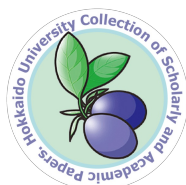




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# GENETICAL STUDIES ON THE MALE STERILITY OF SUGAR BEETS (*BETA VULGARIS* L.) AND ITS RELATED SPECIES

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1) Contribution from the Plant Breeding Institute, Faculty of Agriculture, Hokkaido University, Sapporo, Japan.

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## I. Introduction

The use of male sterility in the commercial production of hybrid seeds is progressing in practice in sugar beets as well as in other cross- and self-pollinating crops (KLEY 1954, DUVICK 1968, MCFARLANE et al. 1965, WIEBE 1968).

The beet flower is a hermaphrodite and the pollen is transferred by insects or wind. Large amounts of pollen are produced and are blown to a great distance. Various insects visit beet flowers for nectar and pollen (ARCHIMOWITSCH 1949). However, the nature of inflorescence and floral organ renders the manipulation of artificial emasculation difficult. Therefore, the use of genetical male sterility has an advantage in producing hybrid seeds on a large scale.

Recently, it has been generally accepted that triploid hybrids of sugar beets are superior to parental diploid and tetraploid plants in their gross sugar production (MATSUMURA et al. 1953, KNAPP 1956, HELMERICK et al. 1965). The seeds of pure triploids are efficiently produced by crossing, diploid male sterile plants with tetraploid pollinators or by reciprocal crossing between tetraploid male sterile plants and diploid pollinators.

The present investigation deals with the nature and the mode of inheritance of the male sterile characters both at the diploid and tetraploid levels, together with some information on the application of the cytoplasmic male sterility for triploid breeding. Further an occurrence of male sterile plants was observed in the progeny of the plants grown from the seeds irradiated by gamma rays. A study on inheritance and cytological observations were carried out on the male sterile character induced by the irradiation.

## II. Literature review

According to the reviews by EDWARDSON (1956) and DUVICK (1959): CORRENS (1904, 1908) first demonstrated the influence of the maternal cytoplasm on sex determinations in the common summer savory (*Satureia hortensis* L.). In the fiber or oil seed flax (*Linum usitatissimum* L.), BATESON and GAIRDNER (1921) described a type of male sterility which was inherited only through the female although its expression was controlled by genes which could be contributed by either parent. CHITTENDEN (1927) and GAIRDNER (1929) confirmed that the male sterility was due to sterility-inducing cytoplasm plus a homozygous recessive gene. Similar cases were reported in corn by RHOADES (1931, 1933) and in onion by JONES and CLARKE (1943).

ARCHIMOWITSCH (1931) was the first to describe the phenomenon of

male sterility in beets. OWEN (1942) gave a preliminary report of cytoplasmic-genetic male sterility. In 1945, he reported in detail on the mode of inheritance of male sterility. According to his genic assumptions, the degree of male sterility was controlled by the interaction of two chromogenes  $X$  and  $Z$  in sterile cytoplasm  $S$ . The genic constitutions of the phenotype are summarized as follows;  $S\ xx\ zz$  is responsible for completely male sterile,  $S\ Xx\ zz$  (or  $S\ xx\ Zz$ ) is for semi-male-sterile, usually without viable pollen, and  $S\ Xx\ Zz$  is for semi-male-sterile, usually with some viable pollen, and sometimes indistinguishable in normal plants. Later, OWEN (1950) recognized the difficulty in distinguishing  $S\ xx\ zz$  and  $S\ xx\ Zz$  phenotypically because of the minor effect of  $Z$  factor under certain environmental conditions. OWEN (1952) also found Mendelian inherited or genic male sterility due to the recessive genes,  $a_1$  and  $a_2$ . In order to utilize the male sterility for heterosis breeding, he published schemes for the production of hybrid seeds by a single cross and a double cross (OWEN 1948, 1950, 1954). The results of a preliminary yield test of the male sterile sugar beet hybrids indicated a possibility for utilization of male sterile inbreds in the breeding program (OWEN et al. 1946, 1954, MURPHY et al. 1950, OLDEMEYER and RUSH 1960, THEURER et al. 1969).

Since the monogerm character which contains only one viable seed instead of several, was found by SAVITSKY (1950), the cytoplasmic male sterility was combined with the monogerm character and used for making the monogerm hybrids (OWEN et al. 1954, SAVITSKY 1952, 1954, 1956, 1962, TOLMAN and JOHNSON 1956, BANDLOW 1964 a, b). In triploid breeding, STEWART and GASKIL (1952) made a performance test of triploid hybrids in which a diploid male sterile line was used for making a hybrid. Triploid hybrids some of which are superior to the diploid control variety were found among the various crosses between diploid male sterile lines and tetraploid lines (ELLERTON and HENDRIKSEN 1959, BANDLOW 1964 b). The cross in the reciprocal direction between tetraploid male sterile lines and diploid lines gave equally promising results in a preliminary test (NAGAO and KINOSHITA 1962, BANDLOW 1964 b).

New sources of male sterility were found from a wild leaf beet and *B. macrocarpa* as well as from sugar beets (OLDEMEYER 1957, SAVITSKY 1958). HOGABOAM (1957) added a new dominant modifier, *Sh* which enhances the pollen productivity of  $S\ Xx$  plants to such an extent that they appear to be male fertile. A linkage relation between the gene ( $X$ ) for pollen restoration and the gene ( $m$ ) for monogerm was established (HOGABOAM 1957, NAGAO et al. 1962). A genetic correlation was found between anther pigmentation

and male sterility (STEIN and GABELMAN 1959). SAVITSKY (1963) reported that the inheritance of male sterility in tetraploid sugar beets was controlled by a single recessive gene which was inherited on the basis of chromatid assortment. In diploids, a monofactorial inheritance and a poly-hybrid mode of inheritance were reported in addition to the theory by two major genes (BLISS and GABELMAN 1965, ROHRBACH 1965 b, BOLZ 1968). However, OWEN's assumption on the complementary effect of two major genes for pollen restoration was realized by several experiments (KNAPP 1955, NAGAO and KINOSHITA 1962, NIELSON and NEMAZI 1967, THEURER and RYSER 1969). Environmental effects on the phenotypic expression of the male sterile character were studied by several investigators (OWEN 1950, BANDLOW 1964 a, ROHRBACH 1965 c, CORTESSI 1967). STEIN et al. (1959) described that a reversion of male sterile to a partially fertile phenotype in their materials was not caused by environmental factors alone, but depended on some internal condition of the plant. It was reported that the male sterility had no side effects on seed producing ability, germination, root weight and sugar content (ROHRBACH 1965 d).

In petunia, FRANKEL (1956), EDWARDSON and CORBETT (1961) demonstrated the transmission of male sterility by grafting, while in other crops negative results were obtained (SAND 1960, OHTA 1961, SHUMWAY and BAUMAN 1966, CLEŸ 1967, EDWARDSON 1967, ZEVEN 1967, THEURER et al. 1968, DASKALOFF 1968, LACADENA 1968). ATANASOFF (1964) suggested that cytoplasmic male sterility should be re-examined from a standpoint of virus infection. Recently, CURTIS (1967) reported asexual transmission of cytoplasmic male sterility in sugar beets. Restoration of pollen fertility from cytoplasmic male sterility by heat shock of germinated seeds was obtained in sugar beets (CLEŸ 1967). In *Epilobium*, a permanent heritable change from *S* cytoplasm into *N* cytoplasm occurred during vegetative propagation (JONES and GABELMAN 1965).

The cytological basis of the male sterility in sugar beets was studied with special reference to tapetal abnormality (ARTSCHWAGER 1947, HOSOKAWA et al. 1954, OHTA and MATSUMURA 1960, NAGAO and KINOSHITA 1962, ROHRBACH 1965 a). The mechanism of male sterility was studied histochemically by HOSOKAWA et al. (1963, 1965).

The selective gametocide 'FW-450' (Sodium 2, 3-dichloroisobutyrate) was applied for the induction of male sterility in cotton (EATON 1957). The effectiveness of the chemical was examined and used for production of hybrid seeds in sugar beets (WIT 1960, BUTTERFASS 1960, DUDLEY 1960, RUEBENBAUER and SCHULTIS 1960, DONÀ DALLE ROSE 1962, OHTA and MATSU-

MURA 1962, ISÁK 1963, FÜRSTE 1964, WACHOWIAK-DALKE and BARDZIŃSKI 1966).

### III. Materials and Methods

The strains which were selected for the present studies include not only strains of sugar beets and table beets (*Beta vulgaris* L.) but also strains of wild beets (*Beta maritima* L.), both at diploid and tetraploid levels as listed in Table 1.

In this table the K-strains are the progeny of plants which were selected from a well known Japanese variety, Hon-iku-192 by the late Dr. S. MATSUMURA. The M-strains originally came out of the cytoplasmic male sterile plants in an American hybrid variety '202-H-16'. H-19 is a so-called 'type-O', the most appropriate pollen parent for producing male sterile progeny derived from an introduced monogerm strain 'M-10' and the isogenic strains, H-19 MS carries the complete-sterile and the monogerm characters. BM-2 is the progeny of a male sterile mutant found in *Beta maritima* L. Autotetraploids were induced from M-strains, Hon-iku-192, H-2002, H-2005 MS, H-19 and H-19 MS by applying colchicine solutions; they were named 4M-strains, 4192, H-4002, H-4005 MS, H-19 (4X) and H-19 MS (4X). Dry seeds of H-19 were irradiated by gamma rays at total exposures of 50 kR and 100 kR. Occurrences of male sterile plants were observed in the progeny of the plants grown from the irradiated seeds. The progeny of the male sterile plants were named  $\gamma$ -strains.

The plants were cultured in the experimental field under natural conditions, or in the greenhouse. Most of the plants in sugar beets and table beets was biennial plants. Therefore, the plants were grown under long day conditions following the photo-thermal induction of young seedlings, in order to hasten the generations. In this respect, the actual procedure given by GASKILL (1954) was employed. It was possible to grow two generations within a year if a greenhouse equipped with illumination is available.

Materials used for the cytological observations on microsporogenesis were prefixed in CARNOY's fluid and placed in a modification of KARPECHENKO's fixative. Serial sections were cut at  $12\mu$  and stained in DELAFIELD's haematoxylin followed by safranin. Chromosome count was made at the tip of primary roots of 2 to 3 cm length. They were treated with cold water at 3° to 5°C for 24 hrs., for shortening the chromosomes and rendering the identification of individual chromosomes much easier. FARMER's fluid was employed as the fixative and FEULGEN's reaction as the staining was used after maceration by 1 N HCl. Preparations were made by the usual squash

TABLE 1 The strains used in the study

| Strain           | Type of crop | Type of cytoplasm | Ploidy              | Description                                                                                     |
|------------------|--------------|-------------------|---------------------|-------------------------------------------------------------------------------------------------|
| M-strains        | Sugar beet   | S                 | 2X                  | Male sterile lines selected from '202-H-16'                                                     |
| K-strains        | do           | do                | do                  | Male sterile lines selected from 'Hon-iku-192'                                                  |
| H-19             | do           | N                 | do                  | Type-O plants selected from monogerm strain 'M-10'                                              |
| H-19 MS          | do           | S                 | do                  | Isogenic line of H-19, with male sterility and monogermity                                      |
| Hon-iku-192      | do           | N                 | do                  | Japanese multigerm variety                                                                      |
| Donyu-2go        | do           | do                | do                  | Japanese multigerm variety                                                                      |
| H-2002           | do           | do                | do                  | A selection from 'Donyu-2go'                                                                    |
| 4 M-strains      | do           | S                 | 4X                  | Tetraploids induced by colchicine treatment from M-strains                                      |
| H-19 (4X)        | do           | N                 | do                  | Tetraploids induced by colchicine treatment from 'H-19'                                         |
| H-19 MS (4X)     | do           | S                 | do                  | Tetraploids induced by colchicine treatment from 'H-19 MS'                                      |
| H-4002           | do           | N                 | do                  | Tetraploids induced by colchicine treatment from 'H-2002'                                       |
| H-4005 MS        | do           | S                 | do                  | Tetraploids induced by colchicine treatment from 'H-2005 MS' which contains male sterile plants |
| 4192             | do           | N                 | do                  | Tetraploids induced by colchicine treatment from 'Hon-iku-192'                                  |
| 4398             | do           | N                 | do                  | Tetraploids introduced from Kihara-Institute                                                    |
| Polyrave         | do           | N                 | 2X, 3X, 4X          | Polyploid variety introduced from Netherlands                                                   |
| Trirave          | do           | S                 | 3X (Most of plants) | Triploid variety introduced from Netherlands                                                    |
| Detroit dark red | Table beet   | N                 | 2X                  | Table beet variety introduced from U.S.A.                                                       |
| BM-2X            | Wild beet    | N                 | 2X                  | An annual strain of <i>Beta maritima</i> L.                                                     |
| BM-4X            | do           | do                | 4X                  | Tetraploids induced by colchicine treatment from BM-2X                                          |
| BM-2             | do           | do                | 2X                  | A male sterile mutant found out in BM-2X                                                        |
| BM-2 (4X)        | do           | do                | 4X                  | Tetraploids induced by colchicine treatment from the progeny of BM-2                            |
| γ-lines          | Sugar beet   | S'                | 2X                  | Progenies of male sterile plants induced by gamma irradiation                                   |

method, including final staining with acetocarmine. For the observation of meiosis, small buds were fixed by FARMER's or CARNOY's fluid and stained with aceto-carmine by the usual squash method. Pollens were sampled in order to observe the type of male sterility and pollen sterility by mixing the pollens from several flowers taken from different branches. Pollen grains were stained with cotton blue pigment dissolved in lacto-phenol solution. The total number of pollen grains varied from 200 to 500. The two classes, stained and unstained pollen grains were discrete (Plate II).

