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Flowering Phenology and Evaluation of Pollination Techniques to Achieve Acceptable Fruit Quality of Red-fleshed Pitaya (*Hylocereus polyrhizus*) in Sabah, East Malaysia

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ABSTRACT

As red-fleshed pitaya (*Hylocereus polyrhizus*) is not native to the tropical environment of the Malaysian state of Sabah, Borneo, little is known about its flowering phenology, pollination requirements, and potential pollinators, which has discouraged many farmers from growing this crop. Therefore, this study aimed to examine better pollination techniques to achieve acceptable fruit quality for red-fleshed pitaya production under local climatic conditions. For this purpose, stingless bees (*Tetragonula laeviceps*), self-pollination, natural pollination, and hand pollination were used. Pitayas were planted in the field from January 2018 to February 2022, and 40 flowers were observed to obtain data on flowering phenology and fruit quality. This study observed that anthesis of red-fleshed pitaya took about 24 hours in all treatments, depending on the local climate, starting at 6.30 p.m. and ending at 6.30 p.m. the next day. Besides self-pollination, the pitaya flowers were also successfully pollinated by natural, hand, and stingless bees. However, the fruits pollinated by stingless bees were the heaviest, longest, and thickest, indicating that the integration of pitaya cultivation and stingless bees is likely to improve the yield and quality of the fruits on the farm.

Keywords: Flowering phenology, fruit quality, *Hylocereus polyrhizus*, pollination technique

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INTRODUCTION

The pitaya is a cactus native to Mexico, south and central America, and is one of the fruits suitable for worldwide commercial cultivation. In Malaysia, the white-fleshed pitaya (*Hylocereus undatus*), the red-fleshed pitaya (*Hylocereus polyrhizus*), and the yellow-skinned pitaya (*Hylocereus* *megalenthus*) are considered the most suitable varieties for nationwide cultivation (Then, 2017). However, white-fleshed pitaya and red-fleshed pitaya are grown commercially mainly for their unique fruit shape, attractive red colour and relatively high antioxidant and nutritional value compared to other pitaya varieties (Renfiyeni et al., 2018; Then, 2017). Pitaya cultivation in Malaysia is promising as it benefits local farmers economically and can be exploited commercially. However, little is known about this crop's flowering phenology, pollination requirements, and potential pollinators under hot and humid tropical conditions. From 2000 until recently, hand pollination has been used by pitaya growers on commercial farms as the main technique for obtaining high-quality and large fruits (de Menezes et al., 2015; Li et al., 2022; Moreira et al., 2022; Renfiyeni et al., 2018). Natural pollination is also carried out, but fruiting success is reported to be poor, and the fruit produced is often of poor quality (Renfiyeni et al., 2018; Weiss et al., 1994). In self-pollination and natural pollination, the length between the stigma and the anther of the pitaya flower is considered one of the main obstacles to the success of fruit formation (Renfiyeni et al., 2018). Therefore, to achieve successful pollination of pitaya, it is important that pollination is done either manually or by a pollinator (Joanna & Ding, 2021). As the area under pitaya cultivation has increased exponentially in recent years, a systematic study is needed to understand how to achieve high-quality yields through efficient pollination techniques.

The red-fleshed pitaya is widely grown in Sabah, East Malaysia, because of its sweet taste and more expensive fruit compared to other pitaya varieties (Joanna & Ding, 2021, 2022; Phebe et al., 2009). It has also been observed that some farmers in commercial pitaya farms in Sabah use artificial light to attract nocturnal pollinators such as moths and bats. However, the success rate of pollination is uncertain due to a lack of consistent information. The short duration of the pitaya flower opening, which starts in the evening and closes completely the next morning, also reduces the time for pollination by diurnal pollinators such as bees and ants (Joanna & Ding, 2021; Renfiyeni et al., 2018). Therefore, hand pollination of pitaya has to be done at night as the natural pollinators of the flowers normally come during the day (Moreira et al., 2022). In Peninsular Malaysia, hand pollination on commercial farms is usually done at night from 8:00 p.m. to 2:00 a.m. but is very labour intensive (Ahmad Hafiz et al., 2019; Then, 2017; Then et al., 2020). In Mexico, the country of origin of pitaya, bats have been reported to efficiently pollinate flowers at night (Moreira et al., 2022; Valiente-Banuet et al., 2007), while some studies have found that bees can effectively pollinate flowers during the day and improve yield quality, although they have much less time to visit the flowers (de Oliveira Muniz et al., 2020; Indriyani & Hardiyanto, 2018; Valiente-Banuet et al., 2007). So far, the potential nocturnal and diurnal pollinators for the natural pollination of pitaya flowers in Sabah have not been documented, which is important for studies.

