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Floral biology of three mango clones in relation to their pollination efficiency

(Biologi pembungaan tiga klon mangga dan hubungannya dengan kecekapan pendebungaan)

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Key words: *Mangifera indica*, L., anthesis, stigma receptivity, pollen viability, anther dehiscence, pollination biology

Abstrak

Satu siri penyelidikan untuk menghuraikan biologi bunga bagi tiga klon mangga dari segi kecekapan pendebungaan telah dijalankan.

Hasil daripada penyelidikan ini didapati hampir 80% bunga bagi ketiga-tiga klon mangga terbuka pada pukul 8.00 pagi. Pada hari pertama antesis, stigma berada dalam keadaan reseptiviti yang optimum. Pada waktu tengah hari yang sama, cepudebunga merekah dan mengeluarkan debunga yang menunjukkan berlakunya pertindihan fasa staminat dan pistillat bagi klon-klon tersebut. Ketiga-tiga klon menunjukkan kebolehhidupan debunga yang tinggi (81–87%). Pada satu jambak bunga, antesis bunga-bunga hermafrodit tertumpu pada peringkat awal fasa pembungaan (minggu pertama). Bunga jantan terus terbuka dengan banyaknya hampir sepanjang tempoh pembungaan mencadangkan bahawa sumber debunga adalah mencukupi. Walaupun bilangan bunga hermafrodit adalah lebih rendah daripada bunga jantan dalam satu jambak bunga tetapi kekurangan ini boleh diseimbangkan oleh reseptiviti stigma yang berterusan sehingga 4 hari selepas antesis.

Abstract

A series of experiments was initiated to delineate aspects of floral biology of three mango clones in relation to their pollination efficiency.

Experimental results reveal that about 80% of flowers in all clones opened by 8.00 a.m. On the first day of anthesis, stigmas were at their peak receptive condition, and at noon on the same day, the anthers dehisced and shed pollens; indicating an overlapping of staminate and pistillate phases in these clones. Pollen obtained from all the three clones were highly viable (81–87%). Anthesis of hermaphrodite flowers was concentrated at the earlier phase (first week) of flowering of the inflorescence. Male flowers continued to open in large numbers almost throughout the flowering period suggesting that there is an abundant supply of pollen. The lower number of hermaphrodite flowers compared to the males in an inflorescence could be counteracted by the fact that stigma receptivity of the hermaphrodite flowers persist up to 4 days after anthesis.

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Introduction

Mango occupies a premier place in the fruit growing industry in the tropics. In Malaysia, it is still grown on a relatively small scale but there is considerable potential for expansion especially in the northern states of Peninsular Malaysia.

The mango varieties in Malaysia generally exhibit two flowering peaks in a year i.e. in January – February and in July – September. However a lot of irregularities in the flowering habit of many of our local mango varieties have been observed especially in areas where no distinct drought exists before and during the flowering season (Rukayah 1986). Even when the trees flower profusely, there is a tendency for our local varieties to produce a light crop; either due to failure to set fruit or heavy post-fertilization fruit losses. The most popular commercial mango variety (cv. Harumanis) also suffers from this shortcoming (Mokhtar 1986). The problem of inconsistency in the bearing behaviour and frequent low production in mango are not only localized in Malaysia but is a world wide problem as it is of common occurrence in most mango growing areas such as in India (Singh 1948), Florida (Lynch and Mustard 1954), Philippines (Galang and Lazo 1938) and in Hawaii (Nakasone et al. 1955).

Therefore, research emphasis on mango has always been directed towards areas relating to the reproductive physiology. Researchers overseas have outlined a number of factors contributing to the low reproductive success in mango. These include lack of pollination and failure of fertilization (Singh 1954), ovule abortion, and embryo degeneration (Young 1942), inadequate soil moisture (Hayes 1954), pest and disease (Wagle 1928) and low auxin content during fruit development (Singh and Arora 1957). However, not much emphasis has been paid to the efficiency of the breeding system of mango. Some studies on the floral biology of mango has been carried out overseas by Naik and Rao (1943), Singh (1954) and Randhawa and Damodaran (1961) but not much attempt has been made to relate the breeding system of mango to its pollination efficiency.

The role of the breeding system in determining the reproductive success of a plant was emphasized by Bertin and Sullivan (1988). They showed that limitations in the breeding system of a plant can constrain reproduction considerably and even affect the fecundity of the plant. This hypothesis was also supported by works carried out by Frankel and Galun (1977) and Bawa (1980) where the importance of synchronization and co-ordination of events such as anthesis, stigma receptivity, anther dehiscence and pollen vector activity in achieving successful pollination was stressed. Knowledge on these aspects of the breeding system are considerably lacking in our local mango varieties. Therefore, in this paper, a series of experiments was designed to delineate aspects of pollen viability, anthesis, anther dehiscence and stigma receptivity of three mango clones. An attempt is also made to identify and indicate any existing limitation in the breeding system of these clones which might constrain production.