#### IV. Experimental Results

##### A. Cytoplasmic-genetic male sterility

###### 1. Classification of male sterility

Types of abnormality of anthers and pollen grains from the progenies of K- and M-strains were classified into four groups, namely the normal type, the semi-sterile type-a, the semi-sterile type-b and the complete-sterile type (Table 2, Plates I and II). As shown in this table and plates, the complete-sterile type (C.S.) possesses white and shrunken anthers completely devoid of pollen. Semi-sterile type-b (S.S.b) shows non-dehiscent reddish-yellow anthers in which a large part of pollen grains are abortive and small, though the exine of pollen was well developed. Semi-sterile type-a (S.S.a) seems to be a most variable phenotype. Both well dehiscent and non-dehiscent anthers are contained in the same flower of a single plant, at different rates of mixing. The pollen grains are functional while the pollen fertility varied remarkably in each anther. Normal type (N) has well dehiscent anthers and as high pollen fertility as that of control strains.

TABLE 2 Classification of male sterility

| Type of male sterility            | Complete sterile<br>C.S. | Semi-sterile type-b<br>S.S.b | Semi-sterile type-a<br>S.S.a | Normal (S)*<br>N | Normal (N)*<br>Control |
|-----------------------------------|--------------------------|------------------------------|------------------------------|------------------|------------------------|
| Color of anther                   | White                    | Orange                       | Yellow                       | Yellow           | Yellow                 |
| Dehiscent                         | —                        | —                            | ±                            | +                | +                      |
| Pollen fertility (%)              | 0                        | 0-20                         | 20-70                        | 70-100           | 70-100                 |
| Germination rate of pollen grains | 0                        | 0                            | 30                           | 90               | 100                    |

\* Type of cytoplasm.

###### 2. Variability of phenotypic expressions in male sterility

Variation of pollen sterility during the flowering period was examined

using the strains, H-19 and H-19 MS\* in diploid and tetraploid levels (Table 3). There is a tendency that pollen sterility is lowest in full bloom while increases at the initiation and termination irrespective of male sterile types and ploidy levels. Similar experiments were carried out to compare the pollen sterility in different flowering periods. The plants were grown in the greenhouse under long day conditions and the first flowers were sampled in June. The stalks were cut down after flowering and the second flowers appeared from October to November. As shown in Table 4, the difference of pollen sterility is 14.3% at its maximum in normal cytoplasm strain H-19,

TABLE 3 Variation of pollen sterility during flowering period

| Ploidy | Strain   | Plant No. | Type of cytoplasm | Phenotype of male sterility | Pollen sterility (%) |            |      |
|--------|----------|-----------|-------------------|-----------------------------|----------------------|------------|------|
|        |          |           |                   |                             | Initiation           | Full bloom | End  |
| 2X     | H-19     | 1         | N                 | N                           | 10.1                 | 2.9        | 1.0  |
|        |          | 2         |                   | "                           | 17.6                 | 2.3        | 11.7 |
|        |          | 3         |                   | "                           | 27.5                 | 6.5        | 6.8  |
| 2X     | H-19 MS* | 1         | S                 | N                           | 6.7                  | 2.8        | 2.6  |
|        |          | 2         |                   | "                           | 9.3                  | 1.7        | 2.0  |
|        |          | 3         |                   | "                           | 27.1                 | 14.5       | 3.4  |
|        |          | 4         |                   | S.S.a                       | 27.9                 | 70.5       | 39.8 |
|        |          | 5         |                   | "                           | 72.5                 | 47.2       | 45.9 |
|        |          | 6         |                   | S.S.b                       | 83.0                 | 65.8       | 77.9 |
|        |          | 7         |                   | "                           | 100                  | 100        | 100  |
|        |          | 8         |                   | "                           | 100                  | 100        | 100  |
|        |          | 9         |                   | C.S.                        | 100                  | 100        | 100  |
|        |          | 10        |                   | "                           | 100                  | 100        | 100  |
| 4X     | H-19     | 1         | N                 | N                           | 17.6                 | 8.8        | 22.6 |
|        |          | 2         |                   | "                           | 6.7                  | 27.4       | 19.8 |
|        |          | 3         |                   | "                           | —                    | 29.8       | 25.4 |
| 4X     | H-19 MS* | 1         | S                 | S.S.a                       | 5.7                  | 36.2       | 97.4 |
|        |          | 2         |                   | S.S.b                       | 91.6                 | 92.9       | 92.9 |
|        |          | 3         |                   | "                           | —                    | 99.4       | 100  |

\* The seeds of H-19 MS were produced under open pollination and contained male fertile (N and S.S.a) plants with male sterile (S.S.b and C.S.) plants.

\* The seeds produced under open pollinations were used for this experiment. Therefore, the population contained various types of male sterility.

while a higher variability was observed in the N type and S.S.a type plants of *S* cytoplasm at diploid and tetraploid levels.

Another experiment was conducted to examine the variability of pollen sterility within a single plant (Table 5). Flower buds were sampled in full bloom, two times at an interval of one week and fixed by FARMER's fixative for observation of pollen grains. The buds just prior to flowering were taken at random from five different branches which were chosen at regular intervals

TABLE 4 Comparison of pollen sterility between spring and autumn

| Ploidy | Strain  | Plant No. | Type of cytoplasm | Phenotype of male sterility | Pollen sterility (%) |             | Difference between spring and fall |
|--------|---------|-----------|-------------------|-----------------------------|----------------------|-------------|------------------------------------|
|        |         |           |                   |                             | Spring (June)        | Fall (Nov.) |                                    |
| 2X     | H-19    | 1         | N                 | N                           | 1.4                  | 15.7        | -14.3                              |
|        |         | 2         |                   | "                           | 1.9                  | 6.6         | -4.7                               |
|        |         | 3         |                   | "                           | 3.3                  | 2.2         | 1.1                                |
|        |         | 4         |                   | "                           | 3.5                  | 10.2        | -6.7                               |
|        |         | 5         |                   | "                           | 7.8                  | 14.6        | -6.8                               |
|        |         | 6         |                   | "                           | 10.3                 | 2.8         | 7.5                                |
| 2X     | H-19 MS | 1         | S                 | N                           | 3.8                  | 34.1        | -30.3                              |
|        |         | 2         |                   | S.S.a                       | 18.4                 | 100         | -81.6                              |
|        |         | 3         |                   | "                           | 77.8                 | 26.7        | 51.1                               |
|        |         | 4         |                   | S.S.b                       | 100                  | 100         | 0                                  |
|        |         | 5         |                   | C.S.                        | C.S.                 | C.S.        | 0                                  |
|        |         | 6         |                   | "                           | C.S.                 | C.S.        | 0                                  |
| 4X     | 4M-50   | 1         | S                 | N                           | 14.0                 | 62.3        | -48.3                              |
|        |         | 2         |                   | S.S.a                       | 33.0                 | 55.0        | -22.0                              |
|        |         | 3         |                   | "                           | 45.3                 | 62.3        | -17.0                              |
|        |         | 4         |                   | "                           | 48.1                 | 52.8        | -4.7                               |
|        |         | 5         |                   | "                           | 53.7                 | 22.4        | 31.3                               |
|        |         | 6         |                   | "                           | 54.3                 | 23.2        | 31.1                               |
|        |         | 7         |                   | "                           | 58.2                 | 42.4        | 15.8                               |
|        |         | 8         |                   | S.S.b                       | 100                  | 40.0        | 60.0                               |
|        |         | 9         |                   | "                           | 100                  | 55.3        | 44.7                               |
|        |         | 10        |                   | "                           | 100                  | 98.7        | 1.3                                |
|        |         | 11        |                   | "                           | 100                  | 98.8        | 1.2                                |
|        |         | 12        |                   | "                           | 100                  | 100         | 0                                  |
|        |         | 13        |                   | "                           | 100                  | C.S.        | 0                                  |

TABLE 5 Average difference of pollen sterility between flowers, anthers and lobes of a single plant

| Ploidy | Strain  | Plant No. | Type of cytoplasm | Phenotype of M.S. | Difference of pollen sterility (%) |       |                    |       |                    |       |                    |       |
|--------|---------|-----------|-------------------|-------------------|------------------------------------|-------|--------------------|-------|--------------------|-------|--------------------|-------|
|        |         |           |                   |                   | Between branches                   |       | Between flowers    |       | Between anthers    |       | Between lobes      |       |
|        |         |           |                   |                   | mean <sup>1)</sup>                 | range | mean <sup>2)</sup> | range | mean <sup>3)</sup> | range | mean <sup>3)</sup> | range |
| 2X     | H-19    | 1         | N                 | N                 | 3.2                                | 0-7   | 3.3                | 0-18  | 2.9                | 0-30  | 3.4                | 0-45  |
|        |         | 2         |                   |                   | 8.9                                | 0-16  | 2.2                | 0-18  | 1.8                | 0-18  | 3.9                | 0-55  |
|        |         | 3         |                   |                   | 7.5                                | 0-14  | 2.1                | 0-9   | 3.7                | 0-36  | 3.4                | 0-34  |
| 2X     | H-19 MS | 1         | S                 | N                 | 1.4                                | 0-3   | 2.4                | 0-13  | 2.7                | 0-28  | 2.1                | 0-20  |
|        |         | 2         |                   |                   | 3.3                                | 0-7   | 2.6                | 0-26  | 2.3                | 0-47  | 2.1                | 0-49  |
|        |         | 3         |                   |                   | 7.1                                | 3-14  | 15.2               | 0-71  | 9.9                | 0-100 | 2.9                | 0-91  |
|        |         | 4         |                   | S.S. a            | 32.6                               | 0-63  | 14.6               | 0-52  | 16.3               | 0-85  | 9.0                | 0-76  |
|        |         | 5         |                   |                   | 11.5                               | 1-21  | 17.6               | 2-68  | 14.4               | 1-61  | 15.5               | 0-82  |
|        |         | 6         |                   | S.S. b            | 20.1                               | 2-39  | 15.0               | 1-69  | 16.1               | 0-72  | 13.0               | 0-83  |
|        |         | 7         |                   |                   | 0                                  | 0     | 0                  | —     | 0                  | —     | 0                  | —     |
|        |         | 8         |                   |                   | 0                                  | 0     | 0                  | —     | 0                  | —     | 0                  | —     |
|        |         | 9         |                   | C.S.              | 0                                  | 0     | 0                  | —     | 0                  | —     | 0                  | —     |
|        |         | 10        |                   |                   | 0                                  | 0     | 0                  | —     | 0                  | —     | 0                  | —     |
| 4X     | H-19    | 1         | N                 | N                 | 9.2                                | 1-21  | 6.9                | 0-27  | 8.9                | 0-74  | 6.6                | 0-55  |
|        |         | 2         |                   |                   | 21.8                               | 1-50  | 9.4                | 0-54  | 6.1                | 0-75  | 3.7                | 0-20  |
|        |         | 3         |                   |                   | 9.3                                | 0-14  | 22.4               | 0-66  | 7.5                | 0-21  | 7.0                | 0-55  |
| 4X     | H-19 MS | 1         | S                 | S.S. a            | 42.7                               | 0-89  | 5.0                | 0-37  | 5.0                | 0-86  | 3.6                | 0-62  |
|        |         | 2         |                   | S.S. b            | 3.6                                | 1-7   | 6.6                | 0-22  | 6.6                | 0-39  | 4.0                | 0-26  |
|        |         | 3         |                   |                   | 1.0                                | 0-2   | 1.0                | 0-5   | 0.8                | 0-10  | 0.3                | 0-2   |

1) Average percentage of 20 differences.

2) Average percentage of 30 differences.

3) Average percentage of 90 differences.

from the lowest branch of the plant. In every flower bud, three anthers were picked up at random and split in half along the stomium with the aid of a magnifying glass. Pollen sterility was calculated from an observation of about 200 pollen grains. Thus, about 36,000 pollen grains were examined in every single plant. As shown in Table 5, variation of pollen sterility was not so large in plants of normal types in *N* and *S* cytoplasm, while a remarkable variability was observed in S.S.a type plants and a few plants of S.S.b type in *S* cytoplasm. Pollen sterility was stable in most of S.S.b type and C.S. type plants. The average difference of pollen sterility and range of the variation were examined between every different branch, between every different flower, between every different anther and between the two anther lobes.

In some anthers, pollen sterility differed up to 91% between the lobes, while no difference was observed in another anther, even in the normal type of *N* cytoplasm. A similar tendency was obtained from the variation between anthers, between flowers and between branches. The data indicates that pollen sterility in plants of *N* and S.S.a types is quite unstable within a single plant even in full bloom while most of S.S.b type and C.S. type plants are stable throughout different parts of a single plant. In normal cytoplasm plants, sometimes brown non-dehiscent anthers are found mixed with normal yellow anthers even in a single flower. The pollen sterility was compared between yellow and brown anthers (Table 6). A remarkable variation of pollen sterility was observed both in yellow and brown anthers at diploid and tetraploid levels. The difference of pollen sterility between yellow and brown anthers was significant only in the No. 1 plant of H-19 (4X). It seems that the abnormality of anthers are caused by environmental factors or internal conditions.

