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Floral biology and the compatibility system in ciku cv. Jantung *(Manilkara achras)*

[Biologi bunga and sistem keserasian ciku kv. Jantung *(Manilkara achras)*]

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Key words: *Manilkara achras*, anthesis, anther dehiscence, stigma receptivity, pollen viability, pollen transfer

Abstrak

Penyelidikan ini telah dijalankan untuk mengenal pasti dan memahami biologi reproduktif ciku kv. Jantung. Ujian dilaksanakan untuk menentukan biologi bunga, kecekapan pemindahan debunga dan sistem pendebungaan ciku. Berasaskan perubahan pada morfologi luar kudup bunga, empat peringkat perkembangan bunga telah dikenal pasti iaitu peringkat 1–3 pada peringkat kudup dan peringkat 4 semasa bunga berkembang. Pada peringkat 2, anter merekah dan melepaskan debunga. Debunga hanya menjadi hidup pada peringkat 4 iaitu semasa antesis. Stigma berada dalam keadaan reseptif daripada peringkat 2 hingga 3 tetapi mencapai kemuncaknya pada peringkat 3 iaitu sehari sebelum bunga berkembang. Dengan ini, keputusan menunjukkan terdapat sedikit atau tiada pertindihan fasa staminat dan pistilat (pengasingan masa) bagi ciku Jantung. Keadaan ini seterusnya disahkan oleh kajian pendebungaan yang hanya mendapat 4.8% pembentukan putik buah apabila kudup bunga dibegkan. Adanya pengasingan masa pada bunga ciku Jantung menghadkan pendebungaan sesama sendiri dalam sekuntum bunga dan oleh itu membolehkan pendebungaan silang. Walau bagaimanapun, kadar pembentukan putik buah yang tinggi (65%) daripada kajian pendebungaan sendiri berbantu seterusnya menunjukkan bahawa ciku Jantung bersifat serasi sesama sendiri. Walaupun pendebungaan sesama sendiri pada sekuntum bunga terhad, pendebungaan antara bunga boleh berlaku secara ekstensif kerana ciku Jantung bersifat serasi sesama sendiri.

Abstract

The investigation was initiated to identify and understand fully the reproductive biology of ciku cv. Jantung. Trials were conducted to determine the floral biology, efficiency of pollen transfer and the system of pollination. Based on the changes in the external morphology of ciku flower buds, four stages of floral development were identified; stages 1–3 at bud stage and stage 4 at flower opening. At stage 2, the anthers dehisced and released pollens. The pollens only became viable and functional at stage 4, i.e. at anthesis. The stigma was receptive from stage 2 to 3 but was at its peak at stage 3, i.e. a day before the flower opened. These results therefore showed that there was little or no overlapping between the staminate and the pistillate phases (temporal separation) of ciku cv. Jantung. This was further confirmed with the pollination trial where only 4.8% of fruit set was obtained when the flower buds were bagged. The presence of

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temporal separation in ciku cv. Jantung limits within-flower pollination and therefore favours outcrossing. However, the relatively high fruit set (65%) obtained from the assisted self-pollination trial further suggests that ciku Jantung is self-compatible. Although within-flower pollination is limited, between flower pollination can occur extensively as the flowers are self-compatible.

Introduction

Ciku *(Manilkara achras)*, a delicious tropical fruit indigenous to South America, has presently attained considerable popularity in Malaysia. It is grown widely in smallholdings, mainly for domestic consumption and has yet to make an impact in the commercial sector.

In Malaysia, ciku is cultivated mainly for its fruit. However, in countries like Mexico, Guatemala and British Honduras, it is grown for the commercial production of chicle (chicle is made from a white milky latex obtained from the bark of the ciku tree or from its unripe fruit). Ciku, when compared with other tropical fruits, has not been fully exploited of its potentialities. As it is rich nutritively, has good eating qualities and cosmetic appeal, and comparable with temperate fruits like apple and pear, it can be a good import substitute for these fruits (Anon. 1988).

However, before commercial production of ciku can be lucrative and profitable to the growers, the crop has to be thoroughly understood and its major constraints meet. At present, there is a need to improve the local varieties in terms of its fruit uniformity (shape and size), earliness in bearing, yield stability and increase tree dwarfness. To meet these specifications, either by breeding for superior clones or by efficient cultural management, we need to understand and acquire basic information on its reproductive status and pollination requirements. Information on these aspects is currently lacking for our local ciku varieties, namely Jantung, Betawi and Subang.