Materials and methods

The trial was carried out over two flowering seasons in 1986 and 1987 at the MARDI Research Station in Kuala Kangsar, using 8 to 10-year old mango trees. The experiments were conducted on three mango clones, viz., Karthakalumban, Simpang Empat and Nang Klang Wan. The experimental design used was the complete randomised design and the data were analysed using Duncan's new multiple range test.

Pollen viability

As soon as the anthers dehisced, pollen

was collected and brought back to the laboratory for viability studies. The viability of pollen was assessed by germinating pollen in an artificial media and then determining the percentage of germination. Pollen was considered germinated when the length of the pollen tube was twice the diameter of the pollen grain. This trial was conducted in two stages. The first stage was concerned with determining the most suitable germination medium for mango pollen, while in stage two, this medium was used to determine the pollen viability of the three clones.

Stage I – Standardization of germination medium The germination medium used in this trial is sucrose solution. To standardize the level of sucrose solution needed for testing pollen germination, preliminary trials were conducted with Karthakalumban pollen grains. Sucrose solution of concentrations 0, 10, 20, 25 and 30% were prepared and pollen was germinated using the Hanging Drop Method (Reyes 1934). For each slide, germination counts were made in five different microscopic fields, and at each sucrose level, 30 such estimations were made. The concentration of sucrose that gave the highest percentage of germination was used as the standard germination medium for mango pollen.

Stage II – Comparative pollen viability study The same technique of pollen germination (Stage I) was adopted here. The viability of three mango clones were tested by determining the percentage of pollen germination after 24 h of incubation. Fifty estimations were made for each clone.

Time and pattern of flower anthesis
Counts of flowers were made on inflorescences obtained from a sample of four plants in each of the three clones studied. Three young inflorescences (before anthesis) per tree were selected and labelled. The number of flowers that opened was recorded daily at two-hourly interval from 8 a.m. to 4 p.m., until flowering was complete. After every count, the flowers were gently removed by using forceps to avoid recounting.

A separate set of data was collected to observe the daily opening pattern of male and female flowers in the Karthakalumban inflorescence.

Anther dehiscence
Dehiscence of the mango anther lobes takes place longitudinally. Just after dehiscence, the anthers changed from bright red to blackish, exposing the white pollen grains. This change in colour of the anther lobes was used as an indicator for determining anther dehiscence.

To study the time of anther dehiscence, four inflorescence in each clone were selected and labelled. About 50 freshly opened flowers (irrespective of sex) within each inflorescence were marked early in the morning before the anthers dehisced. The number of marked flowers with dehisced anthers was recorded at hourly intervals from 9 a.m. to 4 p.m. These observations were carried out over four consecutive days.

Effective pollination period/stigma receptivity
The effective pollination period (EPP) is the period during which the stigmas are receptive, that is, capable of holding pollen and stimulating germination (William and Wilson 1970). EPP was measured by carrying out pollination at four different time intervals after flower opening.

Prior to anther dehiscence, freshly opened hermaphrodite flowers were emasculated and the remaining flowers removed. The panicles were then bagged. At the time of anther dehiscence, the bags were removed and the flowers were hand-pollinated. Similar pollination treatments

were carried out on the second, third and fourth day after flower opening. After 24 h, styles from the hand-pollinated flowers were removed, fixed in ethyl-acetate alcohol and stained in safranin-analine blue (Dionne and Spicer 1957). The styles were then assessed by light microscopy for pollen germination on the stigmatic surface. Stigma receptivity was based on the success of pollen germination on the stigmatic surface.

Results

Pollen viability

Stage I The sucrose solution at 25% concentration gave the highest percentage of pollen germination *(Table 1)* showing its suitability as a standard germination medium. Higher concentrations than this had a retarding effect on pollen germination.

Table 1. Percentage pollen germination at various sucrose concentrations

Sucrose concentration (%)	Mean percentage of pollen germination
0	0 c
10	46.7 d
20	52.7 c
25	83.7 a
30	76.0 b
C.V. (%)	14.8

Means with the same letters are not significantly different from one another at $p < 0.05$

At 0% sucrose no germination occurred; after 2–3 h of incubation, bursting of mango pollen was observed.