TABLE 6 Pollen sterility of yellow anther (dehiscent) and brown anther (non-dehiscent) in H-19

| Ploidy | Strain | Plant No. | Type of cytoplasm | Phenotype | Pollen sterility (%) <sup>1)</sup> |               |
|--------|--------|-----------|-------------------|-----------|------------------------------------|---------------|
|        |        |           |                   |           | Yellow anthers                     | Brown anthers |
| 2X     | H-19   | 1         | <i>N</i>          | S.S.a     | 69.6 (2-100) <sup>2)</sup>         | 69.1 (14-100) |
|        |        | 2         |                   | S.S.a     | 55.3 (3-100)                       | 69.2 (15- 99) |
| 4X     | H-19   | 1         | <i>N</i>          | S.S.a     | 6.8 (1-14)                         | 39.4 ( 3-100) |
|        |        | 2         |                   | S.S.a     | 12.2 (1-38)                        | 20.3 ( 4-43)  |

1) Average of 15 anthers.

2) Range of variation.

### 3. *Modification of male sterility by environmental conditions*

Two extreme conditions, low temperature and no fertilizer, were used for examining the influence of environmental factors on phenotypic expression of male sterility. In order to examine the effect of low temperature during the microsporogenesis, plants were shifted into a low temperature cabinet (3° to 5°C) with illumination, and stored for 3 days after the onset of first flowering. Pollen sterility was observed just before the treatment and at every two days after completion of the treatment. As shown in Table 7, some plants were affected severely even in normal cytoplasm plants. In the male sterile strains, H-19 MS and 4M-50, most of the plants except for complete sterile plants were affected remarkably and changed their phenotypic expression. The influence of low temperature increased rapidly over 5 days after treatment in the plants of N and S.S.a types. The results indicated that the complete sterile type is most stable, whereas other types are affected remarkably by the low temperature.

TABLE 7 Modification of pollen sterility after treatment by low temperature

| Ploidy | Strain  | Type of cytoplasm | Change of M.S. type | Number of plants | Maximum increase of pollen sterility (%) |
|--------|---------|-------------------|---------------------|------------------|------------------------------------------|
| 2X     | H-19    | N                 | N → N               | 7                | 19.1                                     |
|        |         |                   | N → S.S.a           | 3                | 51.3                                     |
|        |         |                   | N → S.S.b           | 1                | 93.0                                     |
| 2X     | H-19 MS | S                 | N → N               | 1                | 22.0                                     |
|        |         |                   | N → S.S.a           | 2                | 60.5                                     |
|        |         |                   | N → S.S.b           | 4                | 80.8                                     |
|        |         |                   | N → C.S.            | 1                | 89.0                                     |
|        |         |                   | S.S.a → S.S.a       | 1                | 28.0                                     |
|        |         |                   | S.S.a → S.S.b       | 1                | 77.0                                     |
|        |         |                   | S.S.b → S.S.b       | 13               | 2.1                                      |
|        |         |                   | S.S.b → C.S.        | 9                | 7.1                                      |
|        |         |                   | C.S. → C.S.         | 4                | 0                                        |
| 4X     | 4M-50   | S                 | N → S.S.b           | 1                | 86.0                                     |
|        |         |                   | S.S.a → S.S.b       | 4                | 48.2                                     |
|        |         |                   | S.S.b → S.S.b       | 6                | 6.4                                      |
|        |         |                   | S.S.b → C.S.        | 1                | 0                                        |

TABLE 8 Modification of pollen sterility by deficiency of fertilizer

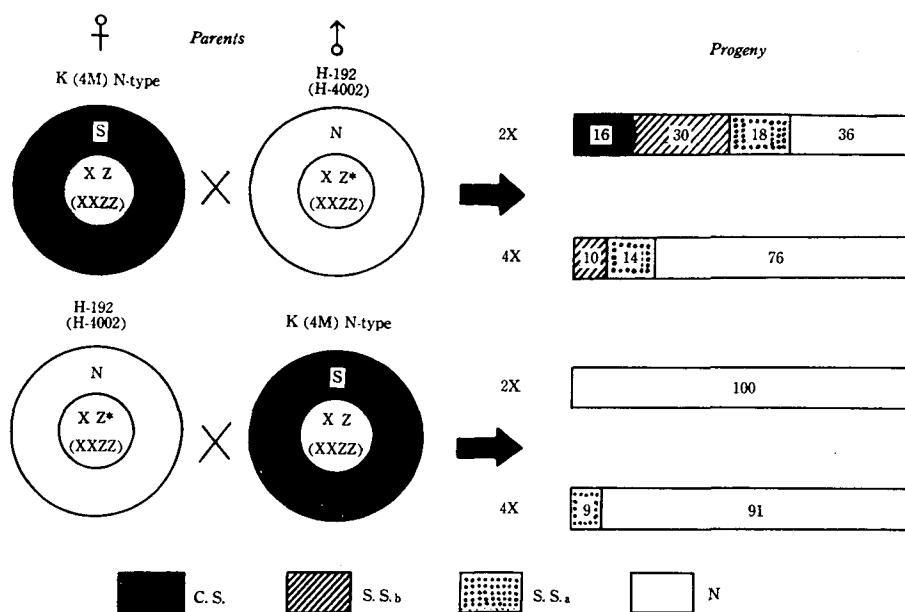
| Ploidy | Strain  | Type of cytoplasm | Phenotype of M.S. |              | No. of plants | Maximum difference of pollen sterility (%) |
|--------|---------|-------------------|-------------------|--------------|---------------|--------------------------------------------|
|        |         |                   | Fert. soil        | Vermiculite* |               |                                            |
| 2X     | H-19    | N                 | N                 | N            | 7             | 21                                         |
|        |         |                   | N                 | S.S.a        | 1             | 38                                         |
|        |         |                   | N                 | S.S.b        | 1             | 92                                         |
| 2X     | H-19 MS | S                 | N                 | N            | 1             | 5                                          |
|        |         |                   | S.S.b             | C.S.         | 1             | 0                                          |
|        |         |                   | C.S.              | C.S.         | 1             | 0                                          |
| 4X     | H-4002  | N                 | N                 | N            | 4             | 17                                         |
|        |         |                   | S.S.a             | N            | 2             | 13                                         |
|        |         |                   | S.S.a             | S.S.a        | 4             | 15                                         |
|        |         |                   | N                 | S.S.a        | 2             | 59                                         |
| 4X     | 4M-50   | S                 | N                 | S.S.a        | 1             | 35                                         |
|        |         |                   | S.S.a             | S.S.a        | 2             | 21                                         |
|        |         |                   | S.S.a             | S.S.b        | 1             | 31                                         |
|        |         |                   | S.S.b             | S.S.b        | 9             | 4                                          |

\* Fertilizer was not applied.

The effect of nutritional conditions was examined for the plants of H-19, H-19 MS, H-4002 and 4M-50. The roots of the plants were severed right in two. One of the halves was cultured in vermiculite without the application of fertilizer. The other half was cultured in heavy fertilized soil. The effect of nutritional condition was not so large that the changing of phenotypic expression from male fertile (N or S.S.a) to male sterile (S.S.b) was rarely indicated both in *N* and *S* cytoplasm (Table 8).

#### 4. Mode of inheritance

First a study was made to determine if the male sterile character was affected by cytoplasmic factors. Reciprocal crosses were made between normal plants from the male sterile strains, K- and 4M-strains and normal cytoplasm strains, Hon-iku-192 and H-4002. As shown in Figure 1, complete sterile and semi-sterile type-b plants occurred only from the mother plants of *S* cytoplasm at diploid and tetraploid levels. Plants of the progenies of the reciprocal combinations were all male fertile (N and S.S.a types). It follows from this fact that the male sterility of K-strains and 4M-strains



\* All genotypes ( $XZ$ ,  $Xz$ ,  $xZ$  and,  $xz$ ) in diploids are included.  
Parenthesis means their tetraploids.

Figure 1. Cytoplasmic inheritance of male sterility.

depends upon a male sterile cytoplasm. It is also assumed that some modifiers are responsible for the restoration of pollen fertility in the sterile cytoplasm.

In order to ascertain the presence of these sorts of modifying genes in the author's materials, complete sterile plants from H-19 MS (equivalent to M-2-8), were crossed with pollinators from the variety of normal cytoplasm, Hon-iku-192-5. The  $F_1$  plants from a cross of H-19 MS  $\times$  Hon-iku-192-5 were all male fertile (N and S.S.a types) showing the highest degree of pollen restoratoion (Table 17 a). Three plants which were phenotypically classified as N type were isolated to make sib-mating and their seeds were planted as separate progenies, viz. as  $F_2$  populations. Further,  $B_1$ s were produced from crosses between H-19 MS and the four  $F_1$ s. With regard to the phenotypic variation of the male sterile character in  $F_2$ s and  $B_1$ s, they were classified into four types as shown in Table 9 a and b.

Assuming that two dominant genes  $X$  and  $Z$  are responsible for the restoration of pollen fertility when they co-exist with a plasmagene  $S$ , one may expect to find that the genotypes of the semi-sterile type-a (S.S.a), semi-sterile type-b (S.S.b) and complete sterile type (C.S.) are governed by the double dominant, single dominant and double recessive condition of the said

genes respectively, in an  $F_2$  segregation ratio of 9:6:1. In addition to this, if an another modifier is involved in these cross combinations and exerts its enhancing effect on restoration of pollen fertility—that is it turns S.S.a into N—, a monogenic ratio of 3:1 would be expected between normal (N) and semi-sterile type-a (S.S.a). Thus, combining the above two ratios, one may reasonably expect a segregation ratio of  $N:S.S.a:S.S.b:C.S.=27:9:24:4$ . In actual observation of the  $F_2$  plants, the chi-square test indicated a good fit to the expected ratio throughout each of the cross combinations. In the case of  $B_1$  segregation, a ratio of  $N:S.S.a:S.S.b:C.S.=1:1:4:2$  should be expected from the genic scheme of the above three genes. Actual observation of  $B_1$  did not show a close fit to the above ratio; however if  $B_1$  plants

TABLE 9 Inheritance of male sterility in the crosses between  
H-19 MS (C.S. type) and Hon-iku-192-5

a.  $F_2$ : '56-106 H-19 MS (C.S.) $\times$ Hon-iku-192-5

| Cross Combination           | Phenotype in $F_2$ |             |             |             |               | Goodness of fit |      |           |
|-----------------------------|--------------------|-------------|-------------|-------------|---------------|-----------------|------|-----------|
|                             | N                  | S.S.a       | S.S.b       | C.S.        | Total         | $\chi^2$        | d.f. | P         |
| 1. Obs.<br>Cal.             | 79<br>87.33        | 25<br>29.11 | 84<br>77.63 | 19<br>12.94 | 207<br>207.01 | 4.739           | 3    | 0.10-0.20 |
| 2. Obs.<br>Cal.             | 25<br>21.09        | 9<br>7.03   | 13<br>18.75 | 3<br>3.13   | 50<br>50.00   | 2.853           | 2    | 0.20-0.30 |
| 3. Obs.<br>Cal.             | 38<br>27.84        | 9<br>9.28   | 17<br>24.75 | 2<br>4.13   | 66<br>66.00   | 7.100           | 2    | 0.02-0.05 |
| Cal. ratio<br>(9:6:1) (3:1) | 27                 | 9           | 24          | 4           |               |                 |      |           |

b.  $B_1$ : H-19 MS $\times$ '56-106  $F_1$  [H-19 MS (C.S.) $\times$ Hon-iku-192-5]

| Cross combination                 | N     | S.S.a | S.S.b | C.S.  | Total  |
|-----------------------------------|-------|-------|-------|-------|--------|
| H-19 MS $\times$ '56-106 $F_1$ -4 | 13    | 5     | 17    | 6     | 41     |
| " $\times$ " $F_1$ -7             | 12    | 4     | 19    | 7     | 42     |
| " $\times$ " $F_1$ -8             | 4     | 0     | 5     | 7     | 16     |
| " $\times$ " $F_1$ -9             | 9     | 1     | 8     | 6     | 24     |
| Total                             | 38    | 10    | 49    | 26    | 123    |
| Cal. (1:1:4:2)                    | 15.38 | 15.38 | 61.50 | 30.75 | 123.01 |
| Cal. (3:1)                        |       | 92.25 |       | 30.75 | 123.00 |

Goodness of fit (1:1:4:2) . . .  $\chi^2 = 38.477$ , d.f. = 3,  $P < 0.01$ .

(3:1) . . . . .  $\chi^2 = 0.978$ , d.f. = 1,  $P = 0.30-0.50$ .

were classified into two groups as (N, S.S.a, S.S.b):(C.S.), an expected digenic ratio of 3:1 was fairly well satisfied in this examination.