In contrast to common honeybees, studies on stingless bees as main pollinators of pitaya flowers are relatively rare worldwide, probably because they are restricted to subtropical and tropical regions (Jalil, 2017; Michener, 2007; Rahman et al., 2015; Rasmussen, 2008, 2013; Roubik, 1989; Sakagami, 1982; Sakagami et al., 1990). Like honeybees, stingless bees are effective pollinators because they have special structures for collecting pollen called corbicula that facilitate the transfer of pollen to other flowers. Previous studies have shown that stingless bees are used as primary pollinators in several tropical crops such as chilli (Putra et al., 2016), mustard (Atmowidi et al., 2007), *Brassica oleraceae* (Wulandari et al., 2017), *Foeniculum vulgare* (Layek et al., 2022), and rock melon (Azmi et al., 2019). In addition, pollination by stingless bees contributes significantly to crop production by increasing the number of pods, seeds per pod, seed weight per plant, and seed germination of mustard (Atmowidi et al., 2007) and kale (Wulandari et al., 2017) as well as improving the production quality of melon (Bahlis et al., 2021) and the quality and quantity of okra pods (Djakaria et al., 2022). Pollination by stingless bees has also been reported to increase fruit set, fruit size, and weight of strawberries (Alpionita et al., 2021; Roselino et al., 2009; Widhiono et al., 2012), so they may be suitable pollinators for pitaya flowers. This study selected *Tetragonula laeviceps*, one of Malaysia's most common species of stingless bees (Atmowidi et al., 2007), as the model organism for pitaya pollination. Previous studies have shown that *T. laeviceps* is suitable for integration into crops as it can cope better with hot and humid tropical weather conditions and can easily build its nests in new habitats (Agus et al., 2019; Atmowidi et al., 2007; Azmi et al., 2019; Bahlis et al., 2021; Djakaria et al., 2022; Layek et al., 2022; Roselino et al., 2009; Wulandari et al., 2017).

The lack of information on potential pollination and pollinator requirements for pitaya has limited understanding of the agronomic requirements for growing this non-native crop in Sabah, East Malaysia. As farmers learn more about this crop, its flowering phenology, pollination requirements, and potential pollinators, yields will likely increase, but information is currently limited. This study assessed the flowering phenology and quality of red-fleshed pitaya fruit obtained through stingless bee pollination, self-pollination, natural pollination, and hand pollination.

MATERIALS AND METHODS

Location of Study and Preparation of Materials

This study was conducted at the Insectarium, Faculty of Sustainable Agriculture, Universiti Malaysia Sabah (UMS), from January 2018 to February 2022. Sixteen polybags were planted with red-fleshed pitaya (*H. polyrhizus*), and all fertilisations were applied at the same rate and time. In this study, the red-fleshed pitaya plants took about two years to mature and produce flowers. Colonies of stingless bees, *T. laeviceps*, were kept in the field for about three months under natural climatic conditions (22 to 31°C, 83% humidity) and acclimatised. Environmental parameters such as temperature (°C) and relative humidity (%) were recorded with the HOBO® Pro V2 data logger (USA).

