Investigations into Causes of Sterility

III. Gametocide-induced male-sterile *Vicia faba* L.

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Vicia faba L. plants were treated with three gametocides namely 2,2-dichloropropionic acid (Dalapon), sodium 2,3-dichloroisobutyrate (Mendok) and 1,2-dihydropyridazine 3,6-dione (Maleic hydrazide), with an aim of inducing pollen sterility. Various concentrations of these gametocides were applied as aqueous foliar sprays at several stages of plant growth. Of the various applications of these gametocides tried, only 2,2-dichloropropionic acid at 0.3 per cent and sodium 2,3-dichloroisobutyrate at 0.25 and 0.4 per cent levels were found to be capable of causing complete pollen abortion. In order to determine the exact stage at which pollen abortion is caused by these gametocides, meiosis was studied in 2,2-dichloropropionic acid- and sodium 2,3-dichloroisobutyrate-treated plants and their controls. It has been observed that while the former gametocide brings about a variety of meiotic irregularities, latter does not do so. The following pages bear the details of meiosis in untreated and 2,2-dichloropropionic acid-induced male sterile plants.

Material and methods

Flower buds from untreated and 0.3 per cent 2,2-dichloropropionic acid-treated plants were harvested and fixed at comparable stages of development in acetic-alcohol (1:3) with acetic acid component saturated with ferric acetate. Following overnight fixation the buds were transferred to 70 per cent alcohol. Pollen mother cells were squashed in 0.5 per cent acetocarmine.

All observations and microphotographs were made from temporary preparations, while only a few slides were made permanent for future record, by passing through acetic acid-tertiary-butyl alcohol series.

Observations

Meiosis in untreated plants

Early prophase chromosomes consisting of differentially stained segments kept overlapped and were, therefore, not countable. Meiotic chromosomes could, however, be best studied at diakinesis, prometaphase and metaphase stages (Figs. 1, 2). At all these stages, six bivalents with varying number of

chiasmata were easily countable. One of these bivalents was almost double the size of other bivalents and along with one smaller bivalent kept associated with the nucleolus (Fig. 1). Pairing of chromosomes through all these stages was perfect.

Distribution of chromosomes at anaphase I was regular with six chromosome moving to each pole (Fig. 3). The longest bivalent was usually the last

Figs. 1-4. Meiosis in untreated plants of *Vicia faba*. 1, diakinesis, 6 II. $\times 837$. 2, metaphase, 6 II. $\times 837$. 3, anaphase I, 4 II + 4 I. $\times 1,225$. 4, anaphase II, 6-6-6-6. $\times 655$.

to separate. The second division commenced with a brief prophase II followed by aggregation of chromosomes at two metaphase II plates. Distribution at anaphase II was synchronous with six chromatids moving to each of the four poles (Fig. 4). Wall formation was simultaneous, resulting into tetrahedral microspore tetrads.

Meiosis in treated plants

In 70 per cent of the total pollen mother cells studied, meiosis was regular. The remaining cells were characterized by varying degrees of stickiness, which resulted in multiple chromosome associations and in extreme cases

clumping of entire complement.

At prophase I, scoring was not possible on account of the difficulty encountered in the interpretation of various chromosome configurations. At prometaphase and metaphase stages, varying number of bivalents were found to keep associated with each other in some cells (Table 1, Figs. 5, 6). In

Table 1. Meiotic instability at metaphase I in treated plants

Nature of PMC	Frequency	Per cent of grand total	Per cent of total abnormal PMC
Normal	195	73.6	—
Partial clumping	25	10.9	41.4
Complete clumping	20	7.5	28.6
Fragments	21	7.9	30.0
Grand Total	265	100.0	—

others, all chromosomes were clumped into a single mass, rendering the identification of individual bivalents impossible (Fig. 7). Chromosome fragments were observed in nearly 8 per cent cells scored at this stage (Figs. 6-8).

Figs. 5-12. Meiosis in treated plants. 5, prometaphase, multiple chromosome associations. $\times 1,000$. 6, metaphase I, chromosome stickiness and fragments. Note fine threads of agglutinated chromosomes. $\times 700$. 7, completely clumped metaphase I with a fragment. $\times 1,850$. 8, metaphase I, 6II+1f. $\times 1,000$. 9, anaphase I, a chromosome bridge. $\times 1,000$. 10, anaphase I, a fragment. $\times 1,000$. 11, late anaphase I, 4 fragments. $\times 1,000$. 12, anaphase I, intense chromatin agglutination. $\times 700$.