The theoretical assumption was testified in another cross between a complete sterile sugar beet and a normal table beet (Table 10 a, b). In  $F_1$  plants of the cross, H-19 MS  $\times$  Detroit dark red-3, two types, normal and semi-sterile type-b appeared. Two plants of N type and two plants of S.S.b type were isolated to make sib-crossing between plants of S.S.b type and N type and crossing within plants of N type. Two populations from the cross of N-type  $\times$  N-type and two populations from the cross of S.S.b type  $\times$  N type were produced from their seeds. If one assumes that a normal plant of Detroit dark red was heterozygous for *X* or *Z* genes, viz. *N Xx ZZ* or *N XX Zz*, two genotypes of  $F_1$  plants should be *S Xx Zz* and *S xx Zz* showing male fertile (N or S.S.a) and semi-sterile type-b respectively, as expected from the cross, *S xx zz*  $\times$  *N Xx ZZ*. A segregation ratio of N or

TABLE 10 Inheritance of male sterility in the crosses between H-19 MS (C.S. type) and Detroit dark red-3

a.  $F_2$ : '56-107 H-19 MS (C.S.)  $\times$  Detroit dark red-3

| Cross combination      | Crossing type in $F_1$ | Phenotype in $F_2$ |       |        |       |        | Goodness of fit |      |            |
|------------------------|------------------------|--------------------|-------|--------|-------|--------|-----------------|------|------------|
|                        |                        | N                  | S.S.a | S.S.b  | C.S.  | Total  | $\chi^2$        | d.f. | P          |
| 1. N $\times$ N        | Obs.                   | 80                 | 11    | 61     | 12    | 164    | 0.320           | 2    | 0.80-0.90  |
|                        | Cal.                   | 92.25              |       | 61.50  | 10.25 | 164.00 |                 |      |            |
| 2. N $\times$ N        | Obs.                   | 56                 | 7     | 33     | 14    | 110    | 9.155           | 2    | 0.01-0.02  |
|                        | Cal.                   | 61.88              |       | 41.25  | 6.88  | 110.01 |                 |      |            |
| Cal. ratio (3:1) (3:1) |                        | 9                  |       | 6      | 1     |        |                 |      |            |
| 3. S.S.b $\times$ N    | Obs.                   | 60                 | 31    | 157    | 39    | 287    | 4.110           | 2    | 0.10-0.20  |
|                        | Cal.                   | 107.63             |       | 143.50 | 35.88 | 287.01 |                 |      |            |
| 4. S.S.b $\times$ N    | Obs.                   | 109                | 29    | 183    | 66    | 387    | 7.341           | 2    | 0.001-0.01 |
|                        | Cal.                   | 145.13             |       | 193.50 | 48.38 | 387.01 |                 |      |            |
| Cal. ratio (3:1) (1:1) |                        | 3                  |       | 4      | 1     |        |                 |      |            |

b.  $B_1$ : '56-107  $F_1$ -S.S.b (H-19 MS  $\times$  D.D.R.-3)  $\times$  H-19

| Cross combination                             | N | S.S.a | S.S.b | C.S. | Total | $\chi^2$ | d.f. | P       |
|-----------------------------------------------|---|-------|-------|------|-------|----------|------|---------|
| '56-107 $F_1$ -S.S.b Obs.<br>( <i>SXxxz</i> ) | 0 | 4     | 43    | 36   | 83    | 1.458    | 1    | 0.2-0.3 |
| $\times$<br>H-19                              |   |       | 47    |      |       |          |      |         |
| ( <i>Nxxxx</i> ) Cal.<br>(1:1)                |   | 41.5  |       | 41.5 | 83.0  |          |      |         |

S.S.a:S.S.b:C.S.=9:6:1 is expected in  $F_2$  plants from the cross of  $N \times N$ , while a ratio of 3:4:1 is given for  $N$  or S.S.a:S.S.b:C.S. in  $F_2$  plants from the cross of S.S.b  $\times$   $N$ , depending upon the genic state of two genes,  $X$  and  $Z$ . Leaving a problem on a monogenic difference between S.S.a and  $N$  later on, actual observation was made in comparison with the theoretical number calculated from the above two ratios. In most of the crosses an expected digenic ratio was fairly well satisfied. A monogenic ratio of 3:1 was examined for  $N$  and S.S.a in all of the five crosses in  $F_2$ s. Cross combinations of 3 and 4 showed a good fit to 3:1 ratio while considerable deviation from 3:1 ratio was observed in the two other crosses, 1 and 2. A plant of semi-sterile type-b in  $F_1$  was crossed with a type-O plant of H-19. In such a case of  $B_1$  generation, a ratio of S.S.b:C.S.=1:1 should be expected from a segregation of  $X$  or  $Z$  genes. Actual observation showed a close fit to the ratio when a few plants of S.S.a were put together with S.S.b plants.

On the whole therefore, from the experimental results of two crosses, it was confirmed that the complete sterile type is dependent upon the double recessive state of the pollen restoring genes,  $X$  and  $Z$ . As to an enhancer which has to do with the effect of  $X$  and  $Z$ , there remains a possibility of the presence of a modifying gene or genes. Taking into consideration the variability of character expression which was described in the preceding sections of this chapter, proof of such gene or genes requires progeny tests to obtain further informations. Thus, the complementary action of  $X$  and  $Z$  genes is appropriate for the pollen restoration under the condition of co-existence with a plasmagene  $S$  at diploid level; they are designated as  $X$

TABLE 11 Linkage relation between the gene ( $m$ ) for monogerm and the gene ( $X$ ) for pollen restoring in  $S$  cytoplasm

Cross: '56-106 H-19 MS (C.S.)  $\times$  Hon-iku-192-5  $F_2$

| Germ type              | Multigerm                                        |                            | Monogerm                                         |                            | Total  |
|------------------------|--------------------------------------------------|----------------------------|--------------------------------------------------|----------------------------|--------|
| Male sterility         | Male fertile & semi-sterile<br>(N, S.S.a, S.S.b) | Complete sterile<br>(C.S.) | Male fertile & semi-sterile<br>(N, S.S.a, S.S.b) | Complete sterile<br>(C.S.) |        |
| Genotype               | $XZM$ , $XzM$ , $xZM$                            | $xzM$                      | $XZm$ , $Xzm$ , $xZm$                            | $xzm$                      |        |
| Obs.                   | 156                                              | 9                          | 32                                               | 10                         | 207    |
| Cal.<br>(R.C.V.=21.7%) | 150.24                                           | 5.01                       | 43.82                                            | 7.93                       | 207.00 |

Recombination value=21.7  $\pm$  5.58%.  
 $\chi^2=7.134$ , d.f.=3,  $P=0.05-0.10$ .

$\chi^2$ -analysis

| Component             | d.f. | $\chi^2$ | P         |
|-----------------------|------|----------|-----------|
| Germ type (3:1)       | 1    | 2.449    | 0.10-0.20 |
| Male sterility (15:1) | 1    | 3.030    | 0.05-0.10 |
| Linkage               | 1    | 15.097   | <0.01     |
| Total                 | 3    | 20.576   |           |

TABLE 12 Linkage relation between the gene (*m*) for monogerm and the gene (*X*) for pollen restoring in *S* cytoplasmCross: '56-107 H-19 MS (C.S.) $\times$ Detroit dark red-3  $F_2$ 

| Germ type              | Multigerm                  |                                      | Monogerm                   |                                      | Total  |
|------------------------|----------------------------|--------------------------------------|----------------------------|--------------------------------------|--------|
| Male sterility         | Male fertile<br>(N, S.S.a) | Male sterile<br>(S.S.b, C.S.)        | Male fertile<br>(N, S.S.a) | Male sterile<br>(S.S.b, C.S.)        |        |
| Genotype               | <i>XZM</i>                 | <i>XzM</i> , <i>xZM</i> , <i>xzM</i> | <i>XZm</i>                 | <i>Xzm</i> , <i>xZm</i> , <i>xzm</i> |        |
| Obs.                   | 89                         | 53                                   | 12                         | 27                                   | 181    |
| Cal.<br>(R.C.V.=24.5%) | 87.22                      | 48.53                                | 14.59                      | 30.66                                | 181.00 |

Recombination value=24.5 $\pm$ 3.61%. $\chi^2=1.345$ , d.f.=3, P=0.70-0.80. $\chi^2$ -analysis

| Component            | d.f. | $\chi^2$ | P         |
|----------------------|------|----------|-----------|
| Germ type (3:1)      | 1    | 0.015    | 0.90-0.95 |
| Male sterility (9:7) | 1    | 1.151    | 0.20-0.30 |
| Linkage              | 1    | 11.360   | <0.001    |
| Total                | 3    | 12.526   |           |

and *Z* adopting OWEN's gene symbols.

## 5. Linkage relation

As shown in Table 11, a linkage relation was established between the gene, *X* for pollen restoration and the gene, *m* for the monogerm character. The recombination value calculated in the coupling phase was 21.7%. In another cross between a sugar beet and a table beet, H-19 MS $\times$ Detroit dark red-3, a combined ratio of two characters, male fertile vs. male sterile and multigerm vs. monogerm, indicated the linkage relation between *X* and *m*. A recombination value calculated in the coupling phase was 24.5% (Table 12).

### 6. Genetic association between male sterility and color characters

It has been known that the red coloration of table beets depends upon the genic constitution of *RR GG* and a sugar beet with red-hypocotyl and white root possesses the genotype of *Rr gg* or *RR gg*. In addition to this, there is a close linkage between *R* and *G* (*Y*)\* with approximately 7.5% crossing overs (KELLER 1936). In  $F_2$ 's of the cross, H-19 MS (red-hypocotyl white root)  $\times$  Detroit dark red (table beet), a monogenic inheritance due to the gene *G* was confirmed for the coloration of root. Independent association was recognized between the genes, *X* and *Z* for pollen restoring and the gene, *G* for the coloration of the plants (Table 13 a, b).

According to the experiments by STEIN and GABELMAN (1959), in table beets, a correlation was observed between anther pigmentation and male sterility. In  $F_2$ s and  $B_1$  of the cross, H-19 MS  $\times$  Detroit dark red, red root beets produced both red-pigmented and non-pigmented anthers while white

TABLE 13 Independent relationship between the genes (*X* and *Z*) for pollen restoring in *S* cytoplasm and the gene (*G*) for red root

a.  $F_2$ : '56-107 H-19MS (C.S.)  $\times$  Detroit dark red-3

| Progeny No.            | Male sterility | Male fertile (N, S.S.a) |             | Male sterile (S.S.b, C.S) |             | Total         | Goodness of fit |      |           |
|------------------------|----------------|-------------------------|-------------|---------------------------|-------------|---------------|-----------------|------|-----------|
|                        | Root color     | Red                     | White       | Red                       | White       |               | $\chi^2$        | d.f. | P         |
| 1                      | Obs.<br>Cal.   | 31<br>29.95             | 7<br>9.98   | 26<br>23.30               | 7<br>7.77   | 71<br>71.00   | 1.318           | 3    | 0.70-0.80 |
| 3                      | Obs.<br>Cal.   | 51<br>46.41             | 12<br>15.47 | 37<br>36.09               | 10<br>12.03 | 110<br>110.00 | 1.598           | 3    | 0.50-0.70 |
| Cal. ratio (9:7) (3:1) |                | 27                      | 9           | 21                        | 7           |               |                 |      |           |

b.  $B_1$ : '56-107  $F_1$ -S.S.b (H-19 MS  $\times$  D.D.R.-3)  $\times$  H-19

| Progeny No.      | Male sterility | Semi-sterile (S.S.a, S.S.b) |             | Complete sterile (C.S.) |             | Total       | Goodness of fit |      |           |
|------------------|----------------|-----------------------------|-------------|-------------------------|-------------|-------------|-----------------|------|-----------|
|                  | Root color     | Red                         | White       | Red                     | White       |             | $\chi^2$        | d.f. | P         |
| 6<br>(1:1) (1:1) | Obs.<br>Cal.   | 19<br>20.75                 | 28<br>20.75 | 18<br>20.75             | 18<br>20.75 | 83<br>83.00 | 3.410           | 3    | 0.30-0.50 |

\* Some investigators employ the genic symbol '*Y*' instead of '*G*'. However, the author used *G* based on the priority by KAJANUS (1917).

TABLE 14 Correlation between male sterility and pigmentation of anthers in segregating populations

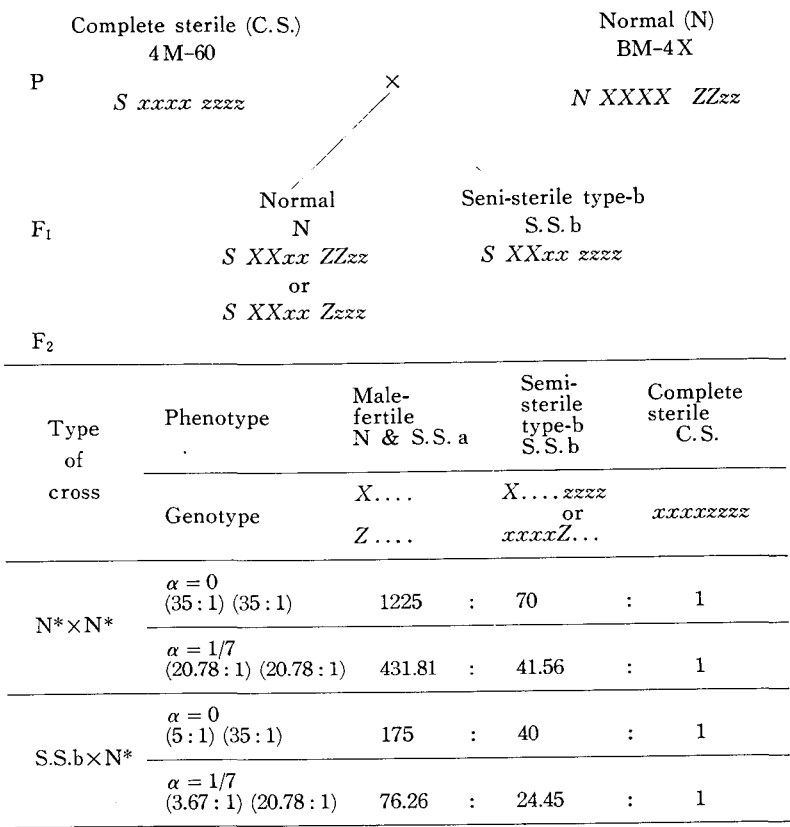
| Progeny No. | Root color                | Red       |       |      | Red or white  |       |      | Total | Test of independence |     |           |
|-------------|---------------------------|-----------|-------|------|---------------|-------|------|-------|----------------------|-----|-----------|
|             | Anther color              | Pigmented |       |      | Non-pigmented |       |      |       | $\chi^2$             | d.f | P         |
|             | Male sterility            | N, S.S.a  | S.S.b | C.S. | N, S.S.a      | S.S.b | C.S. |       |                      |     |           |
| 2           | F <sub>2</sub> population | 4         | 10    | 4    | 48            | 22    | 2    | 90    | 15.621               | 2   | <0.01     |
| 3           |                           | 6*        | 5     | 11   | 45            | 20    | 1    | 88    | 33.542               | 2   | <0.01     |
| 6           | B <sub>1</sub> population | 0         | 14    | 9    | 0             | 5     | 9    | 37    | 2.204                | 1   | 0.10-0.20 |

\* One of the six plants possessed a white root.

root beets showed all non-pigmented anthers except for a single plant of pigmented anthers. Although the mode of inheritance of pigmented anther is not clarified, a correlation exists between the phenotypic expression of male sterility and the pigmentation of anthers, as shown in Table 14. It is probable that the correlation of the characters depends upon the linkage relation between the gene for pollen restoration and the gene which controls the pigmentation of anthers.