Under tropical conditions, ciku tree flowers throughout the year. Studies on the reproductive biology of ciku outside Malaysia have produced inconsistent results. In India, Sambamurthy and Ramalingam (1954) concluded that ciku was a wind-pollinated crop while in Phillipines, Gonzalez and Feliciano (1953) showed that insects were the major pollinators. Additional contradiction of results was evident in the findings of Hayes (1957) and Nalawadi et al. (1977) where differences were reported in the time of anthesis, stigma receptivity and anther dehiscence of ciku. Inconsistencies of these results could be due to genetic and/or environmental differences. Either of which makes it necessary for us to investigate the above aspects.

The purpose of this investigation was to identify and understand fully the reproductive biology of ciku tree. Through floral biology studies and assisted pollination, the breeding and the compatibility systems present in ciku Jantung were identified. In addition, attempts were made to determine the most suitable assisted pollination technique because of its importance in future breeding programs.

Materials and methods

This investigation was carried out at MARDI, Serdang during 1989–1990, using 17 year-old ciku Jantung trees. The experimental design adopted in all the sub-trials was completely randomized (CRD) with 6–10 replicates in each sub-trial (single tree plot).

Floral biology

The external morphological changes taking place during the floral development of ciku flowers were closely examined. Fifty young buds (before the emergence of stigma from the corolla) were tagged randomly on five trees. Daily observations and recordings were made to determine the time of emergence of the style from the corolla and time of anthesis.

The viability of pollens was assessed by germinating pollens in an artificial medium composed of sucrose, agar (0.05%), boric acid (0.001%), calcium nitrate (0.003%) and magnesium sulphate (0.002%). To determine the most suitable concentration of sucrose for germination, various concentrations of sucrose solutions (0–6%) were tried. The concentration of sucrose that gave the highest percentage of germination was used as the standard germinating medium in the subsequent trial where the pollen viability at different stages of floral development was determined. About 15–20 estimates were made for each of the 10 replicates at a particular developmental stage. The pollens were considered as germinated only when the length of the pollen tube was twice the diameter of the grain.

Anther dehiscence can be easily observed as the pollens are released in large quantities and can be seen sticking on to the surface of the anthers. In each of the six replicates, 50 young buds (before emergence of stigma from the corolla) were tagged and observed daily from 9.00 a.m. until 12.00 p.m. to determine the period of anther dehiscence. Data were obtained from 2 to 18 April and from 21 to 26 May 1990. Observations continued until all the anthers had dehisced.

Through field observations, the stigma of ciku was seen to secrete a white milky latex at certain times. This was also evident in ciku varieties in the Phillipines (Gonzalez and Feliciano 1953). According to Baker et al. (1973), the major function of stigmatic exudate in flowering plants is assisting in pollen capture, germination, protection and resistance to desiccation. Therefore, the appearance of stigmatic exudate can be used as an indicator for determining stigma receptivity. To study stigma receptivity, another set of 50 young flower buds in each of the six replicates were tagged and observed daily to determine the time and duration of the stigmatic secretion. The experiment was carried out from 3 to 18 April and from 24 May to 2 June 1990.

Natural pollen transfer

To study the efficiency and the peak period for natural pollination, the number of pollen grains deposited on the stigmas at different floral stages were recorded. Two sets of data were obtained, i.e. from 3 to 18 April and from 23 May to 2 June 1990. Fifty stigmas were sampled randomly from each of the six replicates. They were harvested at 9.00 a.m. and then fixed in ethyl-acetate alcohol and stained in safranin-analine blue (Dionne and Spicer 1957). The stigmas were then gently squashed on microscopic slides and observed under a microscope at x 40 magnification. The number of freshly germinated pollen grains that were deposited on the stigmatic surface was counted.