Disjunction of chromosomes at anaphase I was normal in 74 out of the 110 cells scored. Remaining cells were characterized by bridges and laggards (Table 2, Figs. 9-11). Tripolar separation of chromosomes accompanied by 1 or 2 fragments was observed in 6 cells. Anaphasic separation in pollen mother cells exhibiting complete clumping of chromosomes occurred through the elongation of entire chromatin material (Fig. 12), followed by its breakage into two. In some cases the division was complete but in others a chromatin

Table 2. Meiotic instability at anaphase I in treated plants

Nature of PMC	Frequency	Per cent of grand total	Per cent of total abnormal PMC
Normal	74	67.3	—
Bridges	22	20.0	61.1
i. (In cells with bipolar spindles)	(16)	(14.5)	(44.4)
ii. (In cells with tripolar spindles)	(6)	(5.5)	(16.7)
Laggards	14	12.7	38.9
Total	110	100.0	—

strand persisted as a bridge, which occasionally lasted till the cytoplasm exhibited furrowing. This division resulted in the formation of dyads (Fig. 13). In a few cases the formation of dyads was accompanied by chromosome fragments, which were either included into one of the dyad cells to form micronuclei or delimited as separate cells (Fig. 14). The dyad cells did not generally undergo a second division.

Table 3. Meiotic instability at anaphase II in treated plants

Nature of PMC	Frequency	Per cent of grand total	Per cent total abnormal PMC
Normal	62	68.9	—
Bridges	10	11.1	35.7
Laggards	12	13.3	42.8
Tripolar separation	6	6.6	21.4
Grand total	90	100.0	—

In a few mother cells, furrowing started simultaneously at several places. In such cases, the invaginating furrows did not only divide the cytoplasm but also brought about the division of chromatin material (Fig. 15). Rarely, however, furrowing left the chromatin material undivided, which it passed as a whole into one of the daughter microspores. The remaining microspores, therefore, remained enucleate (Fig. 16).

The anaphasic anomalies at telophase I were reflected in the form of micronuclei (Fig. 17). In 7.1 per cent cells, three nuclei probably formed as a result of tripolar separation were observed (Fig. 18).

Figs. 13-21. Meiosis in treated plants. 13, persistent chromatin bridge at dyad stage. $\times 1,000$. 14, a first division tetrad. Note size of microspores and a micronucleus. $\times 1,000$. 15, random furrowing of PMC cytoplasm and chromatin material. $\times 1,000$. 16, lack of movement of chromosomes and the formation of enucleate microspores. $\times 1,000$. 17, telophase I, 3 micronuclei. $\times 900$. 18, PMC showing 3 nuclei and a chromatin fragment. $\times 837$. 19, telophase II, a double chromosome bridge. $\times 837$. 20, a persistent chromatin bridge between 2 microspores at tetrad stage. $\times 1,000$. 21, anaphase II, 3 spindles, unequal separation, bridges and laggards. $\times 837$.

Second nuclear division also showed a number of irregularities. Occurrence of bridges (Fig. 19) and laggards was frequent (Table 3). Bridges sometimes persisted upto sporad stage (Fig. 20). Disjunction of chromatids in 6.6 per cent pollen mother cells was characterized by usually three spindles some of which were multipolar. Distribution of chromatids in such cases was highly irregular and accompanied by thin chromatin bridges and fragments (Fig. 21).

Laggards were ultimately either incorporated in the microspores, where they formed micronuclei or delimited as separate spores. The result of all these abnormalities was the formation of 30 per cent polysporads with 2-8 microspores. Polyads with still larger number of microspores (as many as 10) were probably the result of multipolar separation.

A state of pollen mother cells simulating the phenomenon of "Cytomixis"—the process of extrusion of chromatin from the nucleus of one pollen mother cell into the cytoplasm of adjacent mother cell, was observed in 93 out of a total of 878 cells scored at various stages of meiosis. Often a number of cells were seen joined to each other by means of chromatin connections (Figs. 22-24). Such cells were found intermixed with normally dividing pollen mother cells. The contributing cells were apparently deficient in chromatin material possessing from apparently complete chromosome complements to only a few fragments (Fig. 25).

Nuclei of the "cytotoxic" pollen mother cells were found at various stage of division.

Figs. 22-26. Cytomixis in treated plants. 22-24, PMC showing chromatin connections at interphase, prophase and anaphase I respectively. $\times 400$. 25, two adjacent PMC with different amounts of chromatin material. $\times 700$. 26, long chromosome involved in chromatin connection. $\times 837$.

Some of them at telophase I were observed to possess chromatin bridges as well (Fig. 25). Identity of individual bivalents could be made out in only a small percentage of cells wherein the longest bivalent was seen to be invariably involved, probably due to the greater amount of chromatin material (Fig. 26). In others the chromosomes appeared highly sticky and undifferentiable.

Discussion

The main change induced by dalapon during meiosis is the coalescence of chromosomes, as a result of which they form complex unanalysable associations. The coalescence of chromosomes has been attributed to certain property of "stickiness" and also to the presence of "similar" genes in these regions (Vanderlyn 1949).

Dalapon is already known to cause chromosome stickiness in hair cells of *Tradescantia* (Sawamura 1965). The stickiness might probably be a result of chromosome agglutination produced by the intense action of the chemical substances (Resende and Rijo 1948). This does not seem to differ from the heterochromatin-agglutination reported to occur spontaneously (Resende 1946, Cordario 1946, Resende and Rijo 1948, Snoad 1951, Jain 1960, Khoshoo 1965) and that produced by the action of radiations. This effect of radiations is called "physiologic" and has been attributed to depolymerization of the nucleic acids, which become more fluid (Darlington 1942).

