1.62^{b}	0.79 ± 1.62^{b}
Assisted self-pollination (n=144)	0^{c}	0^{b}	0^{b}	0^{b}	0^{b}	0^{b}
Cross-pollination with 'D24' (n=102)	81.35 ± 28.74^{a}	58.94 ± 34.99^{a}	43.88 ± 37.32^{a}	39.94 ± 34.46^{a}	39.00 ± 34.87^{a}	36.24 ± 36.83^{a}
Autonomous autogamy (n=120)	6.25 ± 14.23^{bc}	0.40 ± 1.79^{b}	0.40 ± 1.79^{b}	0.40 ± 1.79^{b}	0.40 ± 1.79^{b}	0.40 ± 1.79^{b}
F-value	62.51	56.55	31.34	29.77	27.28	21.24

*Means with the same letter vertically are not significantly different at $P \leq 0.05$ using Duncan's Multiple Range Test (DMRT).
*Total number of 'Musang King' plants: 6 plants

Distinct pollination behaviours in ‘Musang King’ durian contrasted with those noted in other varieties, like the successful pollination in ‘Monthong’ durian reported by Jutamanee and Sirisuntornlak (2017). This highlights the diversity within durian varieties and the necessity of tailored agricultural practices for optimal fruit sets.

Our study reveals a preference for cross-pollination in ‘Musang King’, especially with the ‘D24’ variety. This finding is crucial for durian cultivators. It can guide them in selecting appropriate pollination techniques and variety pairings to maximise yield and maintain genetic diversity. Ei and Ismail (2022) also noted the presence of herkogamy in ‘Musang King’ flowers, emphasising the importance of cross-pollination in durian cultivation.

Cross-pollination treatments with ‘D24’, ‘D168’, ‘D190’ and ‘D200’ showed variable fruit set results. For instance, cross-pollination with ‘D190’ initially achieved the highest average fruit set of 92.86% during the second week but reduced to 11.90% by the twelfth week (*Table 2*), followed by cross-pollination with ‘D168’, ‘D200’ and ‘D24’ with their fruit setting of 87.59 %, 68.33 % and 52.04 % at the second week. However, in the twelfth week after pollination, when fruit was nearly ready for harvest, the highest fruit set was recorded in cross-pollination with ‘D200’ (16.67 %), followed by ‘D168’ (12.86 %), ‘D24’ (12.2 %) and ‘D190’ (11.9 %).

The findings from the large-scale study validate the preliminary results of the pilot study on the self-incompatibility of the ‘Musang King’ durian variety (*Table 3*). The absence of fruit setting following self-pollination confirms the variety’s self-incompatibility.

Cross-pollination treatments resulted in successful fertilisation, suggesting the potential of varieties like ‘D24’, ‘D190’ and ‘D200’ as viable pollen sources. No significant variations were observed among these different pollen sources toward the end of the fruit development stage, especially at week 10th after pollination, offering flexibility for farmers. The percentage of fruit setting were lower than the pilot study during the previous year, which could be due to environmental factors that may be further studied in future. This can be seen where at week 10th after pollination, cross-pollination with ‘D24’ only set 8.78 % fruit compared to 39.0 % during the pilot study. However, it also could mimic real situations that may occur for pollination in large-scale farms. The data could be used as a minimum to maximum guide for potential fruit settings when similar pollination happens at large-scale farms.

Table 2. Fruit set percentages at weeks after anthesis following pollination with different pollen sources (Pilot Study 2, 2019)

Treatment (Sample size)	Weeks after anthesis					
	2nd	4th	6th	8th	10th	12th
Assisted self-pollination (n=7)	0	0	0	0	0	0
Cross-pollination with ‘D24’ (n=7)	52.04 ± 47.86	23.47 ± 35.59	9.18 ± 12	9.18 ± 12	9.18 ± 12	7.14 ± 12.2
Cross-pollination with ‘D168’ (n=7)	68.33 ± 34.03	17.62 ± 19.31	12.86 ± 18.9	12.86 ± 18.9	12.86 ± 18.9	12.86 ± 18.9
Cross-pollination with ‘D190’ (n=7)	92.86 ± 13.11	32.14 ± 16.96	19.05 ± 20.25	19.05 ± 20.25	19.05 ± 20.25	11.9 ± 20.89
Cross-pollination with ‘D200’ (n=7)	87.59 ± 15.67	40.14 ± 37.35	30.95 ± 29.55	30.95 ± 29.55	23.81 ± 30.21	16.67 ± 28.87

*Total number of ‘Musang King’ plants: 1 plant

Table 3. Fruit set percentages at weeks after anthesis for the large-scale study (2020)

Treatment (Sample size)	Weeks after anthesis		
	6th	8th	10th
Open pollination (n=180)	0^{c}	0^{b}	0^{b}
Assisted self-pollination (n=144)	0^{c}	0^{b}	0^{b}
Cross-pollination with ‘D24’ (n=171)	15.635 ± 21.082^{b}	9.799 ± 18.416^{a}	8.425 ± 17.027^{a}
Cross-pollination with ‘D190’ (n=147)	25.859 ± 25.697^{a}	9.884 ± 19.582^{a}	8.78 ± 17.415^{a}
Cross-pollination with ‘D200’ (n=147)	27.523 ± 29.451^{a}	12.335 ± 20.863^{a}	9.917 ± 17.456^{a}
Autonomous autogamy (n=180)	0^{c}	0^{b}	0^{b}
F-value	30.63	10.89	9.87

*Means with the same letter vertically are not significantly different at $P \leq 0.05$ using Duncan’s Multiple Range Test (DMRT).
*Total number of ‘Musang King’ plants: 12 plants

Our study revealed that open pollination did not lead to fruit establishment, possibly due to incompatibility of available pollen, insufficient pollination, or adverse climatic conditions. However, other flower clusters in the orchard set fruits until harvest, suggesting that open pollination is viable under certain conditions.

Our study supports the concept of self-incompatibility in the 'Musang King' variety, emphasising the necessity of cross-pollination for fruit development. The results support Ei and Ismail's (2022) recommendation for 'Musang King' farmers to implement mixed-variety cultivation. Our findings indicate that 'D24', 'D190', and 'D200' are effective pollen sources for 'Musang King', validating Ei and Ismail's (2022) findings on the importance of cross-pollination due to self-incompatibility traits in durians.

Conclusion

In conclusion, this study confirms the self-incompatibility of the 'Musang King' durian variety, highlighting the critical need for cross-pollination for fruit production. The inability of the 'Musang King' to produce fruit via self-pollination supports the self-incompatibility hypothesis. The study identified 'D24', "D168', 'D190' and 'D200' as effective pollen donors for 'Musang King'. Thus, durian farmers should adopt strategic cross-pollination with suitable varieties, considering factors like taste, market demand, and resilience. The benefits of a multi-variety planting system over a mono-variety system for increased yield are also emphasised. This research underlines the importance of further studies to improve cross-pollination techniques and evaluate the effectiveness of durian varieties under varying pollination and orchard conditions to ensure consistent fruit production. Future research should focus on identifying optimal varieties for cross-pollination with 'Musang King' to maximise yield and profitability, offering promising directions for enhancing the durian farming industry.

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