Treatments and Experimental Design

The experiment was conducted with a complete randomised design (CRD), and the treatments for the plant in this study were (1) self-pollination (4 polybags), (2) natural pollination (4 polybags), (3) hand pollination (4 polybags), and (4) pollination by stingless bees (*T. laeviceps*) (4 polybags). Figure 1 shows the treatments for pollination of pitaya flowers. For self-pollination, flower buds were bagged in fine muslin bags (1 mm × 1 mm) 24 hr before anthesis to avoid visits by pollinators. In hand pollination, pollen was manually transferred from the stamens or the male part of the flower, to the pistil or the female part. The flower was bagged in the evening (4.00 p.m.) and hand-pollinated the next day at 6.00 a.m. in the morning. After manual pollination, the flower was bagged until the anthesis closed at 6.30 p.m. to avoid other pollinators. The flower was not bagged in natural pollination, so all pollinators could pollinate it. The types of potential pollinators visiting the flower during the anthesis period were visually observed twice within 24 hr (from 4.30 p.m. to 12:00 a.m. and from 4:00 a.m. to 6:30 a.m.) using the method described by de Oliveira Muniz et al. (2019). For pollination by stingless bees, three hives with strong colonies of *T. laeviceps* were placed next to the pitaya at least seven days before flowering to allow the stingless bees to acclimatise. The plants and hives were covered with mesh netting the evening before flowering and left covered for 24 hr. The foraging activity of the bees was observed at the three hives in front of the hive entrance tube for ten

Figure 1. Treatment for pitaya pollination: (a) self-pollination; (b) natural pollination; (c) hand pollination; and (d) *T. laeviceps* pollination

minutes every hour from 6.30 a.m. to 4.30 p.m. on rain-free days, using the method described by de Oliveira Muniz et al. (2019).

Collection of Data

Flower Phenology Observation

For pitaya, each flower phenology was assessed on five consecutive days during several flowering phases from March 2020 to March 2021. For the study of flowering phenology, 40 pitaya flowers were randomly selected for data collection from all buds that had successfully flowered. All flower buds were observed throughout their development, and the length of the bud, the diameter of the open flower, the length of the stigma lobe and the length of the stamens were measured.

Fruit Quality Parameters Evaluation

Data were measured from 40 fruits (10 fruits × 4 treatments) for the fruit quality study. Pollination efficiency of pitaya was measured after fruit harvest, which usually occurred 28 to 33 days after the successful pollination of flowers. Fruit quality parameters were assessed, including weight, length, diameter, harvest age, and set formation. The value of fruit set formation was determined using the following formula:

$$\text{Fruit set rate (\%)} = \frac{\text{Total no. of fruitlets}}{\text{Total no. of flowers}} \times 100\%$$

(Renfiyeni et al., 2018)

Data Analysis

Statistical analyses were carried out using SPSS (version 26). One-way analysis of variance (ANOVA) was used to compare the morphological characteristics of the assigned flowers for each pollination type, the fruit characteristics and pollination types, and the fruit weight, length, and diameter.

RESULTS AND DISCUSSION

Flower Phenology of Red-fleshed Pitaya

In this study, pitaya was found to flower between one and a maximum of three clusters per month about two years after planting, which is also confirmed by Then et al. (2020). However, depending on climatic conditions, the flowering pattern and yield are not uniform. This study observed that a moderate temperature of about 32°C during the dry season is the most important factor influencing the flowering of red pitaya, compared to the rainy season with high and low temperatures.

In Figure 2a, the pitaya flowers appear at the edges of the stems and develop into flower buds in about 10 to 13 days following the emergence of spherical buttons from the stem margins. The whitish-green and cylindrical flower buds reach a length of about 30.55 cm, 3 to 4 weeks after the flower buds start to appear when anthesis takes place. The pitaya flowers started to open at 6.30 p.m. and were fully open by 8.00 p.m. on the first day. The next day, the pitaya flowers closed slightly around the afternoon (1.30 p.m.) and were fully closed by 6.30

Figure 2. Floral morphology and anatomical structure of *Hylocereus polyrhizus* at the time of anthesis, different types of sepaloid and petaloid tepals: (a) Both male and female organs coexist in a flower; and (b) lateral view of a flower, stigma, and anther
Note. Scale bar represents 1 cm

p.m. (Figure 2b). The antithesis of pitaya flowers begins at 6.30 in the late afternoon in Sabah, one to two hours earlier than in Peninsular Malaysia (Then et al., 2020). It is probably related to the situation in the state of Sabah, where it gets dark at 6.30 p.m., whereas in Peninsular Malaysia, it usually starts at 7.30 p.m.

