MEGASPOROGENESIS AND FEMALE FERTILITY IN THREE EDIBLE TRIPLOID BANANAS

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Introduction

The wild bananas are highly seed fertile and show regular development of a Normal type of monosporic eight-nucleate embryosac. The edible bananas are variably seed sterile as consequence of several distinct but complementary causes; their female cytology is so far known in several edible diploids and in their diploid derivatives. In them, varying proportions of viable spores are formed but multiple archesporia and various cytological irregularities result in a low frequency of mature embryo-sacs (2-36%). Subsequently, failure of fertilization and failure of post-fertilization development lead to a high degree of seed sterility (Dodds, 1945).

Edible triploid bananas are variably seed sterile and the number of seeds produced is characteristic of the clone though very variable as between bunches (Cheesman & Dodds, 1942; Shepherd, 1954, 1959). Some clones (e.g. members of the Cavendish group) have never yielded seeds at all; others produce one or several, or even many seeds per bunch. These seeds usually yield polyploid plants. With haploid pollination, the majority of seedlings are tetraploid; minorities are heptaploid, diploid, triploid and aneuploid. Evidently the functional female cells are most commonly triploid or hexaploid (Cheesman & Dodds, 1942; Larter, 1948; Shepherd, 1959). Banana breeding rests upon this fact or, more precisely, upon the fact that the triploid 'Gros Michel' banana produces rare functional embryo-sacs of which the majority of those that bear viable zygotes prove to have had triploid eggs (Larter, 1948, Table 1). The cytology of polyploid spore production in diploid hybrids of seeded bananas is quite well known (Dodds & Pittendrigh, 1946; Dodds & Simmonds, 1946); whether or not the cytology of the process is similar in the edible triploids is a question of some importance, as will appear below.

There are, therefore, two immediate problems, namely: the morphological expression of female sterility and the origin of polyploid spores. The results presented in this paper have some bearing upon these problems.

Materials and Methods

Three edible triploid banana clones ($2n = 3x = 33$) were chosen for this investigation, primarily on the basis of their relatively high female fertilities. They were: 'Bluggoe' (I.C.T.A. Type No. 11); 'Pisang awak' ('Awak legor' of earlier publications — I.C.T.A.

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Type No. 12); and 'Mysore' (I.C.T.A. Type No. 6). All three are of *acuminata-balbisiana* hybrid origin, the first two being members of the ABB Group, the third a member of the AAB Group (Simmonds and Shepherd, 1955; Simmonds, 1959, Ch. V).

The outer bracts of young inflorescences were removed as the inflorescence emerged from the pseudostem. Ovaries were taken from several hands then and at daily intervals until after "receptivity" (i.e. readiness for pollination). Thus series of samples ranging between −5 and +5 days in age (receptivity = 0) were used. Ovaries were skinned, and the axes, bearing the ovules embedded in mucilage, were fixed in Carnoy and Craf, embedded, cut at 30 μ and stained in Heidenhain's haematoxylin. The quantitative data given below were based on one inflorescence of each clone but some qualitative results and drawings have been based on other fixations.

Results

(*a*) *Morphological*

The normal banana embryo-sac is of the monosporic eight-nucleate type. The megaspore mother cell (MMC) lies towards the micropylar end of the nucellus some distance away from the characteristic columnar cells of the micropylar pad (Dodds, 1945, Fig. 6). Growth of the megaspore takes up the space between the MMC and the pad and the presence or absence of this space (conveniently called the "micropylar gap") is a useful index of the occurrence of growth. With this character in mind, the following classification of ovules was used (cf. headings in Tables 1-3):

A: premeiotic MMCs and meiotic stages.

B: micropylar gap present (i.e. little or no growth had occurred); degenerate micropylar spore or spores present (i.e. meiosis, partial or complete, had taken place); chalazal (potentially functional) spore morphologically good or degenerate, according to age.

C: micropylar gap present; degenerate micropylar spores absent (i.e. meiosis had not occurred); surviving cell morphologically good or degenerate.