#### 7. Tetrasomic inheritance

In tetraploids, cytoplasmic inheritance of male sterility was demonstrated in the crossing between normal plants from 4M-50 and normal cytoplasm plants, H-4002. This is as shown in the data of the preceeding section of this chapter (Figure 1). Although the pollen fertility generally decreases in tetraploid plants, the difference of fertility between diploids and tetraploids is fairly smaller than that between N and S.S.a in diploid strains. Therefore, the abnormality of anthers and pollen grains was classified into four types, as in the case of diploids (Table 2 and Plate II). 4M-60, a complete sterile plant of tetraploid sugar beet, was crossed with a normal plant of tetraploid wild beet, BM-4X (Figure 2). Two types, normal and semi-sterile type-b plants, appeared in F<sub>1</sub> plants. Assuming that the gene expression at tetraploids is equivalent to that at diploids, then the genotypes of the parents may be estimated to be *S xxxx zzzz* for a complete sterile plant of 4M-60 and *S XXxx ZZZZ* or *S XXXX ZZzz* for a normal plant of BM-4X. Thus, the genotypes appearing in F<sub>1</sub> plants may be estimated as *S XXxx ZZzz* or *S XXxx Zzzz* for normal plants and *S XXxx zzzz* for semi-sterile type-b plants respectively. If one may assume that the genotype of the N



\* The genotype is assumed as *S XXxx ZZzz*.

**Figure 2.** Tetrasomic inheritance of male sterility in the cross between tetraploid male sterile beet (4M-60) and tetraploid wild beet (BM-4X).

type in F<sub>1</sub> is *S XXxx ZZzz*\*, then theoretical ratios in the progenies from the crosses, N×N and S.S.b×N, in F<sub>1</sub> plants may be calculated on the basis of chromosome assortment ( $\alpha=0$ ) and chromatid assortment ( $\alpha=1/7$ ) for two genes, X and Z as shown in Figure 2. Actual observations indicated that two basic genes control the pollen restoration for the existence of *S* cytoplasm (Table 15). The segregation ratio arrived at intermediate values of the two theoretical ratios based on parameter  $\alpha=0$  (minimum value) and  $\alpha=1/7$  (maximum value). SAVITSKY (1963) assumed a monofactorial inheritance on the basis of chromatid assortment at the tetraploid level. However, in the author's inves-

\* In consideration to the low frequencies of C.S. type in F<sub>2</sub> of the crosses, N×N and S.S.b×N, the genotype of the N types in F<sub>1</sub> could be estimated as *S XXxx ZZzz*.

TABLE 15 F<sub>2</sub> segregations for male sterility in the cross between 4M-60 and BM-4X

| Type of cross | Year | Phenotype              | Normal<br>N        | Semi-ster.<br>type-a<br>S.S. a | Semi-ster.<br>type-b<br>S.S. b | Complete<br>sterile<br>C.S. | Total              | Goodness of fit |        |                        |
|---------------|------|------------------------|--------------------|--------------------------------|--------------------------------|-----------------------------|--------------------|-----------------|--------|------------------------|
|               |      | Genotype               | X....<br>Z....     |                                | X....zzzz<br>xxxxZ....         | xxxx<br>zzzz                |                    | $\chi^2$        | d. f.  | P                      |
| N×N           | 1966 | Obs.<br>$\alpha = 0$   | 238<br>282         | 44                             | 19                             | 0                           | 301                |                 |        |                        |
|               |      | Cal.<br>$\alpha = 1/7$ | 284.51<br>273.99   |                                | 16.26<br>26.37                 | 0.23<br>0.63                | 301.00<br>300.99   | 0.40<br>2.61    | 1<br>1 | 0.50-0.70<br>0.10-0.20 |
|               | 1967 | Obs.<br>$\alpha = 0$   | 1409<br>1595       | 186                            | 130                            | 1                           | 1726               |                 |        |                        |
|               |      | Cal.<br>$\alpha = 1/7$ | 1631.44<br>1571.15 |                                | 93.23<br>151.22                | 1.33<br>3.64                | 1726.00<br>1726.01 | 14.86<br>4.04   | 1<br>1 | <0.01<br>0.02-0.05     |
| S.S. b×N      | 1966 | Obs.<br>$\alpha = 0$   | 37<br>58           | 21                             | 28                             | 0                           | 86                 |                 |        |                        |
|               |      | Cal.<br>$\alpha = 1/7$ | 69.68<br>64.48     |                                | 15.93<br>20.68                 | 0.40<br>0.85                | 86.01<br>86.01     | 10.31<br>2.60   | 1<br>1 | <0.01<br>0.10-0.20     |
|               | 1967 | Obs.<br>$\alpha = 0$   | 273<br>401         | 128                            | 114                            | 1                           | 516                |                 |        |                        |
|               |      | Cal.<br>$\alpha = 1/7$ | 418.06<br>386.89   |                                | 95.56<br>124.04                | 2.39<br>5.07                | 516.01<br>516.00   | 3.67<br>2.06    | 1<br>1 | 0.05-0.10<br>0.10-0.20 |

tigation, the digenic hypothesis proposed in studies of diploid beets, hold good at tetraploid level also.

#### 8. Selection of pollen parents

Under the genic assumption of the existence of  $X$  and  $Z$ , the genotype  $xx\ zz$  with  $N$  cytoplasm (designated as type-O) is desirable for the pollen parent crossed with complete sterile plants ( $S\ xx\ zz$ ). This type of plants was selected from some commercial varieties by some workers (OWEN 1948, PETERSON 1952, KNAPP 1955, TAKEDA and IMANISHI 1963, NIELSON and NEMAZI 1967). In a population of  $N$  cytoplasm, nine genotypes are expected to exist under the genic assumption of the existence of  $X$  and  $Z$ . They are classified into six types (O to V) by the mode of segregation expected from the crosses, C.S. type ( $S\ xx\ zz$ )  $\times$  Pollinators ( $N$  cytoplasm) (Table 16). Complete sterile plants of K- and M-strains were crossed with some pollinators which was selected at random from the Japanese varieties, Hon-iku-192 and Hon-iku-399. The frequencies of the male sterile types in the progenies of the crosses are shown in Table 17. The genotypes of pollinators were estimated from the theoretical ratios given in Table 16, taking into consideration the variability of phenotypic expression of male sterility. As shown in Table 17 a, the heterozygous conditions of pollinators (I, II and III) were found more frequently than the homozygous conditions of pollinators (O, IV and V). Besides this, in the case where 192-11 and 192-12 were used as pollinators, the genotype of complete sterile plants seemed to affect the segregation mode of the progenies more strongly than the environment. It is probable that minor modifying genes extend their effect on the expression of male sterility as proposed by several investigators (OWEN 1948, ROHRBACH 1965 b, NIELSON and NEMAZI 1967). In addition to that, various ratios for

TABLE 16 Segregating ratios expected from the crosses C.S. type ( $S\ xx\ zz$ )  $\times$  Pollinator ( $N$  cytoplasm)

| Genotype of pollinator  | Phenotypic ratio of offspring (%) |        |           |
|-------------------------|-----------------------------------|--------|-----------|
|                         | C.S.                              | S.S. b | S.S. a, N |
| O $N\ xxxz$             | 100                               | —      | —         |
| I $N\ Xxxz$ or $xxZz$   | 50                                | 50     | —         |
| II $N\ XxZz$            | 25                                | 50     | 25        |
| III $N\ XxZZ$ or $XXZz$ | —                                 | 50     | 50        |
| IV $N\ XXzz$ or $xxZZ$  | —                                 | 100    | —         |
| V $N\ XXZZ$             | —                                 | —      | 100       |

S.S.a to N indicate that the assumed modifying gene does not satisfactorily interpret the enhancing effect of *X* and *Z*. This indicates that the modifying gene or genes interact in a complicated manner with environmental conditions. The frequencies of the male sterile types in the progenies from the same complete sterile plants pollinated with the plants of Hon-iku-192 and

TABLE 17 Phenotypic ratios of male sterility observed in the progenies of the crosses between C.S. type (*S xx zz*) and Pollinator (*N* cytoplasm)

a.

| Cross combination | Percentage of male sterile type |       |       |    | Number of plants | Expected genotype of pollinator |
|-------------------|---------------------------------|-------|-------|----|------------------|---------------------------------|
|                   | C.S.                            | S.S.b | S.S.a | N  |                  |                                 |
| M-2-8×192-1       | 77                              | 23    | 0     | 0  | 31               | 0                               |
| K-3-6×192-2       | 40                              | 56    | 4     | 0  | 45               | I                               |
| M-19 × "          | 61                              | 39    | 0     | 0  | 18               | "                               |
| K-3-2×192-13      | 34                              | 60    | 6     | 0  | 35               | "                               |
| M-2 ×399-3        | 63                              | 23    | 13    | 0  | 30               | "                               |
| M-2 ×399-7        | 50                              | 50    | 0     | 0  | 16               | "                               |
| K-3-6×192-1       | 51                              | 35    | 8     | 5  | 37               | I or II                         |
| " ×192-4          | 74                              | 16    | 0     | 11 | 19               | "                               |
| M-19 ×192-10      | 51                              | 43    | 3     | 3  | 35               | "                               |
| K-3-6×192-12      | 73                              | 27    | 0     | 0  | 22               | "                               |
| M-19 × "          | 70                              | 16    | 11    | 3  | 37               | "                               |
| M-2 × "           | 33                              | 38    | 10    | 19 | 21               | "                               |
| M-17 × "          | 23                              | 59    | 9     | 9  | 22               | "                               |
| K-3-6×192-3       | 26                              | 52    | 15    | 7  | 27               | II                              |
| K-3-2×192-15      | 20                              | 71    | 6     | 3  | 35               | "                               |
| K-3-6× "          | 25                              | 57    | 11    | 7  | 44               | "                               |
| M-2 "             | 0                               | 89    | 7     | 4  | 27               | "                               |
| K-3-8×192-20      | 8                               | 69    | 12    | 12 | 26               | "                               |
| " ×192-21         | 18                              | 45    | 9     | 27 | 22               | "                               |
| " ×192-26         | 57                              | 25    | 10    | 7  | 67               | "                               |
| M-2-7× "          | 39                              | 39    | 11    | 11 | 18               | "                               |
| M-2 ×399-2        | 13                              | 73    | 3     | 10 | 30               | "                               |
| " ×399-4          | 13                              | 78    | 7     | 2  | 46               | "                               |
| K-5-2×192-11      | 15                              | 37    | 32    | 17 | 41               | III                             |
| M-19 × "          | 3                               | 73    | 10    | 15 | 40               | "                               |
| M-2 × "           | 0                               | 41    | 12    | 46 | 41               | "                               |
| M-16 × "          | 0                               | 50    | 29    | 21 | 24               | "                               |
| M-3 × "           | 0                               | 35    | 25    | 40 | 20               | "                               |
| K-3-8×192-22      | 0                               | 73    | 19    | 8  | 26               | "                               |
| M-2-1× "          | 7                               | 50    | 0     | 43 | 14               | "                               |
| K-3-8×192-25      | 3                               | 45    | 21    | 31 | 29               | "                               |
| M-2 ×399-8        | 14                              | 31    | 23    | 31 | 35               | "                               |
| K-3-6×192-6       | 0                               | 70    | 20    | 10 | 10               | IV                              |
| M-19 × "          | 0                               | 88    | 13    | 0  | 24               | "                               |
| M-2-8×192-5       | 0                               | 0     | 25    | 75 | 12               | V                               |

b.

| Cross combination | Number of pollinators | Percentage of male sterile type |       |       |     | Number of plants |
|-------------------|-----------------------|---------------------------------|-------|-------|-----|------------------|
|                   |                       | C.S.                            | S.S.b | S.S.a | N   |                  |
| K-3-6 (C.S.)×192  | 7                     | 41                              | 45    | 8     | 6   | 204              |
| M-19 (C.S.)×192   | 5                     | 37                              | 52    | 7     | 4   | 154              |
| M-2 (C.S.)×399    | 5                     | 31                              | 51    | 9     | 9   | 157              |
| Mean              |                       | 36.3                            | 49.3  | 8.0   | 6.3 |                  |