Pollination

To test whether self-fertilization occurred in ciku, three pollination treatments were performed. There were five replicates in each of the treatments. In each treatment, 50 young flower buds (before the emergence of stigma from the corolla) were bagged to exclude pollinators. The bags were removed after about 1 week. The bags were made large enough so that minimum contact occurred with the stigmas. In the second treatment, the same number of flower buds was bagged and later pollinated (when the stigmas were receptive) using pollen from flowers of the same individuals. Simultaneously, 50 flowers were selected, tagged and left to be naturally pollinated. After a period of 1 month, the number of fruit set in each of the pollination treatment was recorded. This experiment was carried out from 4 June to 15 July and from 6 August to 18 September 1990.

Results

Morphology of ciku flower

Ciku flowers are bisexual in nature and are borne on cymes. Each flower has six sepals (calyx) which are arranged in two whorls. The petals (corolla) are of the same number as the sepals but arranged in only one whorl and are coalescent to each other. In the next whorl, the staminodes and the stamens are arranged alternately. The stamens are attached to the petals *(Figure 1).*

Floral biology

Examinations of changes in external morphology during the floral development in ciku cv. Jantung revealed four distinct floral stages *(Plate 1).*

- Stage 1 – onset of flower opening; the calyx started to open and exposed the tip of the corolla.
- Stage 2 – the style elongated and the tip of the stigma emerged from the corolla.
- Stage 3 – the stigma emergence became more pronounced (2–3 mm from tip of corolla.
- Stage 4 – floral anthesis.

The average time taken from stages 1 to 2 and from stages 2 to 3 was 2 days each while the time taken from stages 3 to 4 was 1 day. Therefore, it takes 5 days for a bud at the “bud-break” stage to reach anthesis.

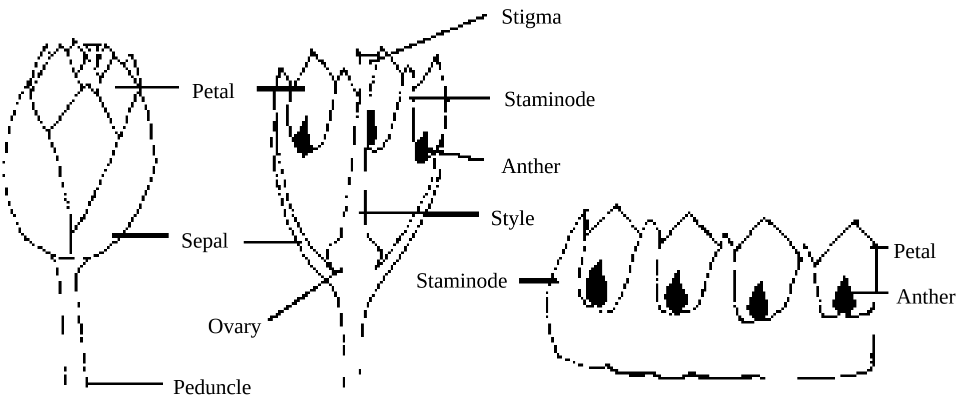

Figure 1. Structural detail of ciku Jantung flower

Plate 1. The four floral development stages in ciku Jantung

Studies on anther dehiscence showed that anthers started to dehisce at stage 1 and continued to do so until it reached stage 2 *(Table 1)*, after which very little dehiscence occurred. At stage 4, no dehiscence was observed. In the statistical analysis of percentage of anther dehiscence(x), the data were transformed using the square root transformation (x + 0.5) to homogenise the variability in the variances. Hartley's Test of Homogeneity was used to test the homogeneity of the variances. On carrying out Duncan's multiple range test, significant differences ($p < 0.005$) between the different developmental stages were evident. Anther dehiscence at stage 2 was significantly higher than that of other stages; thus indicating that maximum dehiscence in ciku occurs at stage 2, i.e. 3 days before anthesis.

The pollen viability test showed that 4% sucrose was the most suitable germinating medium for ciku Jantung. The 4% sucrose medium exhibited significantly higher germination rates ($p < 0.001$) compared with the other media tested *(Table 2)*. When this medium was tested on pollen from the different floral developmental stages, some interesting results were revealed. On the day of anther dehiscence (stage 2), the pollen viability was very low and increased negligibly the next day. Ciku pollens only became functional and viable in stage 4 (at anthesis) as the pollen viability increased significantly ($p < 0.001$) by 82% *(Table 3)*.