D: micropylar gap absent; immature embryo-sacs bearing 0-5 nuclei; morphologically normal or more or less abnormal (e.g. 1, 3 or 5-nucleate or cell shape or staining abnormal).

E: as D but cells 6-8 nucleate; variously abnormal, ranging to 8-nucleate cells that were virtually complete embryo-sacs except for details of cell-shape or size, nuclear disposition, etc.

F: mature embryo-sacs.

Difficulties of classification were not great except that group A overlaps group C; the former (A) includes premeiotic MMCs which could not certainly be distinguished from cells (C) which were old enough to have gone through meiosis but in fact had not done so. In practice, cells were classified as A if they were morphologically good and were associated with meiotic stages (e.g. line 1 of Table 2), as C if they were morphologically abnormal (e.g. much increased in size, shrivelled, enucleate, densely staining) and were associated with post-meiotic phases.

Examples of the various behaviours are shown in Figs. 1 and 2 and results are summarized in Tables 1-3 from which the conclusions are: (1) that embryo-sac development in many ovules is markedly delayed with respect to ovary development and that

Table 1. *Megasporogenesis in an edible triploid banana, 'Mysore'. For conventions see text.*

Day	Frequencies percent of cells in various stages						Cells
	A	B	C	D	E	F	
−2	0	56	40	4	0	0	50
−1	0	54	40	6	0	0	50
0	0	47	46	5	2	0	100
1	0	40	50	8	1	0	50
2	0	40	58	2	0	0	50
3	0	28	52	18	1	0	50
4	0	24	56	12	8	0	25

Table 2. *Megasporogenesis in an edible triploid banana, 'Bluggoe'. For conventions see text.*

Day	Frequencies percent of cells in various stages						Cells
	A	B	C	D	E	F	
−4	95	5	0	0	0	0	80
−3	33	62	3	3	0	0	39
−2	10	85	2	4	0	0	52
−1	0	100	0	0	0	0	32
0	0	75	11	14	0	0	92
1	0	66	9	24	2	0	58
2	0	76	0	15	9	0	34
3	0	63	3	25	9	0	32
4	0	59	5	23	14	0	22
5	0	23	14	36	27	0	22

it sometimes ceases altogether; (2) that mature embryo-sacs are very rare or absent except in 'Pisang awak'; (3) that the three clones differ from one another in respect of frequencies and timing of the various morphological events.

Table 3. *Megasporogenesis in an edible triploid banana, 'Pisang awak'. For conventions see text.*

Day	Frequencies percent of cells in various stages						Cells
	A	B	C	D	E	F	
−5	0	23	23	43	10	0	30
−4	0	22	22	40	16	0	50
−3	0	15	40	18	25	2	55
−2	0	6	30	12	45	6	33
−1	0	21	21	21	26	12	43
0	0	10	38	14	31	7	29
1	0	10	20	37	33	0	30
2	0	4	39	27	23	8	26

The behaviour of the three clones may be summarized thus:

'Mysore': premeiotic and post-meiotic failure high; embryo-sac development rare, irregular and late.

'Bluggoe': premeiotic failure slight; meiosis late; post-meiotic failure very high; embryo-sac development late and irregular.

'Pisang awak': premeiotic failure high; meiosis early (correctly timed?); post-meiotic failure moderate; embryo-sac development irregular but correctly timed and sometimes successful.

A note may conveniently be added here on the subject of the occurrence of antipodal walls. In *Musa acuminata* and related edible diploids, they were not recorded by Dodds (1945) and have not been seen by the writer. Nor were they seen in 'Mysore'; but in 'Bluggoe' and 'Pisang awak' they did sometimes occur and examples are shown in Fig. 2. They occur also in wild *Musa balbisiana* (though could not be certainly identified

