POLLEN ANALYSIS IN *MANGIFERA* IN RELATION TO FRUIT-SET AND TAXONOMY*

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THE number of flowers in a panicle of mango (*Mangifera indica* L.) varies between 2,000 to 6,000 according to variety, of which 1–35 per cent. are bisexual, the rest being functionally male. About 13–28 per cent. of these bisexual flowers set fruits, but only 0·1–0·25 per cent. reach maturity and are harvested (Sen, 1939; Naik, 1943). Bijhouwer (1937) in Java also observed that approximately 99 per cent. of the bisexual flowers drops either before or after fertilisation. In order to find out the cause for such a huge drop in the percentage of fruit setting in proportion to the number of flowers, the pollen grains of a number of varieties were examined to determine if there is any male sterility.

Wodehouse (1935) and others have stressed on the importance of pollen morphology in taxonomy, as they have found characteristic variations in the form of pollen grains correlated with the evolution of the angiosperms. Consequently, pollen morphology has been utilised as an important criterion in the classification of some genera and families. With a view to elucidate the interrelationship among the species of *Mangifera*, an examination of the pollen grains of 14 species of the genus (including some varieties of mango) was undertaken to find out the taxonomic significance of pollen morphology, if any, in *Mangifera*.

MATERIALS AND METHOD

The mature pollen-grains of the mango varieties were mounted on slides in field in Methyl-green Glycerine jelly, prepared according to Wodehouse's method (1935). These were subsequently examined in the laboratory under the microscope.

The pollen-grains of 13 species of *Mangifera*, belonging to both the 'Sections' were examined from herbarium specimens, obtained from the Buitenzorg and Singapore Herbaria.

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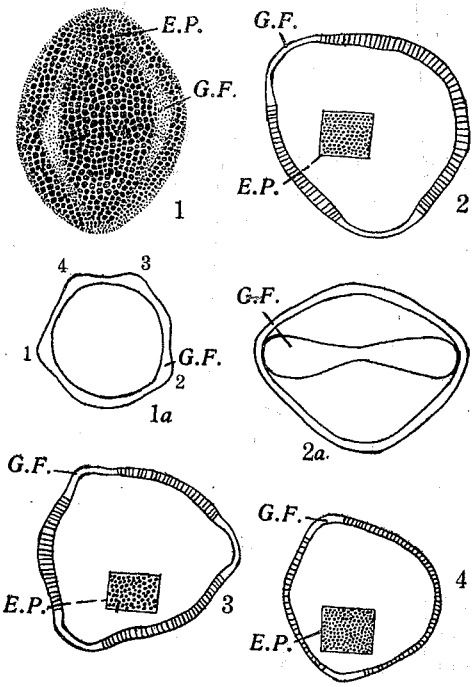
The measurements were made using a Zeiss Ocular micrometer. More than 500 grains were always counted in each case to determine the percentage of sterile grains and the average size. The drawings were made with the help of a Zeiss Drawing Prism.

OBSERVATIONS

The mature pollen grains in all the species of *Mangifera*, including the varieties of mango (*M. indica*), show similar morphology in their elliptic shape and in possessing a closely pitted exine with 3 long tapering, sharply defined furrows, containing a large germpore at the centre of each (*tricolpate type*; Figs. 1–4). The average size of the grains is almost same; exceptions are found in two varieties of mango (*Bhuto Bombai* and *Safdar Pasand*) where 3 per cent. of the grains are of giant size (42–45 μ) and possess 4 germpores instead of 3 (Fig. 1 *a*).



FIGS. 1–4. Fig. 1. Surface view of pollen of *M. indica*, showing pitted exine and germinal furrows; Fig. 1 *a*. Optical section of a grain with 4 germinal furrows of *M. indica* var. *Kalapahar*; Fig. 2. O.S. of *M. cæsia*; Fig. 2 *a*. Side view of *M. cæsia*; Fig. 3. O.S. of *M. lagenifera*; Fig. 4. O.S. of *M. odorata*. G.F., Germinal furrow; *E.P.*, Exine pattern. Fig. 1 *a*, ×600; rest, ×1,000.

Observations on the size variation of the grains in different species, and the percentage of crushed, crumpled or empty grains are tabulated below:—

TABLE I

Size variation and percentage of sterile pollen grains in different species of Mangifera *and varieties of Mango* (M. indica)*