TABLE 18 Phenotypic ratios of male sterility in the progenies of the crosses between S.S.b type and pollinators (*N* cytoplasm)

| Ploidy | Cross combination  | Percentage of male sterile type |       |       |      | Number of plants |
|--------|--------------------|---------------------------------|-------|-------|------|------------------|
|        |                    | C.S.                            | S.S.b | S.S.a | N    |                  |
| 2X     | K-3-11 (S.S.b)×192 | 9                               | 68    | 18    | 5    | 22               |
|        | " -12 ( " )× "     | 2                               | 81    | 14    | 4    | 57               |
|        | " -16 ( " )× "     | 25                              | 25    | 40    | 10   | 20               |
|        | Mean               | 12.3                            | 58.3  | 24.0  | 6.3  | —                |
|        | M-8 (S.S.b)×192    | 8                               | 78    | 11    | 3    | 36               |
|        | " -21 ( " )× "     | 0                               | 93    | 7     | 0    | 15               |
|        | " -30 ( " )× "     | 5                               | 70    | 10    | 15   | 20               |
|        | " -32 a ( " )× "   | 27                              | 73    | 0     | 0    | 11               |
|        | " -32 b ( " )× "   | 15                              | 65    | 8     | 12   | 26               |
|        | " -32 c ( " )× "   | 0                               | 35    | 25    | 40   | 20               |
|        | Mean               | 9.2                             | 69.0  | 10.2  | 11.7 | —                |
| 4X     | 4M-48 (S.S.b)×4398 | 0                               | 94    | 0     | 6    | 16               |
|        | " ( " )×4192       | 3                               | 80    | 3     | 13   | 61               |
|        | 4M-50 ( " )×4398   | 7                               | 89    | 2     | 2    | 135              |
|        | " ( " )×4192*      | 0                               | 89    | 11    | 0    | 37               |
|        | " ( " )×4192*      | 5                               | 76    | 7     | 13   | 128              |
|        | 4M-59 ( " )×4398   | 4                               | 91    | 4     | 0    | 45               |
|        | 4M-60 ( " )× "     | 0                               | 90    | 5     | 5    | 21               |
|        | Mean               | 2.7                             | 87.0  | 4.6   | 5.6  | —                |

\* Observation in different populations.

Hon-iku-399 were summed up and the respective average frequency of each sterile type is shown in Table 17b. It is noted that there was a similar frequency of occurrence of the male sterile types in the progenies from the

three kinds of cross combinations, regardless of whether K- or M- was used as the female parent, and regardless of whether Hon-iku-192 or Hon-iku-399, was used as the male parent.

Another series of cross experiments was conducted with the semi-sterile type-b plants, too. Semi-sterile type-b plants in diploids and tetraploids were crossed with normal plants randomly sampled from Hon-iku-192 at diploids and 4192 and 4398 at tetraploids (Table 18). Though the segregation mode in progenies is different depending upon the genotypes in S.S.b plants which were used as female parents, the total average frequency of C.S. and S.S.b was more than 70% in diploids and about 90% in tetraploids. These results show that even if plants of random samples from the above varieties are used as the pollinators, a relatively high percentage of C.S. and S.S.b types which are safe in pollen shedding can transmit to progenies through mother plants of complete sterile and semi-sterile type-b both at diploid and tetraploid levels. However, in a case where it is desired to obtain 100% incidence of male sterile plants, the type-O plants should be selected and employed as the pollinator.

#### 9. *Maintenance of male sterility in the original population*

As to the maintenance of K-strains, it is rather difficult to breed true the type-O plants from a population of the original variety, Hon-iku-192. Therefore, as a short cut, the author tentatively isolated the K-strains and made inter-crossing between "male sterile group (C.S. and S.S.b)" and "male fertile group (S.S.a and N)" within the same strains. A similar method was applied for M-5 strain in diploids and H-4005 MS and 4M-60 strains in tetraploids. In the original population, 6 to 9 plants from the male sterile group (C.S. and S.S.b) were chosen at random and the frequency of the said four types was examined in the progeny of the male sterile plants (Table 19). Although the segregation mode varies remarkably among the progenies of male sterile plants depending upon the genotypes of the female parents, the average frequency of the male sterile group (C.S. and S.S.b) in the progenies was not so different from that in the original populations of each strain. It is noted that a high percentage of male sterility (90%) in the original population of 4M-60 was transmitted to progenies through S.S.b plants at the tetraploid level. The recovery of the male fertile plants is a beneficial phenomenon for repeated intercrossing in the strain without applying other plants or strains as pollinators.

Through these experiments, it seems that this type of crossing, from a practical point of view, would serve the purpose of keeping the cytoplasmic-genetic male sterility within the respective strains or populations.

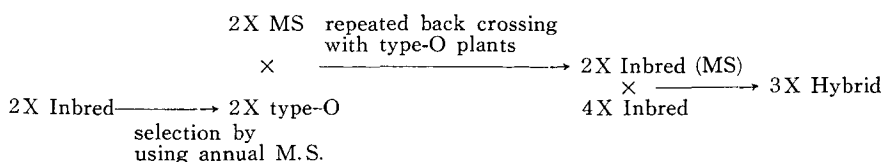
TABLE 19 Phenotypic ratios of male sterility observed in the progenies of the crosses between male sterile types (S.S.b and C.S.) and the normal type of *S* cytoplasm

| Ploidy | Cross combination             | Phenotype of offsprings (%) |       |       |      | Number of plants |
|--------|-------------------------------|-----------------------------|-------|-------|------|------------------|
|        |                               | C.S.                        | S.S.b | S.S.a | N    |                  |
| 2X     | K-3-7 (S.S.b)×(N)             | 0                           | 42    | 13    | 46   | 24               |
|        | " -8 ( " )×( " )              | 10                          | 80    | 10    | 0    | 10               |
|        | " -12 ( " )×( " )             | 0                           | 14    | 38    | 48   | 21               |
|        | " -16 ( " )×( " )             | 0                           | 42    | 42    | 16   | 19               |
|        | " -17 ( " )×( " )             | 16                          | 32    | 16    | 36   | 25               |
|        | " -21 ( " )×( " )             | 0                           | 52    | 34    | 15   | 27               |
|        | " -23 ( " )×( " )             | 0                           | 28    | 28    | 43   | 21               |
|        | mean                          | 3.7                         | 41.4  | 25.9  | 29.1 |                  |
|        | K-3 Original population       | 2                           | 30    | 17    | 51   | 84               |
|        | M-5-3 (S.S.b)×(N)             | 14                          | 43    | 14    | 29   | 21               |
|        | " -5 ( " )×( " )              | 33                          | 13    | 13    | 40   | 15               |
|        | " -1 (C.S.)×( " )             | 21                          | 32    | 15    | 32   | 34               |
|        | " -4 ( " )×( " )              | 56                          | 17    | 11    | 17   | 18               |
|        | " -6 ( " )×( " )              | 18                          | 64    | 9     | 9    | 11               |
|        | " -7 ( " )×( " )              | 6                           | 35    | 6     | 53   | 17               |
|        | " -14 ( " )×( " )             | 33                          | 27    | 13    | 27   | 15               |
|        | mean                          | 25.9                        | 33.0  | 11.6  | 29.6 |                  |
|        | M-5 Original population       | 41                          | 14    | 16    | 29   | 51               |
| 4X     | H 4005 MS-4 (S.S.b)×(N)       | 16                          | 68    | 16    | 0    | 19               |
|        | " -5 ( " )×( " )              | 8                           | 71    | 4     | 17   | 24               |
|        | " -6 ( " )×( " )              | 0                           | 53    | 13    | 33   | 15               |
|        | " -7 ( " )×( " )              | 15                          | 85    | 0     | 0    | 13               |
|        | " -9 ( " )×( " )              | 0                           | 29    | 35    | 35   | 17               |
|        | " -10 ( " )×( " )             | 7                           | 79    | 14    | 0    | 14               |
|        | " -16 ( " )×( " )             | 5                           | 50    | 15    | 30   | 20               |
|        | " -17 ( " )×( " )             | 0                           | 0     | 33    | 67   | 12               |
|        | " -1 (C.S.)×( " )             | 14                          | 57    | 14    | 14   | 7                |
|        | mean                          | 7.2                         | 54.7  | 16.0  | 21.8 |                  |
|        | H 4005 MS Original population | 10                          | 42    | 14    | 34   | 177              |
|        | 4M-60-1 (S.S.b)×(N)           | 0                           | 92    | 8     | 0    | 25               |
|        | " -2 ( " )×( " )              | 0                           | 81    | 10    | 10   | 21               |
|        | " -4 ( " )×( " )              | 31                          | 62    | 0     | 8    | 13               |
|        | " -6 ( " )×( " )              | 19                          | 71    | 5     | 5    | 21               |
|        | " -10 ( " )×( " )             | 6                           | 92    | 2     | 0    | 49               |
|        | " -11 ( " )×( " )             | 17                          | 83    | 0     | 0    | 12               |
|        | mean                          | 12.2                        | 80.2  | 4.2   | 3.8  |                  |
|        | 4M-60 Original population     | 0                           | 90    | 5     | 5    | 21               |

#### 10. Application of male sterility in triploid breeding

Triploid hybrids are usually produced by a single cross between diploid and tetraploid inbreds or strains. Based on the author's experimental results,

## A. USE OF 2X MALE STERILITY



## B. USE OF 4X MALE STERILITY

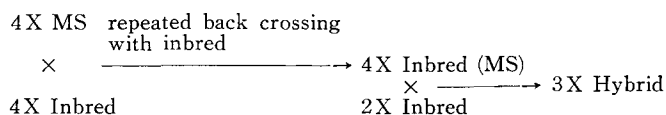


Figure 3. Breeding scheme on triploid hybrids by the use of male sterility.

a breeding scheme of triploid hybrids by the use of male sterility both at the diploid and tetraploid levels may be postulated. This is diagrammatically shown in Figure 3. In the use of diploid male sterility, type-O plants must be selected from a normal cytoplasm strain by progeny tests of the crosses between an annual complete sterile plant and pollinators from the normal strain. Then diploid male sterile plants are back-crossed with type-O plants as recurrent parents to produce diploid inbreds or strains with male sterility. Pure triploid hybrids are obtained by crossing between a diploid male sterile inbred or strain and a tetraploid inbred or strain. If the seed productivity of a diploid male sterile inbred is insufficient, a single cross hybrid can be replaced for the crossing with a tetraploid inbred.

In the use of tetraploid male sterility, tetraploids of type-O plants are not necessary because of the fact that more than 90% of the plants show practical male sterility in the progeny of the crossing between male sterile plants and pollinators chosen at random from the strain. The male sterile character is introduced to desirable inbreds or strains by successive back crossing. Thus, pure triploid hybrids are produced by crossing between a tetraploid male sterile inbred or strain and a diploid inbred or strain. Finally combining ability should be testified in triploid hybrids which was produced by the use of diploid or tetraploid male sterility.

Trial seeds were produced from crossing between normal diploid and tetraploid plants and from crossings by use of diploid or tetraploid male sterility. Normal tetraploids, H-4002, and normal diploids, H-2002, were mix-planted in a ratio of 3:1 and the seeds were harvested separately from diploids and tetraploids. Tetraploid male sterile strains, 4M-50, 4M-59 and 4M-60 which were produced by the crossing with the plants randomly chosen from the normal strain 4192, and which consisted of male sterile

plants in excess of 90%, were mix-planted with normal diploid plants of H-2002 in a ratio of 3:1, and the seeds were obtained from tetraploid male sterile plants. Diploid male sterile strains, M-14, M-35 and M-41 which were produced by the crossing with the plants randomly chosen from Hon-iku-192 without the use of type-O plants and which consisted of lower frequencies of male sterile plants (57-78%), were mix-planted with normal tetraploids, H-4002 or 4192 in a ratio of 3:1 and the seeds were obtained from diploid male sterile plants.

Frequency of diploid, triploid and tetraploid individuals in the eight populations were examined in the seedlings by counting the chromosome numbers (Figure 4). 100% or 97% triploid seeds were obtained from the crossings between tetraploid male sterile strains and diploid pollinators, whereas a smaller percentage of triploid seeds (34-57%) was produced from diploid male sterile strains. In the progenies of the crossing between normal tetraploids and diploids, the frequency of triploids was 77%, while it decreased to 48% in the progeny of the reciprocal direction of the cross, diploids  $\times$  tetraploids. These results indicate that a higher percentage of triploids produced with tetraploid male sterile plants is due to a higher percentage of male sterile plants in 4M-strains and a faster growth of haploid pollen

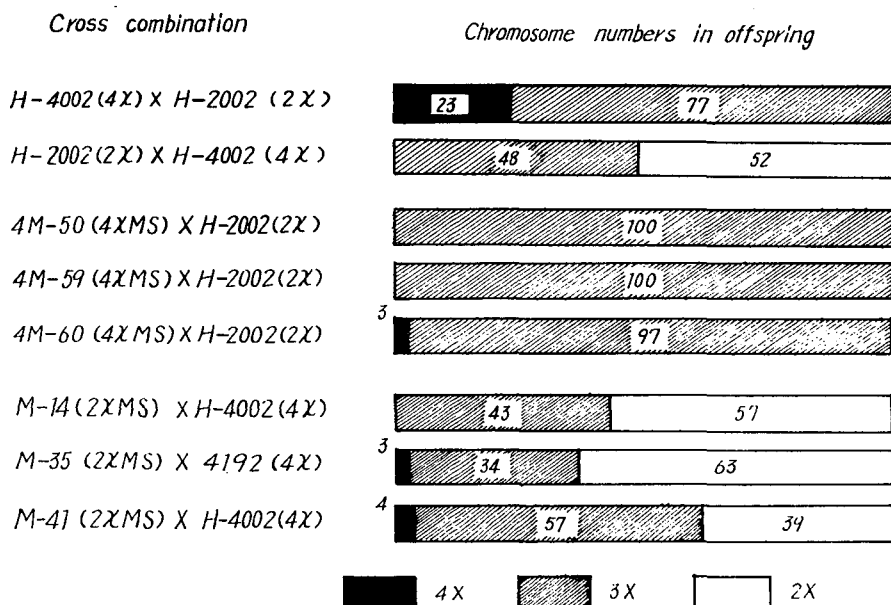


**Figure 4.** Frequency of diploids, triploids and tetraploids in various populations of triploid hybrids.

tubes from diploid pollinators than that of diploid pollens from normal tetraploids which are contained in male sterile tetraploids in 4M-strains. It is also shown that the use of imperfect male sterility in diploids is not effective for the production of a higher proportion of triploids.