Fig. 1. Examples of normal and aberrant embryo-sac development; **a**—'Bluggoe', x 3100; **b-d**—'Pisang awak', x 750; **e-m**—'Mysore', x 800. In the following notes, the capital letters (A-F) in brackets refer to the classification of the cell as in section 3(*a*) of the text and Fig. 3. **a**—the first division of meiosis, an irregular (triploid) anaphase with lagging chromosomes and a non-congressed univalent (A, −4 days); **b**—two large micropylar nuclei (probably hexaploid) (D, −5 days); **c**—young embryo-sac with only seven nuclei (contrast nuclear size with preceding example) (E, −5 days); **d**—a semblance of cellular organization but failures of polarity and nuclear division have occurred (E, −3 days); **e**—a single large nucleus in an enlarged cell, probably hexaploid (D, −1 day); **f**—at least part of a meiosis has occurred but the arrangement of the micropylar "spores" suggests that one or more of them was a microcyte (B, −1 day); **g**—a 1-nucleate cell in which meiosis has not occurred (C, 0 days); **h**—8-nucleate but polarity and nuclear differentiation have failed (E, 0 days); **i**—compare with the preceding example—this cell is morphologically nearly normal but is in a state which should have occurred several days earlier (E, 0 days); **j**—a morphologically normal but probably hexaploid embryo-sac (F, 0 days); **k**—2-nucleate, showing failure of polarity and cell division (D, +1 day); **l**—2-nucleate, the nuclei having the appearance of the polars whose position they occupy (D, +3 days); **m**—complete failure of polarity and undefined mitotic abnormalities (E, +3 days).