Stage II All the three clones exhibited high pollen viability of 80% to 87%. *(Table 2).* Pollen viability in Nang Klang Wan (84.5%) and Karthakalumban (87.4%) were significantly higher than that of Simpang Empat (80.7%).

Anthesis

Time of anthesis Flower opening from 6 a.m. to 8 a.m. was high and consistent in all the three clones. However, after 8 a.m., flower opening became very inconsistent and indefinite with high variation between samples *(Table 3).*

Pattern of flower opening The daily pattern of flower opening in the inflorescences of the three mango clones

Table 2. Percentage of pollen germination in three mango clones

Clone	Mean percentage of pollen germination
Simpang Empat	80.7 b
Nang Klang Wan	84.5 a
Karthakalumban	87.4 a
C.V. (%)	8.4

Means with the same letters are not significantly different at $p < 0.05$

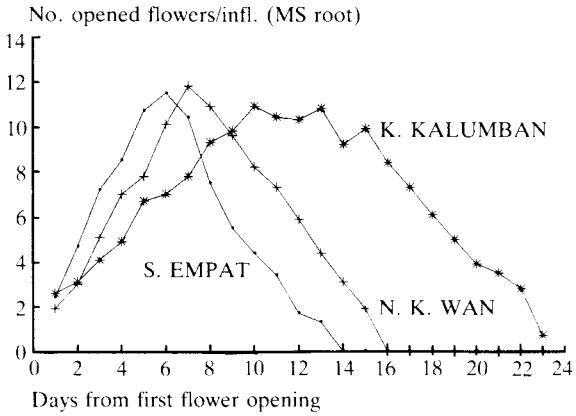

Figure 1. Pattern of flower opening in three mango clones

Table 3. Mean percentage of flower anthesis at two-hour intervals in three mango clones

Clone	Mean percentage of flowers opened at				
	6–8 a.m.	8–10 a.m.	10–12 a.m.	12–2 p.m.	2–4 p.m.
Simpang Empat	84.1 ± 5.3	4.2 ± 2.6	2.9 ± 2.5	7.5 ± 1.9	1.3 ± 0.8
Nang Klang Wan	88.5 ± 5.4	8.7 ± 2.9	2.6 ± 1.4	1.5 ± 0.7	1.7 ± 1.9
Karthakalumban	80.3 ± 5.6	5.5 ± 2.7	4.8 ± 1.5	4.9 ± 2.6	4.2 ± 2.7

is shown in *Figure 1*. The flowering behaviour of the clones was compared using the Kolmogorov-Smirnov two-sample test. Results indicate significant differences between the flowering patterns of Karthakalumban and Simpang Empat and between Karthakalumban and Nang Klang Wan (both significant at $p < 0.05$) *(Figure 1)*. A more symmetrical curve was obtained for flower opening in Karthakalumban inflorescences compared to those of Simpang Empat and Nang Klang Wan. In addition to this, Karthakalumban inflorescences were longer lived and opened flowers reached their peak abundance 4–5 days later than the other two clones.

The opening pattern of male and hermaphrodite flowers in inflorescences of Karthakalumban is shown in *Figure 2 (a and b)*. The flowering curves of the two flower types were found to be significantly different from each other in both years (Kolmogorov-Smirnov two-sample test, $p < 0.05$). The male flowers reached their peak abundance later than the hermaphrodite flowers and were present in the inflorescence for a longer period of time. At the earlier phase of flowering, a relatively greater number of hermaphrodite flowers (42.8%) appeared. However, towards the end of the flowering period, many more male flowers were observed (88.9%).

Anther dehiscence

The pattern of anther dehiscence at hourly interval after 9 a.m. is presented in *Table 4*. The experiment showed that anthers start dehiscing at 9 a.m. and continued to do so until 3 p.m. Maximum dehiscence took place between 11 a.m. and 12 noon. By 12 noon, 70% of the anthers had already dehisced. Similar patterns of dehiscence were observed in all three clones. This was confirmed by carrying out analysis of variance of the data which showed that there were no significant differences among the means of the three clones. However, there were significant differences ($p = 0.05$) among the time intervals as discussed above.

Effective pollination period/stigma receptivity

Results *(Table 5)* indicate that flowers were most receptive on the first day of anthesis (81.3%). Receptivity decreased as the flower aged. This trend was maintained in all three clones as no significant differences were obtained among them. However, means between different days of anthesis were highly significant ($p < 0.05$). Consistency in results was only detected on the first and second day of anthesis *(Table 5)* indicating that it is only possible to generalize on receptivity during this particular period.