In 1960 and 1961, yield trials were conducted at the experimental field of the Hokkaido University in Furano, which is located in the central part of Hokkaido. The triploid hybrids which were produced experimentally by using diploid and tetraploid male sterility as described in the preceding section, were planted together with their parental diploid and tetraploid strains. The experimental design was a randomized block with four replications. The plot size was 5 m<sup>2</sup> and spacings were 50 cm between rows and 23 cm between plants within rows. Farming and application of fertilizer were in the usual manner.

Throughout the results of two years, the triploid hybrids which were produced with tetraploid male sterile plants, showed higher yields in both root weight and sugar content in comparison with triploid hybrids in similar combinations which were produced with diploid male sterile plants (Figure 5). This seems to depend on the high proportion of triploids in the triploid hybrids produced with tetraploid male sterile plants. As suggested by BANDLOW (1964b), the combining ability of pure triploid hybrids is as important

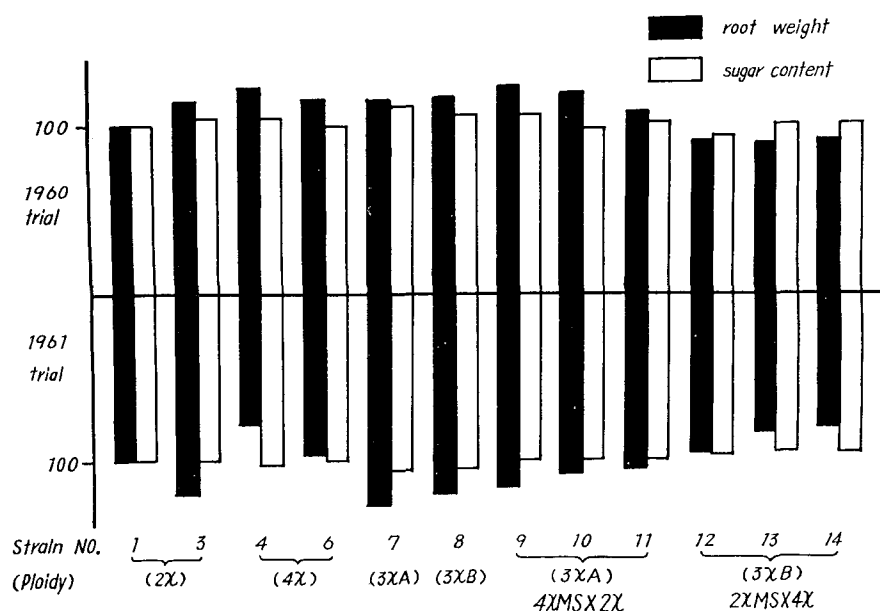


Figure 5. Yield test of various triploid hybrids.

for the yield as the proportion of triploids.

## B. Genetic male sterility\*

### 1. Source of the materials

In 1962, a complete male sterile plant was found in an annual wild beet, *Beta maritima* L. The mutant was named as BM-2. In the progeny of the mutant, BM-2, various degrees of male sterile plants and normal plants appeared. In 1963, nine plants were chosen at random from the male sterile plants in the progeny of BM-2 and crossed with BM-2-13 which were chosen from the normal plants in the progeny of BM-2. Nine populations were grown from the crosses between male sterile plants and a normal plant. The mode of inheritance was examined in 1964. In addition, BM-2 was crossed with a normal cytoplasm strain of sugar beet, H-19 in 1962.  $F_1$  plants were isolated in different greenhouses. Two  $F_1$  plants were sib-crossed in a greenhouse and six  $F_1$  plants were inter-crossed in another greenhouse. The seeds were planted as separate progenies, viz.  $F_2$  populations. As the sugar beet was a biennial plant, photo-thermal induction was applied for  $F_2$  populations and most of the plants flowered within summer of 1964. A sugar beet strain of the Mendelian male sterility, CT-5, was introduced from the U.S.A. by courtesy of the Sugar Beet Institute in Japan. In 1967, a complete male sterile plant, BM-2-1 from the progeny of BM-2 was crossed with a normal plant 'CT-5-1' which was contained in a population grown from the seeds of 'CT-5'.  $F_1$  plants were grown in the greenhouse with illumination during the winter of 1967, after treatment of the seedling by low temperature.  $F_1$  plants were inter-crossed by pairs to produce the seeds of  $F_2$ s in the early spring of 1968.  $F_2$  populations of the cross, BM-2-1  $\times$  CT-5-1, were grown in the experimental field under long day conditions with an application of photo-thermal induction and the onset of flowering was seen during the summer of 1968. Male sterile types were examined in each  $F_2$  populations.

### 2. Phenotypes of male sterility

Types of male sterility appeared in the progeny of BM-2 were classified into six types because, in the variation of male sterility, the intermediate types between complete sterile (C.S.) and semi-sterile type-b (S.S.b) and between semi-sterile type-b (S.S.b) and type-a (S.S.a) were found. They are named as modified C.S. and modified S.S.b. Four types from C.S. to modified S.S.b are tentatively denoted as MS-I, MS-II, MS-III and MS-IV in which

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\* Male sterility depending on a single gene, synonymous with "genic male sterility".

TABLE 20 Classification of male sterility derived from BM-2

| Type of male sterility                               | Complete sterile MS-I | Modified C.S. MS-II | Semi-sterile type-b MS-III | Modified S.S.b MS-IV | Semi-sterile type-a S.S.a | Normal (Control) N |
|------------------------------------------------------|-----------------------|---------------------|----------------------------|----------------------|---------------------------|--------------------|
| Color of anther                                      | White                 | White & orange      | Orange                     | Orange               | Yellow                    | Yellow             |
| Dehiscent                                            | —                     | —                   | —                          | ±                    | ±                         | +                  |
| Pollen fertility (%)                                 | 0                     | 0-20                | 0-20                       | 20-50                | 20-70                     | 70-100             |
| Classification in cytoplasmic-genetic male sterility | C.S.                  | C.S. or S.S.b       | S.S.b                      | S.S.b or S.S.a       | S.S.a                     | N                  |

MS-I and MS-III are equivalent to C.S. and S.S.b (Table 20). Modified C.S. (MS-II) is characterized with the mixture of white shrunken anthers and non-dehiscent reddish-yellow anthers which contain abortive pollens. In the modified S.S.b (MS-IV), dehiscent and non-dehiscent yellow anthers exist within a flower and shows an intermediate type between S.S.b and S.S.a. Thus, the male sterility in the progeny of BM-2 indicates a continuous variation between MS-I and MS-IV while they were discrete with the normal type.

### 3. Mode of inheritance.

As shown in Table 21 a, progenies of the male sterile plants which were crossed with BM-2-13 produced the said six types of male sterility. In each of the nine crosses which includes seven crosses of MS-I×N, one cross of MS-II×N and one cross of MS-III×N, nearly half of the plants indicated the normal type, while the other half belonged to the male sterile types, (MS I-IV). S.S.a type showed a relatively small number or did not exist. If the phenotypes were grouped into two groups, viz. male sterile (MS I-IV) and male fertile (N and S.S.a), the segregation ratio in the nine progenies, showed a close fit to the ratio of 1:1 irrespective of the type of crosses, MS-I×N, MS-II×N and MS-III×N (Table 21 b). Therefore, it was assumed that a single recessive gene is responsible for the phenotypic expression of the male sterility. The genotype of the male sterile plant was in a recessive homozygous state for the said gene, while the genotype of the N type plant was heterozygous condition of the said gene. When BM-2-13 was self-fertilized, it gave 30 plants of male fertile type (N and S.S.a) and 16 plants of male sterile types (MS I-IV) indicating a good fit to the segregation ratio of 3:1 ( $\chi^2=2.348$ ,  $P=0.10-0.20$ ). The progenies of the crosses, MS-II×N and MS-III×N, showed a similar mode of segregation of the male sterility when compared with those of the cross MS-I×N. It follows from

TABLE 21 Inheritance of male sterility in the crosses within the progeny of a mutant, BM-2 of wild beet

a.

| Cross combination | Parental phenotype | Phenotype of male sterility |       |                      |       |       |    | Total |
|-------------------|--------------------|-----------------------------|-------|----------------------|-------|-------|----|-------|
|                   |                    | MS-I <sup>1)</sup>          | MS-II | MS-III <sup>2)</sup> | MS-IV | S.S.a | N  |       |
| BM-2-1×13         | MS-I×N             | 17                          | 2     | 1                    | 3     | 1     | 12 | 36    |
| 3× "              | MS-I×N             | 16                          | 10    | 3                    | 1     | 2     | 33 | 65    |
| 4× "              | MS-I×N             | 8                           | 4     | 1                    | 3     | 0     | 9  | 25    |
| 7× "              | MS-I×N             | 34                          | 17    | 11                   | 2     | 0     | 43 | 107   |
| 8× "              | MS-I×N             | 13                          | 3     | 2                    | 4     | 1     | 22 | 45    |
| 12× "             | MS-I×N             | 19                          | 6     | 5                    | 4     | 1     | 43 | 78    |
| 14× "             | MS-I×N             | 10                          | 0     | -6                   | 6     | 2     | 26 | 50    |
| 5× "              | MS-II×N            | 32                          | 7     | 3                    | 1     | 1     | 55 | 99    |
| 6× "              | MS-III×N           | 18                          | 4     | 0                    | 1     | 0     | 18 | 41    |

b.

| Cross combination | Phenotype of male sterility |          |       | Goodness of fit (1:1) |           |
|-------------------|-----------------------------|----------|-------|-----------------------|-----------|
|                   | MS (I-IV)                   | S.S.a, N | Total | $\chi^2$              | P         |
| BM-2-1×13         | 23                          | 13       | 36    | 2.778                 | 0.05-0.10 |
| 3× "              | 30                          | 35       | 65    | 0.385                 | 0.50-0.70 |
| 4× "              | 16                          | 9        | 25    | 1.960                 | 0.10-0.20 |
| 7× "              | 64                          | 43       | 107   | 4.121                 | 0.02-0.05 |
| 8× "              | 22                          | 23       | 45    | 0.022                 | 0.80-0.90 |
| 12× "             | 34                          | 44       | 78    | 1.282                 | 0.20-0.30 |
| 14× "             | 22                          | 28       | 50    | 0.720                 | 0.30-0.50 |
| 5× "              | 43                          | 56       | 99    | 1.707                 | 0.10-0.20 |
| 6× "              | 23                          | 18       | 41    | 0.610                 | 0.30-0.50 |

Homogeneity  $\chi^2 = 13.468$ , d.f. = 9, P = 0.10-0.20.

1) Equivalent with C.S. type.

2) Equivalent with S.S.b type.

this that the phenotypic difference of male sterile types (MS I-IV) are possibly due to environmental variations.

In order to introduce the male sterile character to sugar beets, a male sterile mutant, BM-2 was crossed with a normal plant of a monogerm strain, H-19.  $F_1$  plants were normal and  $F_2$  populations were assorted into the said six phenotypes (Table 22 a). The segregation mode of all  $F_2$  populations came

up to expectation, segregating as male fertile (N and S.S.a) : male sterile (MS I-IV)=3:1 (Table 22b). Thus, it was confirmed that this male sterile mutant behaved as a single Mendelian recessive to the normal type. The symbol of its causal gene is designated as 'ms'.