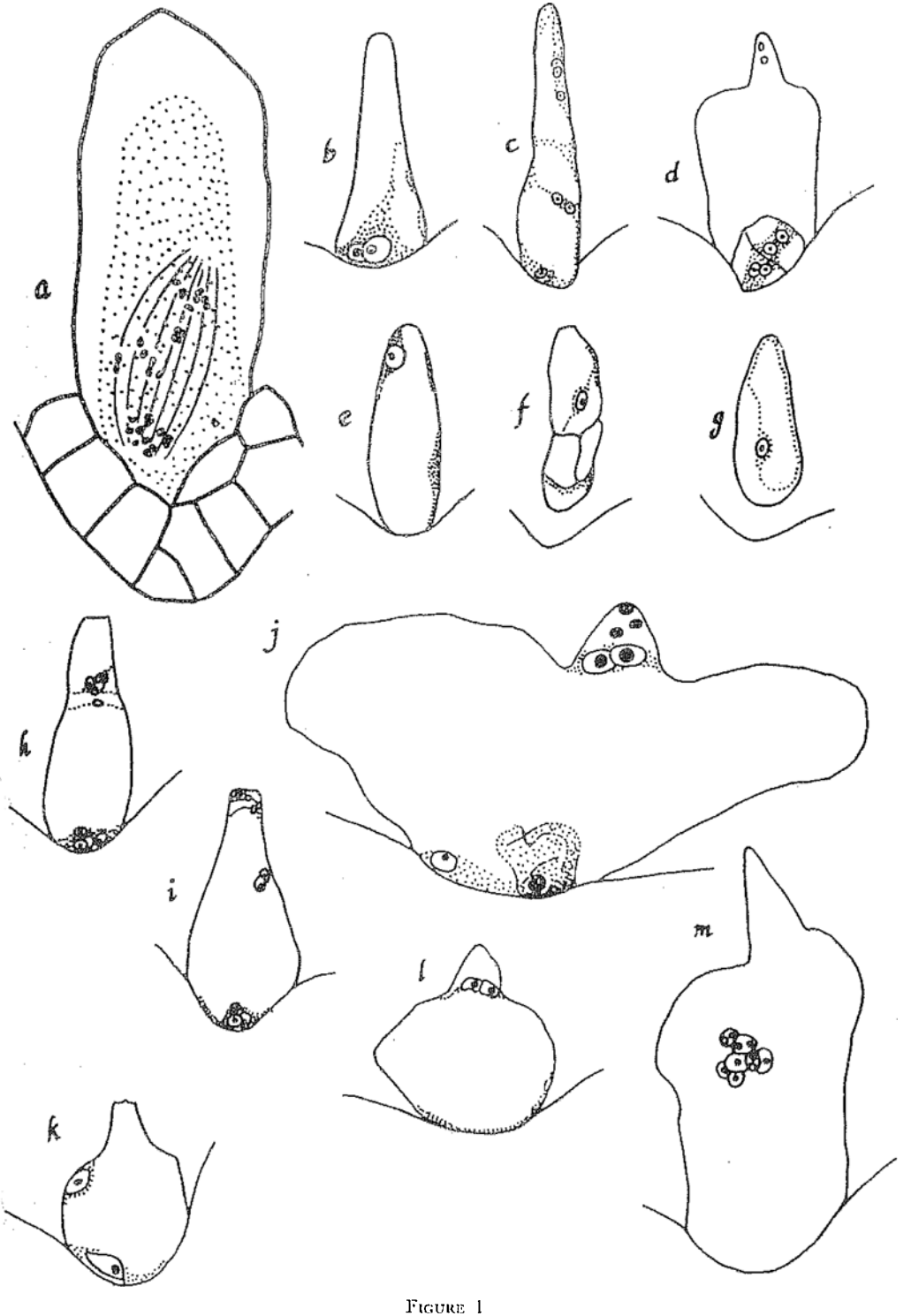

FIGURE 1

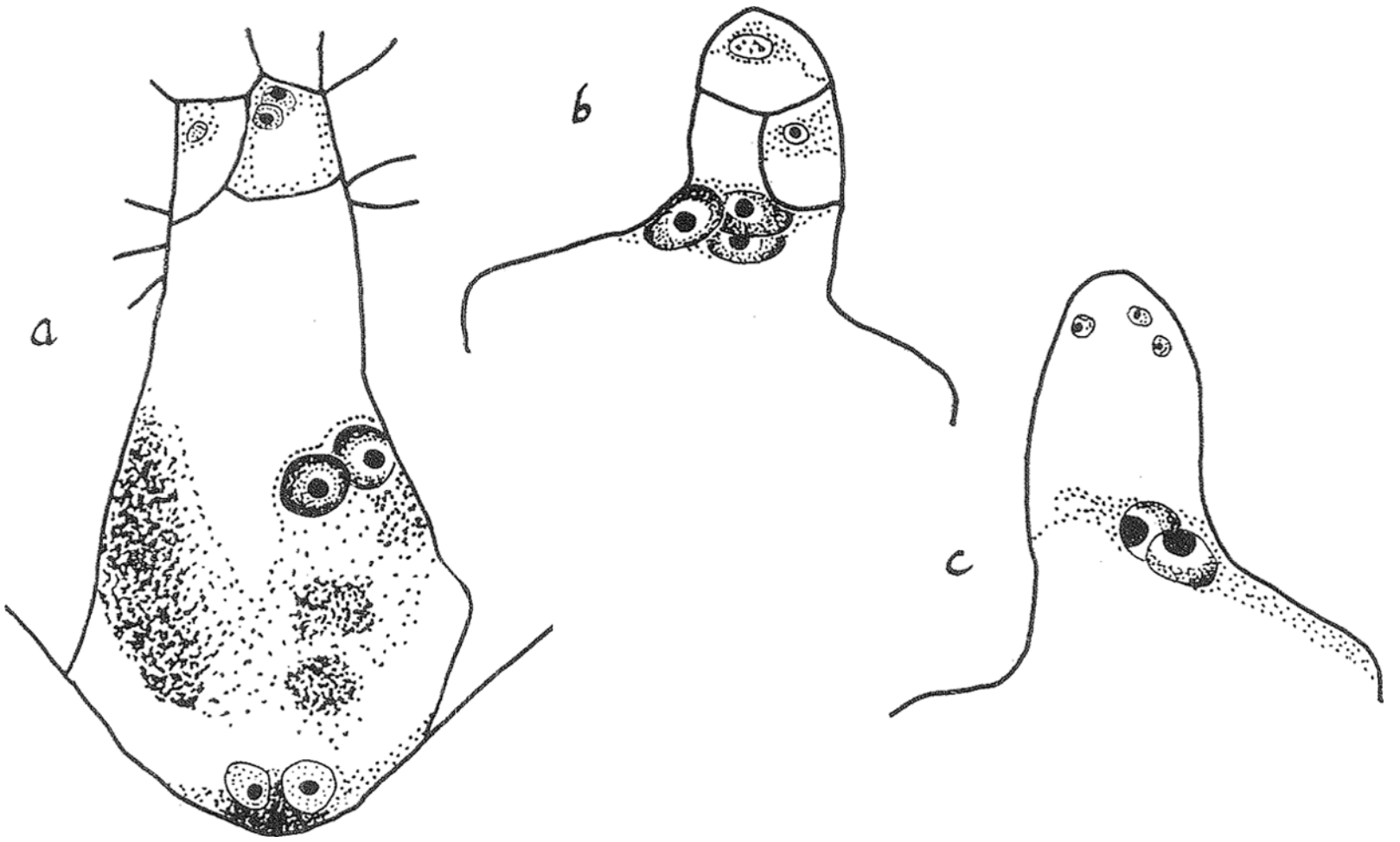

Fig. 2. Antipodal walls in 'Bluggoe' (x 2100). **a**—young embryo-sac, apparently 7-nucleate showing two antipodal cells and three nuclei, many days late in development (+5 days); **b**—chalazal group showing three polars, two antipodals and antipodal walls (+1 day) (cf. Dodds (1943), p. 170 and Text-fig. 9); **c**—normal chalazal group without antipodal walls (+5 days).

in all cells examined) and their presence in the two edible clones presumably reflects the origins of these clones; both are triploid members of the ABB group having two genomes from *Musa balbisiana*, one from *M. acuminata*. The cell shown in Fig. 2*b* supports Dodds's (1945) contention that extra polar nuclei arise from misplaced antipodals.

(*b*) *Seed fertility in relation to female cytology*

Rounded figures for seed fertility are given in Table 4. In 'Mysore' and 'Bluggoe', relatively fertile clones as edible bananas go, less than a tenth of one percent of ovules yield seeds; in 'Pisang awak', an exceptionally fertile clone, only 2·2 percent of ovules develop to maturity. For comparison, 'Gros Michel' (AAA group) is less than half as fertile as 'Mysore'. In 'Mysore' and 'Bluggoe' no mature embryo-sacs were seen at

Table 4. *Female fertility of three edible bananas*

Clone	Ovules per ovary	Fruits per bunch	Ovules per bunch	Good seeds per bunch	Seeds per 1000 ovules
'Mysore'	200	160	32,000	8·5	0·27