In this study, about four or five spherical buttons emerged from the areoles of the mature stems, but only two to three developed into flower buds, which took 3 to 4 weeks to grow and develop into mature flowers, which is also consistent with the observations of Then et al. (2020) in a pitaya farm in Peninsular Malaysia. The flowers of *H. polyrhizus* are white and sessile (Figure 2b), monoecious, with male and female organs and a long flower tube (Figure 2a), averaging 30.55 cm in length. Characteristics of *H. polyrhizus* are the typical white-coloured, sepaloid tepals, which may serve to increase the visibility of the flower to nocturnal and diurnal pollinators by maximising light reflection (Joanna & Ding, 2021). The perianth of the flower consists of two types of tepals: sepals, which cover the flower tube, and petaloid tepals, which sit on the edge of the flower cup. The flowers are zygomorphic as the pistil and stamens are in the ventral part of the flower. The average length of the filament is 19.75 cm. The flowers have a long stigma lobe with an average length of 25.90 cm (Figure 2b; Table 1). The length of the petals corresponds to the length of

Table 1
Pitaya flowers' morphological characteristics

Flower characteristics	**Morphological measurements**
Total flowers (N)	40
Mean of bud length (cm)	30.55±0.04
Mean of the diameter of opened flowers (cm)	27.40±0.06
Mean of the length of the stigma lobe (cm)	25.90±0.18
Mean of length of filament (cm)	19.75±0.13

the flower tube. The pistil connects the stigma on the upper side with the ovary on the lower side.

Fruit Quality Parameters Evaluation

The data for self-pollination were excluded from the one-way ANOVA analysis because pollination was unsuccessful (Table 2). Apart from unsuccessful fruit pollination by self-pollination, no abnormal fruits were observed in other pollination techniques in this study (Table 2). This result is thus consistent with earlier studies by de Menezes et al. (2015), which reported that self-incompatible pitaya species only achieve the highest fruit quality through cross-pollination. The stigma of the red-fleshed pitaya flowers is higher than the anthers, which may have hindered self-pollination (Hadiati & Umjunidang, 2019; Joanna & Ding, 2021; Renfiyeni et al., 2018). This circumstance results in the pollen's inability to reach and adhere to the stigma. It is called self-incompatibility and is also due to genetic factors (Renfiyeni et al., 2018; Ruwaida, 2007). For this reason, pollination of this crop should be assisted by insects as pollinators or by humans through artificial pollination to maximise fruit set and quality (Merten, 2003; Nadila, 2014; Renfiyeni et al., 2018). Pollination and fertilisation of flowers are two interrelated processes whose success depends on the compatibility of pollen and stigma (Renfiyeni et al., 2018).

The fruit for each pollination treatment is shown in Figure 3. The different types of pollination had a significant effect on the trait's fruit weight ($F_{2,27}$ = 16.57, p<0.001), fruit length ($F_{2,27}$ = 66.91, p<0.001), and fruit diameter ($F_{2,27}$ = 59.46, p<0.001), but showed no effect on harvesting age ($F_{2,27}$ = 0.052, p>0.05) (Table 2 and Figure 3). As self-pollination of red pitaya flowers has been reported to be ineffective, pollination by natural or artificial pollination is strongly recommended to improve the efficiency of the pollination process (de Oliveira Muniz et al., 2020). De Oliveira Muniz et al. (2020) reported that pitaya appears to

Table 2
Fruit weight, fruit length and diameter, fruit set rate, and harvesting age of pitaya