In mild cases, only a few bivalents are associated and such cases may be mistaken for secondary associations. In fact the multivalent formation in the treated plants appears to result from the association of bivalents on account of stickiness.

At anaphase I, the agglutinated chromosomes give rise to sticky chromatin bridges. Due to random breakage of such bridges, the resulting univalents are likely to be deficient or duplicate for some regions (c.f. Vaarama 1954). This is likely to effect the pollen sterility even if, the second division is normal. But in practice, the second division exhibits a higher frequency of bridges and laggards which are largely constituted of chromosome fragments.

Chromosome fragmentation has widely been reported as a conspicuous after effect of chemical treatment in plants (Ford 1948, Loveless and Revel 1949, Kihlman and Levan 1951, McLeish 1952, Oehlkers 1952, Sharma and Bhattacharya 1956, Sharma and Sharma 1960, Wilson 1960). Ford (1948) studied the effect of NN-dionethylene on *V. faba* and proposed that the breaks did not take place at random but rather showed some specificity for heterochromatin. The hypothesis was based on the distribution of breaks within the chromosome complement. Loveless and Revell (1949), Oehlkers (1952) and Srivastava (1966) also confirm the non randomness of the chromosome breaks. Kihlman and Levan (1951) found apparent localization of the chromosome breaks in the attachment thread of the satellite of the long chromosome of *V. faba*. The point of breakage of *V. faba* chromosomes on

treatment with dalapon seems fairly confined to the terminal chromatin knobs. However, due to highly condensed and sticky state of meiotic chromosomes, it could not be determined whether the breakage is specific to heterochromatic chromosome regions as reported by most other workers for different chemicals.

Multipolar spindles were observed at both the divisions of meiosis. Walters (1958) mentions that aberrant spindles occur at meiosis in plants in which a large number of univalents are present. Raghuvanshi and Joshi (1966) have reported their occurrence in plants containing regularly paired chromosomes. In the present case, the number of univalents at least at first division is low and rarely exceeds three. Walters (1958) has suggested the origin of multipolar spindles from splitting or extra division of spindle organizers in the first or second prophase. Multipolar spindles have presently been observed at both first and second divisions and it is likely that extra divisions of spindle organizers have occurred in the preceding prophase.

Chromatin connections between pollen mother cells simulating cytomixis, as observed in the treated plants of *V. faba*, is a phenomenon of only sporadic and infrequent occurrence in nature (Maheshwari 1950). But occasionally as in case of *Bouchea fluminensis* (Schnack and Fehleisen 1957) is found to be a constant aspect of meiosis of the plants of a given species. Cytomixis is usually presumed to be the result of unknown physiological disturbances, which may themselves be associated with other meiotic irregularities or with hybridization (Bell 1964). Since dalapon is known to cause the stickiness in mitotic cells (Sawamura 1965), the cytomixis in the present case might probably result from such a phenomenon occurring during premeiotic divisions. The bridges thus formed by the agglutinated chromosomes might persist upto the onset of meiosis in the form of chromatin connections. This assumption is probably supported by the fact that chromosomes in cytotoxic mother cells are often found to be coalesced. Cytomixis gives rise to pollen mother cells with varying chromosome numbers and consequently causes the formation of inviable aneuploid gametes (Rana and Swaminathan 1964).

Such abnormalities as unequal separation of chromosomes, occurrence of laggards or various difficulties of polar movement, formation of restitution nuclei during first telophase and the suppression of the second nuclear division as observed in present case, are reported to occur in nature also, which finally result in the formation of non-viable polyad pollen grains (See Jain 1959).

The meiotic irregularities lead to chromosome dearrangements and loss of pieces of genome and offer an explanation of pollen abortion in dalapon-treated plants of *V. faba*. But as the incidence of such abnormalities is not 100 per cent, there should be other conditions associated with complete pollen abortion. A histological and cytochemical investigation of anther development has revealed that besides meiotic abnormalities, various nutritional deficiencies and vagary in the DNA metabolism are also associated with complete pollen abortion. A detailed description of this study will appear elsewhere.

Summary

Meiosis in 2,2-dichloropropionic acid-induced male-sterile plants of *Vicia faba* L. has been studied. Meiosis was regular in nearly 70 per cent of total pollen mother cells studied. The remaining cells were characterized by varying degrees of stickiness, which resulted in multiple chromosome associations and in extreme cases clumping of entire complement at prophase and metaphase I. Disjunction of chromosomes in such cells was characterized by bridges and laggards. Bridges some times persisted upto sporad stage. Multipolar spindles were observed at both the divisions of meiosis. In some cells, a wall was laid soon after the completion of first nuclear division, thereby resulting in the formation of dyads. Dyads did not undergo a second division.

All these abnormalities resulted in the formation of 30 per cent polyads with 2-10 microspores. Some of the pollen mother cells at various stages of division, exhibited the phenomenon of "cytomixis".

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* Not seen in original.