The ciku Jantung stigmas started to secrete the white milky latex thus becoming receptive at stage 2 but only reached its peak (100%) at stage 3 (i.e. 1 day before anthesis). Duncan's multiple range test showed that receptivity at this particular stage (stage 3) was significantly higher (p <0.05%) than that of other stages *(Figure 2)*. On the day of anthesis, most of the stigmas lost their receptivity when their tips changed from white to brown and stopped producing the exudate.

Table 1. Mean square root of anther dehiscence at different stages of flower development in ciku

Stage of flower development	Mean sq. root of dehisced anthers
1	2.9b
2	6.5a
3	0.9c
C.V.(%)	8.17

Mean values with the same letters are not significantly different from one another at $p < 0.005$ according to DMRT.

Table 2. Mean percentage of pollen germination at various sucrose concentrations

Sucrose concentration (%)	Mean percentage of pollen germination
2	24.2b
4	43.2a
6	26.5b
Control (distilled water)	10.3c
C.V. (%)	43.1

Mean values with the same letters are not significantly different from one another at $p < 0.001$ according to DMRT.

Table 3. Mean percentage of pollen viability at different stages of flower development

Stage of flower development	Mean sq. root % of pollen viability
2	0.8b
3	1.1b
4	5.2a

Mean values with the same letters are not significantly different from one another at $p < 0.001$ according to DMRT.

Natural pollen transfer

The number of pollen deposited on the stigmatic surface of ciku Jantung increased slowly from stages 1 to 3. However, from stages 3 to 4, a sharp increase was noticed and maximum number of pollen grains were deposited. After this stage, the stigmas were not able to trap any more pollen grains and therefore lost their receptivity *(Figure 2)*. In both seasons, the number of pollen grains in stage 4 was significantly higher ($p < 0.05$) compared with the other stages. This is in conformity with the receptivity results where

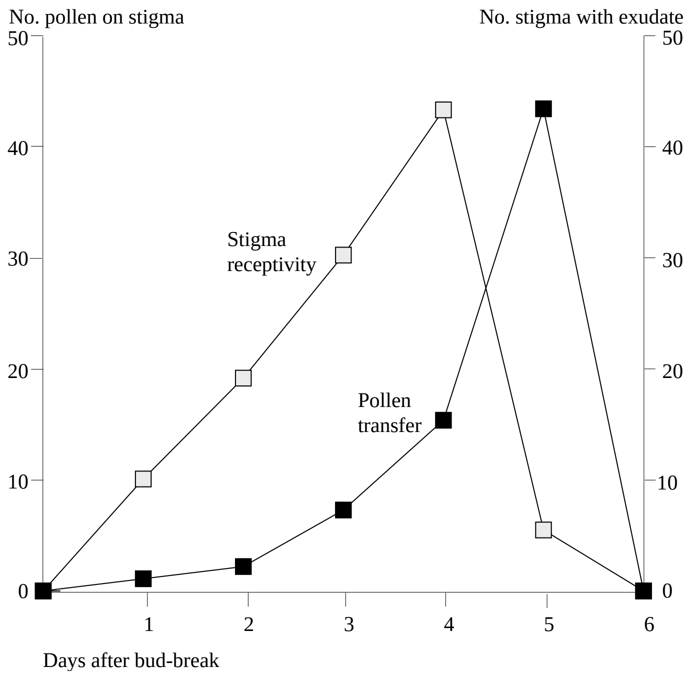

Figure 2. Pollen transfer and stigma receptivity in ciku cv. Jantung

the stigmas were most receptive during the stage 3 period. Thus, when the stigmas were harvested early in the morning on the next day (stage 4), a large number of pollen grains which had already been deposited the day before were present on the stigmas.

Out of 100 stigmas that were examined at stage 4, 96 had at least 810 pollens on them, indicating that there exists a very efficient system of pollen transfer in ciku Jantung (efficiency = 96 %).

Technique of assisted pollination

Prior to any assisted pollination, the ciku Jantung flower buds at stage 1 were bagged with nylon mesh. Pollen grains from flowers at stage 4 were collected in the morning when they were most viable *(Table 3)*. When the flower buds reached stage 3 (the most receptive period), the bags were removed and the pollen grains were brushed on the stigmatic surfaces. The flowers were then rebagged and left as such for a period of 1 month.