Discussion

From recent studies carried out by Bierzychudek (1981) and Rathcke (1983) it was concluded that pollination limitation of flowering plants mediated through the availability of compatible and viable pollen are more frequent than has been generally assumed. However, a high pollen viability of 80–87% displayed by the mango clones studied seem to rule out pollen viability as a contributing factor in

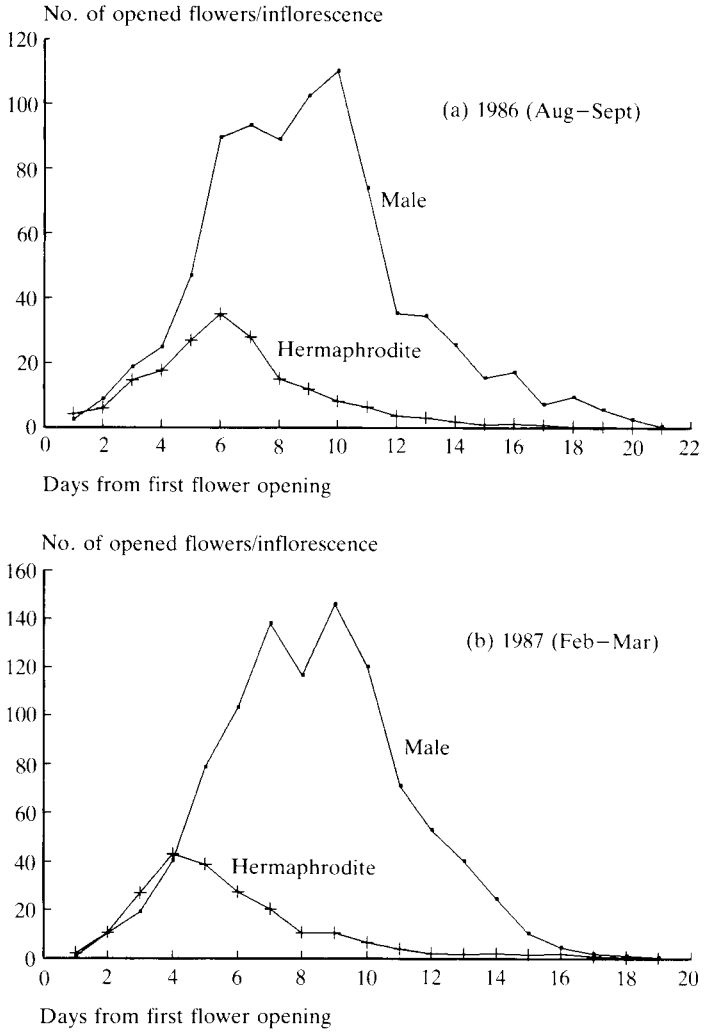

Figure 2. Pattern of male and hermaphrodite flower opening in Karthakalumban inflorescence

Table 4. Mean anther dehiscence at hourly interval in three mango clones

Time	Percentage of anthers dehisces in			
	Simpang Empat	Nang Klang Wan	Karthakalumban	Overall mean
9 a.m.	3.4	0.0	0.5	1.3 cd
10 a.m.	6.3	1.9	18.7	9.0 bc
11 a.m.	28.8	37.7	26.6	31.0 a
12 p.m.	35.7	37.0	34.7	35.8 a
1 p.m.	9.2	1.8	7.7	6.2 cd
2 p.m.	15.7	18.2	11.8	15.2 b
3 p.m.	1.0	3.3	0.0	1.4 cd
4 p.m.	0.0	0.0	0.0	0.0 d

Overall means followed by the same letter are not significantly different from one another at $p < 0.05$ according to Duncan's new multiple range test

Table 5. Percentage stigma receptivity at daily intervals in three mango clones

Day of anthesis	Percentage stigma receptivity in				
	Simpang Empat	Nang Klang Wan	Karthakalumban	Mean	C.V. (%)
1	79.5	84.7	79.7	81.3 a	5.6
2	56.5	50.3	48.4	51.8 b	8.4
3	23.3	20.0	16.7	20.0 c	20.4
4	1.7	3.3	5.0	3.3 d	65.7

Means with the same letters are not significantly different from one another at $p < 0.05$

limiting reproduction. This finding also conformed to that of Spencer and Kennard (1955) where a high percentage of pollen germination ranging from 72–94% was obtained from germinating 15 varieties of mango pollens in 20% sucrose with 1% agar solution. A high initial pollen viability is displayed in most mango varieties, but according to the work of Spencer and Kennard (1955) and Young (1955) mango pollen tend to be susceptible to changes in the environmental conditions which can reduce its effectiveness drastically. This feature is also evident in the present study where the pollens tend to burst after 3 h in water. Thus, implying that continuous rain for 3 h or more in late mornings (when the anthers shed pollens) can permanently destroy mango pollen and thus limit its pollination process.