	Name	Range in size in μ	Size of Majority of grains in μ	Percentage of crumpled or empty grains
1	*M. pentandra* Hook. f. ..	20–23	22	23·5
2	*M. laurina* Bl. ..	20–39	26	1·5
3	*M. longipes* Griff. ..	26–36	30	3·7
4	*M. zeylanica* Hook f. ..	26–30	29	7·9
5	*M. sclerophylla* Hook f. ..	32–45	39	6·9
6	*M. altissima* Blanco ..	18–26	23	8·7
7	*M. similis* Blume ..	29–33	32	3·8
8	*M. quadrifida* Jack. ..	32–39	36	40·0
9	*M. monandra* Merr. .	23–32	26	2·0
10	*M. lagenifera* Griff. ..	26–39	37	2·0
11	*M. fœtida* Lour. ..	36–45	39	1·0
12	*M. odorata* Griff. ..	25–36	32	4·7
13	*M. cæsia* Jack. ..	29–36	35	6·0
14	*M. indica* Linn.			
	var. 1 Himsagar ..	23–32	26	2·0
	,, 2 Jehanara ..	23–29	24	5–7·5
	,, 3 Laskarshikhan ..	23–34	30	4–9
	,, 4 Rogni ..	24–32	28	3–6
	,, 5 Panja Pasand ..	24–32	26	3–4·5
	,, 6 Bhuto Bombai ..	23–42 (3% 42 μ)	27	3–4·5
	,, 7 Kohitur ..	20–32	26	5·2
	,, 8 Safdar Pasand ..	26–45 (2% 45 μ)	28	3·8
	,, 9 Sah pasand ..	23–29	25	4·0
	,, 10 Kishenbhog ..	23–29	26	3·0
	,, 11 Kalapahar ..	23–29	26	4·5
	,, 12 Anupam ..	22–32	26	10·5
	,, 13 Shadwalla ..	23–30	27	3·4
	,, 14 Jhumko Fazli ..	24–32	27	4·6
	,, 15 Pairie ..	26–32	27	6·2
	,, 16 Langra :	26–32	27	4·4
	,, 17 Kalapady :	19–32	27	4·2
	,, 18 Alphonso ..	26–32	29	6·1
	,, 19 Apoos Canara ..	23–32	26	12·3
	,, 20 Myelpellan (Polyembryonic) ..	26–32	29	4·2
	,, 21 Kurukkan (polyembryonic) ..	22–36	26	9·0
	,, 22 Sunderprosad ..	22–30	27	5·7
	,, 23 Daseri ..	22–35	27	7·0
	,, 24 Bombai ..	23–29	26	4·0
	,, 25 Baramasia ..	21–32	26	1·0
	,, 26 Latra ..	23–36	26	5·1
	,, 27 Fazli ..	23–36	30	7·0

* The species 1–9 and 14 belong to *Section I* and 10–13 belong to *Section II*. The varieties 1–14 occur in Bengal and the rest from Bihar, U.P., Bombay and Madras.

From the table it will be seen that the percentage of imperfect grains is generally low, varying between 1·0–12·3 per cent. in the varieties

of mango. In *M. pentandra* and *M. quadrifida* however, the percentages are much higher (23·5 and 40·0). There is some size-variation in the grains but the range is not appreciable, except in *M. sclerophylla*, *M. fœtida* and the giant grains of two varieties of mango (*M. indica*).

Examination of styles from open flowers stained in Acid Fuchsin and Light Green after clearing in lactic acid has shown that the pollen grains germinate on the stigma and give out long tubes, staining red, passing through the transparent style. This supplies direct evidence for the germination of pollen grains on the stigmas of flowers.

In addition to the tests for determining the structural regularity in the pollen grains and their ability to germinate on the stigmas, some observations were made to find out the extent of pollination in nature. A random sampling of 200 flowers on the next day morning after opening, in a fluid mixture of 6 per cent. formalin in 5 per cent. alcohol, gives the following data regarding pollination in 7 varieties.

TABLE II

Percentages of pollinated stigmas in a random sampling of open flowers

Variety	Percentage of stigma pollinated
Kalapahar	6·0
Safdar Pasand	14·3
Enayet Pasand	20·0
Sah Pasand	35·0
Langra	3·3
Laskarshikhan	11·0
Kishenbhog	Nil

The table shows that the percentage of stigmatic surfaces receiving pollen grains is rather low. It may be slightly higher under actual conditions, as some of the grains might have been dislodged during preservation. However there is no doubt that a large number of stigmas remain unpollinated. It has been further observed that the number of stigmas with germinated pollen grains *in situ* is still less.

CONCLUSION

Drop in the fruit-set percentage and its causes.—It is well known that fruit-setting is dependent on the normal development of the male and female reproductive units (the pollen and the ovule), and the successful pollination of the stigmas ultimately effecting the fertilization of the female gametes.

During the course of the present work, an attempt was made to see if there is any defect in the normal development of pollen grains. The study has shown that the majority of the grains in a large number of varieties, occurring in different regions of India, are normal and plump. The percentage of apparently sterile grains is negligible, being only 1·0–12·3 per cent. The formation of normal pollen grains is also indicated by the regular pairing and disjunction of chromosomes during meiosis in PMC (Mukherjee, 1949 *b*). Hence the drop in fruit-set is not due to any defect in the pollen-grains. This view was also expressed by Popenoe (1917).