The percentage of success here was observed to be largely dependent on the bagging procedure. The bags should be at least 20 cm wide by 30 cm in length. While bagging, precautions should be taken as to have minimal contact with the stigmas which are very prone to damage. To avoid further contact, the sides of the bags were stappled to the surrounding leaves nearby as shown in *Plate 2.*

Pollination system

A non-parametric Chi-Square Test was performed on the number of fruit set in the three pollination treatments *(Table 4)*. Results revealed that the treatments were significantly different at $p < 0.05$, thereby suggesting that pollination is necessary in ciku, since negligible number of fruit-set was observed when the flowers were bagged. When the same statistical procedure was tested out on only two pollination treatments, viz. open and assisted self pollination, no significant difference was obtained. Thus, further suggesting that pollen transfer is quite efficient. This is in conformity with the results obtained from the pollen transfer experiment where the

Plate 2. Stapling the sides of bag to the surrounding leaves to avoid damaging the stigma

percentage efficiency was 96%, thus indicating that pollination or pollen transfer in ciku Jantung is not a limiting factor. The overall low percentage of fruit set in both the above treatments further suggest that pest and disease and/or nutrient resource are more probable factors in causing any limitations to fruit set in ciku Jantung as compared with the process of pollen transfer.

Discussion

The investigation revealed that there is minimal overlapping between the male and female sexual phases of the ciku Jantung flowers (presence of temporal separation). This is indicated by the fact that pollens, although released at stage 2 , become viable and functional only at stage 4 and at this stage the receptivity of the stigma had dropped to almost 0, thus minimizing within-flower pollination. Another feature favouring outcrossing in ciku flowers is its unique floral morphology where the stigma is situated way above the anthers, i.e. there is spatial separation of the sexual organs *(Figure 1)*. This phenomenon is supported by the fact that only 5–6% of ciku flowers set fruit when bagged *(Table 4)*. Ciku flowers were also found to be self-compatible as assisted self-pollinated flowers managed to set a relatively high percentage of fruit. Self pollination is therefore one of the main modes of pollination in ciku cv. Jantung. But since within-flower pollination cannot occur, the only other means of self pollination is through between-flower pollination within the same tree (geitonogamy).

Table 4. Fruit set of ciku Jantung under different modes of pollination

Mode of pollination	No. of flowers pollinated	No. of fruit set
Open	99	47
Assisted self	96	62
Bagged	103	5

The breeding system identified in this study for ciku Jantung seemed to differ considerably with that of ciku varieties studied in India and in the Phillipines. In India, Nalawadi et al. (1977) studied the floral biology of four ciku varieties and found that maximum stigmatic receptivity occurred on the day of flower opening. High pollen viability of 96–97% was obtained and maximum pollen germinated in 15% sucrose solution. In addition to this, all the four varieties were found to be self-incompatible and needed cross-pollination for fruit set. Problems of fruit shedding, low fertility and high self-sterility in ciku were also reported in other locations of India (Cheema et al. 1954; Hayes 1957). This phenomenon of self-sterility was again seen in the variety, Ponderosa chico of the Phillipines. According to Gonzalez and Feliciano (1953), low sterility in Ponderosa chico was mainly due to the production of defective and nonviable pollen grains. The stigmatic receptivity in Ponderosa chico was noticed only on the fourth day after the emergence of the style from the corolla. However, in another Phillipines' ciku variety, i.e. the native chico, the pollen grains were found to be fertile and this variety was found to be self-compatible.

The variability found in the breeding system of different ciku varieties (local and abroad) tends to suggest that ciku is either very susceptible to environmental changes and/or is a highly heterozygous crop. Another important aspect found here is the high self-sterility existing in ciku varieties of India and the Phillipines, and therefore cross-pollination is vital for fruit set (Gonzalez and Feliciano 1953; Cheema et al. 1954; Hayes 1957). In our local ciku variety, Jantung, although the morphology and its breeding system favour outcrossing, the pollen grains are still self-compatible and allow between-flower pollination to occur extensively. This could be the main determining factor in explaining as to why ciku Jantung variety is a heavy-bearer while the Indian and the Phillipines ciku varieties are shy-bearers. Since it has been documented that different ciku clones can exhibit different compatibility mechanisms (as in the Phillipines) within the same environment, there is therefore a need to look into the floral biology and the pollination systems of other commercial varieties in Malaysia, namely Subang and Betawi.

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