Results on anther dehiscence and stigma receptivity reveal that there is a distinct overlap in the staminate and the pistillate phases of the mango clones studied. In all the three mango clones, the anthers dehisced at about noon on the first day of anthesis when the stigmas were in the most receptive condition. Similar results were obtained by Wagle (1929) in cv. Alphonso mango and Singh (1952) on cv. Dasheri and Langra. This overlap of sexual phases in mango has an important implication on its breeding system as it indicates a strong tendency of selfing occurring in mangoes. However, the success of pollination by selfing will greatly depend on the existing compatibility mechanism in the plant which unfortunately was not investigated in this present study.

A high pollen viability and high stigma receptivity are not sufficient in attaining successful pollination; there must also exist some kind of co-ordination and synchronization of events like anthesis, pollen shedding and stigma receptivity together with pollen vector activity to ensure successful pollination. Results from the present study and those carried out by Poon, et al. (1982) on insect pollination of mango in Malaysia revealed that co-ordinations of these events are quite distinctly present in mango. Mango flowers tend to open early in the morning (8 a.m.) before the anthers dehisce. As soon as the flower opens, nectar is secreted (Poon et al. 1982) and the stigmas become receptive. The anthers dehisce and shed pollen only 2–3 h after anthesis. At the time of nectar secretion and anther dehiscence i.e. from 8 a.m. to before 12 noon the pollen nectar activity is at its maximum, thus proving that synchronization and co-ordination of events do exist in mango.

Results summarised in *Figure 2 (a* and *b)* show that there is a marked difference in the number and phenology of male and hermaphrodite flowers. The males are produced in large numbers and last longer on the inflorescence compared to the hermaphrodite flowers. Generally, a greater number of male flowers in any inflorescence is necessary to add to the conspicuous floral display in attracting pollinators (Primack and Lloyd 1960). Another advantage of having a greater

number of male flowers is that it increases the chance of pollen removal from flowers by pollinators, thus increasing the efficiency of pollination.

However, there seems to be a limitation in the number of ovules/ hermaphrodite flowers present *[Figure 2 (a* and *b)]*. This low number of hermaphrodite flowers compared to males were also found to occur in a number of other mango varieties found overseas namely cv. Dasheri, Neelum, Bombay and Faigri (Naik and Rao 1943 and Mallik 1957). In the present study the limited number of hermaphrodite flowers on an inflorescence become increasingly prominent after a period of one week *[Figure 2 (a* and *b)]* and this tends to imply that the abundant production of male flowers after this one week period seem to have little contribution in assisting pollination especially if selfing is the main mode of reproduction. In addition, the abundant male flowers present after this one week period will tend to use up valuable energy resources which could have been diverted to the development of the fruitlets.

According to Frankel and Galun (1977) low number of hermaphrodite or female flowers can be counteracted by the persistance of stigma receptivity over a longer duration of time. This feature is evident in all the 3 mango clones as the stigma receptivity in these clones were found to persist up to four days. This is in conformity with the findings of Singh (1954) who worked on cv. Dasheri and Langra. This tendency to persist increases the probability of pollen grains encountering receptive stigmas, thus increasing the efficiency of pollination in mango.

Generally in the theory of sexual selection a positively skewed flowering pattern is favoured where a higher number of flowers open earlier in the flowering period to increase the attractiveness of the inflorescence to pollinators. Since flowers that open earlier are necessary for pollinator attraction but are unlikely to be visited, it is more economical for male flowers to open earlier. The results obtained from the present study *[Figure 2 (a* and *b)]* however does not comply with the above argument. The hermaphrodite flowers reach their peak abundance much earlier than the males *[Figure 2 (a* and *b)]*. Similar findings were also obtained by Jawanda and Singh (1960) who worked on 5 varieties of mango. These results reveal that there may be an initial wastage of female function in mango.

The present findings are also of practical relevance especially in the breeding of new cultivars and proper pollination for mango where seed set is required. The time of anther/stigma maturation is important in determining the effective period during which hand or assisted pollination may be carried out. In mango, necessary manipulations for crossing work should be carried out early in the morning and on freshly opened flowers when receptivity is at its highest. Also, assisted pollination should be carried out in the earlier part of the flowering period of an inflorescence as this is when most of the hermaphrodite flowers open.

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