Characteristics	Pollination types			
	Self-pollination	**Natural pollination**	**Hand pollination**	***Tetragonula laeviceps* pollination**
Total flowers (N)	10	10	10	10
Mean of fruit weight (g)***	n.d.	670±55.31^b	548.50±34.98^a	676.50±51.89^b
Mean of fruit length (cm)***	n.d.	13.60±0.37^b	11.45±0.64^a	14.87±0.63^c
Mean of fruit diameter (cm)***	n.d.	9.45±0.37^b	7.83±0.63^a	11.22±0.83^c
Mean of fruit set rate (%)^{NS}	n.d.	100	100	100
Mean of harvesting age (days after anthesis) ^{NS}	n.d.	30.10±0.60	30.10±0.71	30.20±0.64

Note. *** = Significantly different at p<0.001; Means followed by another letter are significantly different at p<0.05 as measured by the post-hoc Tukey test according to analysis of variance; NS = Not significant; n.d. = No data (unsuccessful pollination)

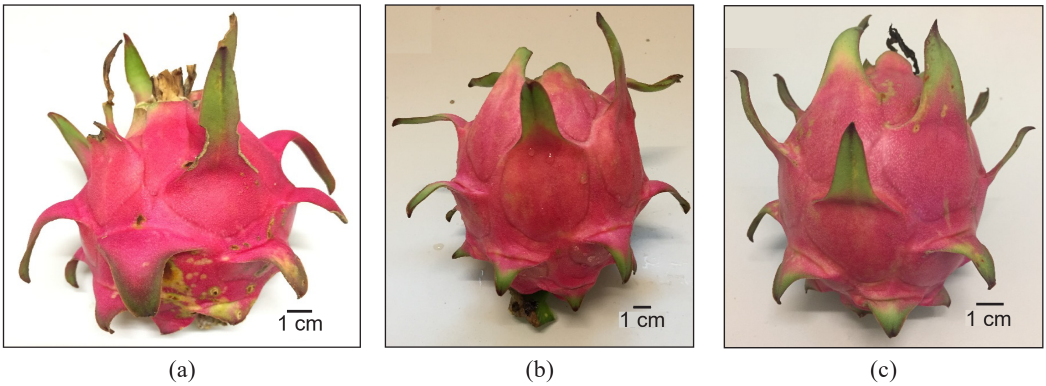

Figure 3. Fruit of pitaya from three treatments: (a) hand pollination; (b) natural pollination; and (c) *Tetragonula laeviceps* pollination

be wholly or partially self-incompatible, and hand pollination is required to achieve commercial yields in cultivation. Pollination is successful when pollen adheres to the stigma and germinates (de Oliveira Muniz et al., 2020). This germinated pollen spreads downwards and penetrates the stigma lobe before reaching the ovary and attaching to the ovule (de Oliveira Muniz et al., 2020). The mature ovary becomes the fruit, and the mature ovule becomes the seed (Darjanto & Satifah, 1990).

In this study, the quality of pitaya fruit produced was also determined by the type of pollination technique (p<0.001). However, average fruit weight, length and diameter were highest when flowers were pollinated by stingless bees (*T. laeviceps*), followed by natural and hand pollination (Table 2). According to Zainudin and Hafiz (2015), pitaya fruits are classified as 'AA' for 500–800 g, 'A' for 350–450 g, 'B' for 250–350 g, and 'C' for less than 250 g. In this study, it was found that hand pollination and natural pollination produced pitaya fruits of the 'AA' class weighing 548 and 670 g, with a length of 11.45 and 13.6 cm, as well as a diameter of 7.83 and 9.45 cm, respectively (Table 2). Hand pollination is a necessary auxiliary method for pitaya production to achieve a high yield (Li et al., 2022). The slightly lower fruit weight, length, and diameter in hand pollination in this study could be due to the late timing of flower pollination, which took place in the morning, 12 to 13 hours after the flowers opened at 6.00 to 7.30 p.m. the previous day. In Guangzhou, China, Li et al. (2022) reported that choosing the right time for hand pollination is an important method for pitaya production in commercial farms to achieve high yields. Li et al. (2022) point out that the best pollination activity of pitaya flowers occurs at night immediately after flowering, between 8.00 p.m. and 2.00 a.m. and that late pollination activity after 4.00 a.m. can reduce fruit size by 10.5%. Therefore, these studies also provide an important basis for choosing the right timing for hand pollination in an open farm to improve the yield and breeding efficiency of red-fleshed pitayas, especially in Sabah, East Malaysia.