'Bluggoe'	500	50	25,000	12·8	0·51
'Pisang awak'	500	80	40,000	880	22
'Gros Michel'*	200	150	30,000	1-4	0·03-0·13

* For comparison, data from Shepherd (1954). In column 5 is given the range of seeds per bunch for four different sites in Jamaica.

receptivity in the cytological material described in the previous section; combining observations for the two clones for days −1 to +5 (Tables 1 and 2), 617 cells were examined. The upper limit of frequency of functional embryo-sacs compatible with these observations is about 0·6 percent (Fisher and Yates, 1943, Table VIII. i., $P=0·025$). This figure may be compared with the observed frequency of seed set of about 0·04 percent (Table 4, mean fertility of the two clones). The conclusion is that at least 7 percent of embryo-sacs (probably far more) can, if fertilized, develop seed. For 'Pisang awak' a similar conclusion holds: Table 3 indicates an embryo-sac frequency of about 10% which is to be compared with a seed fertility of about 2% (Table 4) — evidently, about 20% of embryo-sacs have the potentiality of successful development. For 'Gros Michel', exact information on the frequency of normal embryo-sacs is wanting, but it is probably of the order of several percent (Shepherd, 1954). Since less than 0·01 percent of ovules of this clone develop into seed (Table 4) it is evident that there is a very low frequency of development of embryo-sacs to maturity — almost certainly less than one percent.