The next important factor is the ability of the grains to germinate on the stigma. To find out the percentage of pollen-germination *in vitro*, a large number of observations were made by the 'hanging drop' and plate methods under varying temperature and humidity conditions and using various concentrations of cane-sugar, glucose and maltose in distilled water, as also with agar (1·5–2·0 per cent.) and/or gelatine, along with yeast extract or crushed style; but none of them proved successful in producing long pollen tubes, though short tubes were found to come out in some. It appears therefore, that the pollen-germination requires some specific conditions. Direct observation of the styles, however, has shown that the pollen grains germinate on the stigma and produce long tubes. This, however, is affected by rain, fog (Firminger, 1874), or sudden lowering of temperature at night (Popenoe, 1917).

Another important factor investigated is the percentage of stigmas pollinated under natural conditions. It has been found that only 14–35 per cent. of the stigmas generally receive pollen grains. This corroborates Naik (1943), who has observed that about 66 per cent. of the flowers do not receive any pollen grains. He has also shown that by artificial pollination the fruit-setting can be increased; and the harvested fruits actually increased from 0·1–0·25 to 2·4 per cent. of the bisexual flowers.

The drop in the percentage of fruit-set is therefore primarily due to failure in pollination in more than 66 per cent. of the flowers, and to the climatic conditions affecting pollen germination on the stigma. But from a practical standpoint, the question of pollination is relatively unimportant as the success depends on the percentage of harvested fruits. After fertilization and formation of the young embryo (fruit-set), the development of the fruits to maturity is more a physiological problem connected with nutrition. It is obvious from the fact that only a small percentage of the flowers, which are fertilized, attain maturity. Supply of fresh nutriment to the soil at the proper time and other cultural treatments should help in the development of higher percentage of fruits.

Taxonomic significance of pollen morphology in Mangifera.—The observations on 14 representative species of both the sections of the genus indicate similarity in the morphology of the grains in all the species, with a close range of variation in size, except in *M. sclerophylla* and *M. fœtida*. This similarity in size is significant in view

of the fact that size-difference in pollen grains in the different species is generally correlated with polyploidy or aneuploidy in a genus, as has been found in *Quercus*, *Allium*, *Triticum*, *Euphorbia*, *Rosa*, *Tradescantia*, etc. (*cf.* Darlington and Ammal, 1945; Cain, 1944; Bhaduri, 1942). It therefore suggests a stability in chromosome number ($2n = 40$) in majority of the species of *Mangifera* which is also indicated by the cytological study of some species of the genus (Mukherjee, 1949 *b*).

The taxonomic revision of the genus containing 41 species (Mukherjee, 1949 *a*) has shown that it is rather compact and homogeneous in its floral range, showing variations mainly in the (1) presence or absence of the disc, (2) pentamerous or tetramerous arrangement of the floral parts, (3) presence of 10 or 5 stamens, of which 5, 3 or 1 are fertile, *i.e.*, bear well-developed anthers, (4) nature of ridges on the petals, and (5) nature of pubescence and branching on the panicle. An examination of these characters has shown that they are intergrading in the different species, without any sharp discontinuous change. The pollen morphology also suggests this homogeneous range with intergrading characters in the species comprising the genus, as indicated by the study of external morphology.

The presence of 'giant grains' and its significance.—One of the interesting findings in pollen-analysis is the presence of 'giant grains' in two varieties of mango—*Safdar Pasand* and *Bhuto Bombai*. The presence of such grains in some plants is due to the formation of a restitution nucleus through the failure of second division after meiosis in PMC, ultimately leading to the duplication of chromosomes in the male gamete (Stebbins, 1941; Mehra, 1946). When such a gamete successfully unites with an ordinary female gamete having $n = 20$ chromosomes, it will lead to the formation of a progeny with higher chromosome numbers. When such a plant survives and becomes stable, it is likely to show giganticism. Hence breeding of these two varieties with some other suitable variety as female parent may yield interesting results.

SUMMARY

Pollen analysis of 27 cultivated varieties of mango (*M. indica*) shows similarity in size (23–29 μ) and morphology, and an apparent sterility of 1·0–12·3 per cent. which is negligible considering the huge number of flowers (2,000–6,000) in each panicle.

Direct evidence of germination of pollen grains has been obtained, but it requires specific conditions and is affected by weather changes such as rain, fog or a sharp drop in temperature during night. An examination of stigmas from random sampling in field shows that more than 66 per cent. of the bisexual flowers remain unpollinated.

The huge drop in fruit-set (13–28 per cent. of the bisexual flowers only setting fruit) appears to be due primarily to failure of pollination, and pollen germination caused by adverse weather conditions during the flowering season. The problem of increasing the percentage of harvested fruits is mainly a physiological one, namely, of supplying

nutrition to the developing embryos, as all the fertilized flowers do not develop to mature fruits.

Two varieties of mango (*Bhuto Bombai* and *Safdar Pasand*) show 3 per cent. giant pollen grains with 4 germpores instead of 3, suggesting formation of a restitution nuclei with double the number of chromosomes.

Pollen analysis in 14 other species of *Mangifera* from herbarium specimens shows a similarity in their size and morphology. All the species have *tricolpate* grains, with pitted exine and 3 germinal furrows with a central germpore in each. This suggests the homogeneous nature of the genus, as already indicated by external morphology and chromosome numbers ($2n = 40$).

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