The study on natural pollinators found that diurnal pollinators mainly include hymenopteran insects such as bumblebees, carpenter bees, wasps, hornets, and honeybees. They were observed to visit pitaya flowers in the field only for 10 to 20 s per flower, mainly between 8.00 in the morning and 12.00 noon. Nocturnal pollinators, including moths (Lepidoptera; Sphingidae), were observed actively visiting the flowers between 7.00 and 8.30 at night. However, only ants were observed visiting the flowers both at night and during the day, which was also reported by Sulistiyowati and Putra (2016) for pitaya farms in Java. Fruit weight, length, and diameter were slightly lower when pollinated naturally than when pollinated by stingless bees (Table 2). It is probably related to the short visit and the lack of adaptation of these natural pollinators to the newly introduced plants, which are flowering for the first time in the area studied (Weiss et al., 1994).

Pollination by natural pollinators such as bees can reduce labour costs for pollination services, as has been observed in other crops, e.g., a kiwi farm in China (Zhang et al., 2022) and strawberry farms in Pakistan (Anees et al., 2022). Then et al. (2020) found that the average weight of individual fruits of a naturally pollinated red-fleshed pitaya ranged from 283.60 to 336.50 g at the Tengi Research Station, Selangor, Malaysia. Other studies by Bellec et al. (2006) and Hoa et al. (2006) reported that the average fruit weight of a naturally pollinated red-fleshed pitaya was 300 and 393 g per fruit, respectively. On the other hand, the higher average fruit weight from natural pollination in this study compared to pitaya fruit reported in previous studies could be due to cross-pollination activity by different hymenopteran insects that visited the flowers studied and brought pollen from many different pitaya flowers, even if it was only a short visit. According to Renfiyeni et al. (2018), a large amount of pollen is the most important requirement to allow better results in the cross-pollination of flowers and for the production of high-quality fruits of red-fleshed pitaya.

Compared to the other treatments, the fruits from pollination by the stingless bee, *T. laeviceps*, had the heaviest (676.50 g), longest (14.87 cm), and thickest (11.22 cm) fruits (Table 2). It indicates that *T. laeviceps* can be successfully used as good pollinators and integrated into commercial pitaya farms as they produce the highest quality and average weight of fruits in the 'AA' class. This study found this species to start foraging early at 6.00 a.m. but to be most active between 7.30 a.m. and 12.30 p.m. when the pitaya flowers are still open. This species was also observed actively foraging at relatively low temperatures (25.17 to 27.67°C) and relatively high humidity (85.67 to 94.17%) between 6.00 a.m. and 12.30 p.m. The duration of flower visitation by *T. laeviceps* was longest at 60 to 70 s per flower and shortest at 30 to 40 s per flower. It was observed that the visiting activity of *T. laeviceps* was related to the availability of pollen, which was abundant in pitaya flowers. This result is also in agreement with studies by Alpionita et al. (2021), who found that *T. laeviceps* is a species of stingless bees that generally visited strawberry flowers longer (89.15 s/number) than other insect species, such as honeybees (12.64 s/number), resulting in higher fruit quality in both cases.

CONCLUSION

It was observed that anthesis of red-fleshed pitaya lasted 24 hours in all treatments, depending on the local climate, and started at 6.30 p.m. and ended at 6.30 p.m. the next day. The effectiveness of the pollination method determined the quality of the fruit produced by the pitaya. Apart from unsuccessful fruiting by self-pollination, pitaya flowers were successfully pollinated by natural pollination, hand pollination, and stingless bees, and their fruit quality was acceptable in weight, length, and diameter. However, pollination by *T. laeviceps* generally resulted in better fruit quality than natural pollination and hand pollination of the non-native plant of red-fleshed pitaya in Sabah, East Malaysia.

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