Discussion

The same sort of misbehaviours in embryo-sac formation that Dodds (1945) described for the edible diploid bananas occur also in the three edible triploids described here, namely meiotic irregularities and failures of development at all stages up to maturity. The meiotic irregularities are the consequence of triploidy—i.e. of numerical chromosome

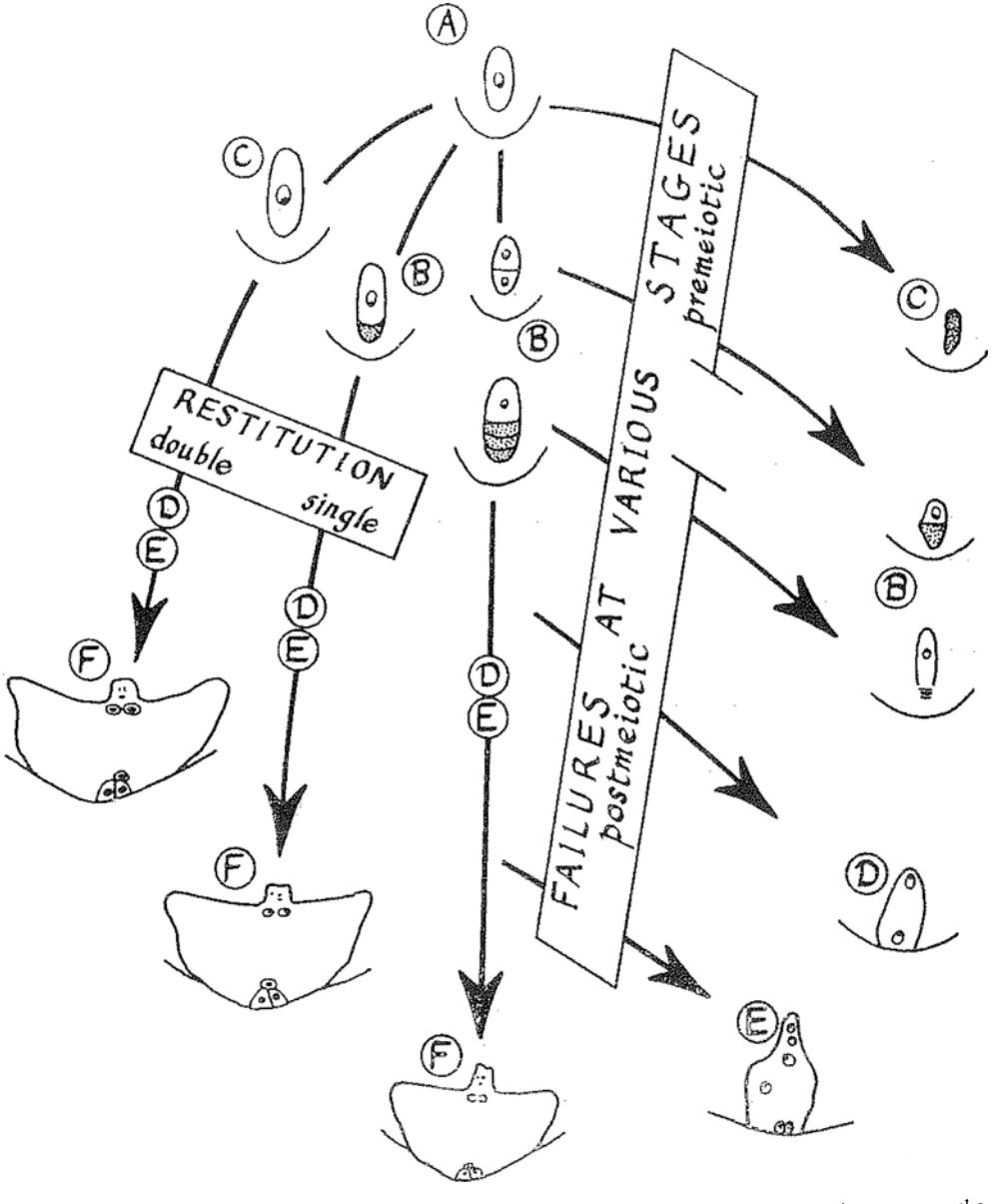

Fig. 3. Summary diagram of the course of female events in edible triploid bananas. Centre—the development of embryo-sacs with reduced chromosome numbers; left—the development of polyploid embryo-sacs; right—arrest of development at various stages.

hybridity; later failure is evidently the result of the physiological milieu of parthenocarpy. The figures of section 2(*b*) suggest that in the three hybrid triploid clones described there is a comparatively slight excess of embryo-sac frequency over seed fertility, particularly when allowance is made for the probable occurrence of morphologically normal sacs which are genetically unbalanced. In these clones, it seems that embryo-sac formation may sometimes be limiting to fertility. For 'Gros Michel' the available figures suggest that embryo-sacs far outnumber seeds and that their occurrence is probably not limiting to fertility. The most fertile recorded bunch of this clone bore 60 seeds which still represents only 0·2 percent of ovules developing, a figure which is to be compared with an embryo-sac frequency that is probably at least five and perhaps ten times greater. This suggests that, for 'Gros Michel', much sterility is to be attributed to failure of fertilization or failure of subsequent events. This conclusion, if correct, perhaps offers some encouragement to the banana breeder inasmuch as it is easier to envisage chemical or other treatments that have favourable effects on pollen tube growth or seed development than it is to envisage treatments which might encourage embryo-sac formation.

The results reported here contribute little to the understanding of the origin of functional polyploid embryo-sacs. The difficulty is mainly statistical: even in the most fertile clone ('Pisang awak') probably only about two percent of ovules contain viable triploid sacs. Furthermore, the stage of the preceding meiosis at which critical observation of the cytological nature of the restitution could be made is transient and technically unfavourable for analysis. Nevertheless some inferences can be drawn. Cheesman and Dodds (1942), Larter (1948) and Shepherd (1959), describing the progeny of triploids under haploid pollination, found that diploids and triploids (sometimes aneuploid), tetraploids (generally euploid) and heptaploids occur in proportions that vary with the clone and its specific origins.

The diploids and triploids are the products of meiosis and the occurrence of aneuploidy reflects the irregular meiosis of the triploid female parents. The mode of restitution that yields tetraploids may be inferred to be primarily of the "Rosenberg" type that results from first divisions nullified by irregular pairing. Behaviours of this kind on the male side of 'Gros Michel' have been recorded by Wilson (1946). This mechanism would lead to the production of predominantly eu-tetraploid progeny. An alternative restitution mechanism is that described by Dodds and Simmonds (1946) for certain diploid hybrids; embryo-sacs having the same chromosome number as the plant that bore them were produced by the failure of the second division following a successful first division. In triploids such a mechanism could not yield embryo-sacs that were precisely triploid and balanced, and it cannot therefore be responsible for the predominantly eu-tetraploid progeny that are obtained. Some of the few aneu-tetraploid seedlings recorded by Larter (1948) from 'Gros Michel' may perhaps have originated thus, however. Finally, heptaploids are virtually certainly produced from hexaploid embryo-sacs that owe their origin to the double-restitution characteristic of *Musa*; both divisions of meiosis fail but the chromosomes divide as usual. Very probably the giant embryo-sac shown in Fig. 1(*j*) of this paper had this origin and is precisely

comparable with the tetraploid sacs of diploid hybrids described by Dodds and Pittendrigh (1946).

SUMMARY

The female cytology of three edible triploid banana clones ('Mysore', 'Pisang awak', 'Bluggoe') is described. Irregularities of meiosis and embryo-sac formation are numerous and development is often delayed in respect to ovary development or altogether arrested. Mature embryo-sacs are therefore rare. Comparison of seed fertilities of the three clones with their cytology suggests that embryo-sac formation is limiting to fertility in at least two of them. In 'Gros Michel', by contrast, embryo sac frequency far exceeds fertility, thus giving some encouragement to the banana breeder's hope of manipulating the seed yield of this clone. The observations, for technical and statistical reasons, give little information about the origin of polyploid progeny; indirect inference suggests that both "Rosenberg" and "*Musa*-type" restitutions are concerned.

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