Further, a study was made to determine if the male sterile character

TABLE 22 Inheritance of male sterility in  $F_2$ s of crosses between a male sterile mutant of wild beet (BM-2) and a sugar beet (H-19)

a.

| F <sub>2</sub> population | Phenotype of male sterility |       |        |       |       |     | Total |
|---------------------------|-----------------------------|-------|--------|-------|-------|-----|-------|
|                           | MS-I                        | MS-II | MS-III | MS-IV | S.S.a | N   |       |
| '63-35 : 1                | 12                          | 6     | 4      | 7     | 2     | 51  | 82    |
| BM-2×H-19: 2              | 6                           | 4     | 6      | 3     | 0     | 30  | 49    |
| F <sub>2</sub> : 3        | 8                           | 4     | 4      | 5     | 3     | 67  | 91    |
| : 4                       | 7                           | 4     | 5      | 6     | 3     | 55  | 80    |
| : 5                       | 9                           | 4     | 3      | 2     | 0     | 36  | 54    |
| : 8                       | 6                           | 2     | 3      | 3     | 2     | 34  | 50    |
| : 9×10                    | 3                           | 4     | 9      | 8     | 5     | 78  | 107   |
| : "                       | 2                           | 5     | 8      | 9     | 3     | 79  | 106   |
| : 10×9                    | 14                          | 6     | 9      | 10    | 2     | 124 | 165   |
| : "                       | 5                           | 3     | 8      | 7     | 3     | 75  | 101   |

b.

| F <sub>2</sub> population | Phenotype of male sterility |          |       | Goodness of fit (1:3) |           |
|---------------------------|-----------------------------|----------|-------|-----------------------|-----------|
|                           | MS (I-IV)                   | S.S.a, N | Total | $\chi^2$              | P         |
| '63-35: 1                 | 29                          | 53       | 82    | 4.699                 | 0.02-0.05 |
| : 2                       | 19                          | 30       | 49    | 4.959                 | 0.02-0.05 |
| : 3                       | 21                          | 70       | 91    | 0.179                 | 0.50-0.70 |
| : 4                       | 22                          | 58       | 80    | 0.267                 | 0.50-0.70 |
| : 5                       | 18                          | 36       | 54    | 2.000                 | 0.10-0.20 |
| : 8                       | 14                          | 36       | 50    | 0.240                 | 0.50-0.70 |
| : 9×10                    | 24                          | 83       | 107   | 0.377                 | 0.50-0.70 |
| : "                       | 24                          | 82       | 106   | 0.314                 | 0.50-0.70 |
| : 10×9                    | 39                          | 126      | 165   | 0.164                 | 0.50-0.70 |
| : "                       | 23                          | 78       | 101   | 0.267                 | 0.50-0.70 |

Homogeneity  $\chi^2 = 12.634$ , d.f. = 9, P = 0.10-0.20.

from BM-2 was affected by cytoplasmic factors or not. A reciprocal cross was made between a normal plant, BM-2-11 which was chosen in the progeny of BM-2 and a normal cytoplasm plant, H-19. As shown in Table 23, there was no remarked difference with the phenotypes of  $F_1$  plants between the crosses, BM-2-13  $\times$  H-19 and the reciprocal, H-19  $\times$  BM-2-13. It is noted that a male sterile plant of MS-IV was obtained from a mother plant of *N* cytoplasm, H-19, as well as from BM-2-13. This may be caused by an environmental variation of a heterozygous state of the gene '*ms*'. On the whole, therefore, it may be ascertained that the male sterility from BM-2 is due to a recessive chromogene which is not affected by a cytoplasmic factor.

As mentioned in the preceding chapter, the pollen restoring gene *X* for *S* cytoplasm is linked with the gene *m* for monogerm character. In connection with this, a genetic relation was examined between the male sterility from BM-2 and the monogerm character by using the  $F_2$  plants from the cross, BM-2 (a multigerm, male sterile mutant)  $\times$  H-19 (a monogerm, normal plant). The actual data on  $F_2$  segregation were given in Table 24, where an independent association was obtained between the gene for male sterility and the gene for monogerm character. This result also indicates that the gene

TABLE 23 Male sterility in the progenies of the reciprocal crosses between *N* type plants (BM-2-11 and H-19)

Cross: '63-36 BM-2-11 (*N*)  $\times$  H-19  $F_1$

| Cross combination                  | Phenotype of male sterility |       |       |        |       |      | Total |
|------------------------------------|-----------------------------|-------|-------|--------|-------|------|-------|
|                                    | N                           | S.S.a | MS-IV | MS-III | MS-II | MS-I |       |
| BM-2-11 ( <i>N</i> ) $\times$ H-19 | 70                          | 3     | 1     | 0      | 0     | 0    | 74    |
| Reciprocal                         | 41                          | 0     | 1     | 0      | 0     | 0    | 42    |

TABLE 24 Independent relationship between the gene (*m*) for monogerm and the gene (*ms*) for male sterility

Cross: '63-35 BM-2 (MS-I)  $\times$  H-19  $F_2$

| Germ type         | Multigerm                |                           | Monogerm                 |                           | Total  |
|-------------------|--------------------------|---------------------------|--------------------------|---------------------------|--------|
| Male sterility    | Male fertile<br>N, S.S.a | Male sterile<br>MS (I-IV) | Male fertile<br>N, S.S.a | Male sterile<br>MS (I-IV) |        |
| Obs.              | 154                      | 62                        | 72                       | 20                        | 308    |
| Cal.<br>(9:3:3:1) | 173.25                   | 57.75                     | 57.75                    | 19.25                     | 308.00 |

$\chi^2 = 5.99$ , d. f. = 3,  $P = 0.10-0.20$ .

'*ms*' exists in a different locus with the gene *X* for the pollen restoration.

4. *Genetic interaction between the gene 'ms' and the pollen restoring genes (X and Z) under S cytoplasm*

In this experiment, it was examined how the gene '*ms*' interacts with *X* and *Z* under *S* cytoplasm. A complete male sterile plant of H-19 MS was crossed with a normal plant, BM-2-13, which was chosen from the progeny of BM-2 and possessed a heterozygous state of the gene '*ms*' with *N* cytoplasm (Figure 6). Therefore, the genotype of the parents are known as *S xxxz* for H-19 MS and *N Msms* for BM-2-13. In  $F_1$  plants from the cross, H-19 MS  $\times$  BM-2-13, two plants of *N* type and two plants of S.S.b type were segregated. The genotypes for  $F_1$  plants were estimated, from the genic constitution of their parents, to be *S XxZz* for *N* type and *S xxZz* for S.S.b type. It was assumed that BM-2-13 possesses a heterozygous state of a pollen restoring gene, *X* or *Z*, viz. *XxZZ* or *XXZz*, which was assumed in the tetraploid wild beet, BM-4X, likewise. In addition to

|                             |           |                                                            |                                                 |                                                                                    |
|-----------------------------|-----------|------------------------------------------------------------|-------------------------------------------------|------------------------------------------------------------------------------------|
| P.                          |           | Complete-sterile<br>H-19 MS (C.S.)<br><i>S xx zz MsMs</i>  | $\times$                                        | Normal<br>BM-2-13 (N)<br><i>N Xx ZZ Msms</i>                                       |
| F <sub>1</sub>              |           | Normal<br><i>S Xx Zz Msms</i><br>or<br><i>S Xx Zz MsMs</i> |                                                 | Semi-sterile (S.S.b or MS-III)<br><i>S xx Zz Msms</i><br>or<br><i>S xx Zz MsMs</i> |
| F <sub>2</sub>              |           |                                                            |                                                 |                                                                                    |
| Type of cross               | Phenotype | Male fertile<br>N & S.S.a                                  | Male sterile<br>MS (I-IV)                       |                                                                                    |
|                             | Genotype  | <i>XZMs</i>                                                | <i>XZms, XzMs, Xzms, xZMs, xZms, xzMs, xzms</i> |                                                                                    |
| N* $\times$ N*              |           | (9:7) (3:1)                                                | 27                                              | : 37                                                                               |
| S.S.b* (MS-III) $\times$ N* |           | (3:5) (3:1)                                                | 9                                               | : 23                                                                               |

\*  $F_1$  plants are assumed to be heterozygous for *Ms* allele (*Msms*).

**Figure 6.** Inheritance of male sterility in the cross between cytoplasmic male sterility (H-19 MS) and a heterozygous plant for Mendelian male sterility (BM-2-13).

TABLE 25  $F_2$  segregations for male sterility in the cross between H-19 MS and BM-2-13

| Type of cross           | Phenotype       | Male fertile<br>N, S.S.a | Male sterile<br>MS (I-IV)             | Total  | Goodness of fit |      |           |
|-------------------------|-----------------|--------------------------|---------------------------------------|--------|-----------------|------|-----------|
|                         | Genotype        | XZMs                     | XZms, XzMs, Xzms,<br>xZMs, xzMs, xzms |        | $\chi^2$        | d.f. | P         |
| N×N                     | Obs.            | 18      12<br>30         | 39                                    | 69     | 0.047           | 1    | 0.80-0.90 |
|                         | Cal.<br>(27:37) | 29.11                    | 39.89                                 | 69.00  |                 |      |           |
| S.S.b<br>(MS-III)<br>×N | Obs.            | 14      30<br>44         | 149                                   | 193    | 2.709           | 1    | 0.05-0.10 |
|                         | Cal.<br>(9:23)  | 54.28                    | 138.72                                | 193.00 |                 |      |           |

this, it was postulated that the gene '*ms*' works in the presence of *X* and *Z* genes under the *S* cytoplasm as well as in the *N* cytoplasm, and the  $F_1$  plants in the crosses,  $N \times N$  and  $S.S.b \times N$  are heterozygous for *Ms* allele (*Ms ms*). If a consideration is made on the environmental variation by the gene '*ms*', phenotypes of  $F_2$  may be assorted into male fertile (*N* and *S.S.a*) and male sterile (*MS I-IV*). Theoretical ratios were calculated for the progenies of two kinds of crosses,  $N \times N$  and  $S.S.b \times N$  in  $F_1$  as shown in Figure 6. The actual observed ratio was a close fit to a trihybrid ratio of 27:37 in the cross,  $N \times N$  (Table 25). In another type of cross,  $S.S.b \times N$ , the theoretical ratio of 9:23 was fairly well satisfied, indicating that the genic assumption of the parent was appropriate. Thus, it was ascertained that the gene '*ms*' inserts the effect in *S* cytoplasm co-existing with *X* and *Z* as well as in *N* cytoplasm. The gene '*ms*' indicates an independent association with *X* and *Z* genes.

##### 5. Genic identification of male sterile characters between the strains, BM-2 and CT-5

The male sterility of a sugar beet strain, CT-5, is governed by a Mendelian recessive gene ( $a_1$  or  $a_2$ ). In a population of CT-5, male fertile plants (*N* or *S.S.a*) and male sterile plants (*C.S.*) occurred in a ratio of 11:13, showing a close fit to a backcrossed ratio of 1:1 ( $\chi^2=0.167$ ,  $P=0.50-0.70$ ). The genic identification was conducted between the genes '*ms*' and '*a*', by using the cross between a complete sterile plant, BM-2-1 and a normal plant, CT-5-1. A large part of  $F_1$  plants were *N*-type, but at the same time, a few *S.S.a* plants and one *MS-III* plant also occurred (Table 26a).

TABLE 26 Genic identification between the genes 'ms' and 'a' for Mendelian male sterility

Cross: BM-2-1 MS-I type (*msms*) $\times$ CT-5-1 N-type (*Aa*)

| a. F <sub>1</sub> | Phenotype        |            | Obs. number |
|-------------------|------------------|------------|-------------|
|                   | Normal           | N          | 150         |
|                   | Semi-sterile-a   | S.S.a      | 6           |
|                   | Semi-sterile-b   | MS-III, IV | 1           |
|                   | Complete sterile | MS-I, II   | 0           |
|                   | Total            |            | 157         |

b. F<sub>2</sub>: N-type (*Aa Msms*) $\times$ N-type (*Aa Msms*)

| Phenotype                     | Genotype    | Cross A   | Cross B   | Total     |
|-------------------------------|-------------|-----------|-----------|-----------|
| N                             | <i>A Ms</i> | 31        | 14        | 45        |
| S.S.a                         |             | 9         | 2         | 11        |
| MS-III, IV                    | <i>A ms</i> | 11        | 1         | 12        |
| MS-I, II                      | <i>a Ms</i> | 27        | 11        | 38        |
|                               | <i>a ms</i> |           |           |           |
| Total                         |             | 78        | 28        | 106       |
| N & S.S.a: MS (I-IV)<br>= 9:7 |             |           |           |           |
| $\chi^2$                      |             | 0.782     | 0.009     | 0.504     |
| P                             |             | 0.70-0.80 | 0.90-0.95 | 0.30-0.50 |

Further, F<sub>1</sub> plants of N-type were inter-crossed by pairs to make F<sub>2</sub> populations. Among the F<sub>2</sub> populations, two populations, A and B segregated into male fertile (N and S.S.a) and male sterile (S.S.b and C.S.) plants in a ratio of 9:7 (Table 26b). Thus, it is ascertained that the gene 'ms' and the gene 'a' are non-allelic and are not located in the same linkage group.

#### 6. Relation between the male sterility and the seed setting

Male sterile plants of MS-I and MS-II types contain underdeveloped flowers in different frequencies, especially in the upper part of inflorescence. The underdeveloped flowers are characterized by diminishing floral organs and imperfect opening of the calyx. This may be a possible causation of producing aborted seeds. In the progeny of BM-2 plant, the percentage of seed setting was compared between the male fertile group (N and S.S.a) and male sterile group (MS I-IV). The seed setting of male sterile plants de-

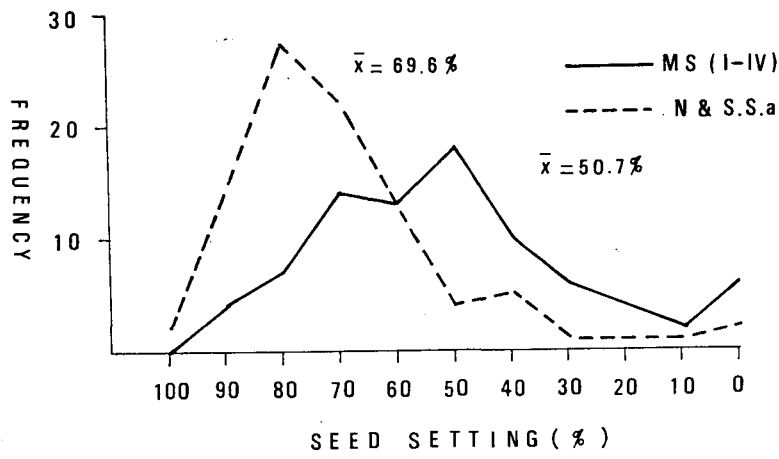


Figure 7. Relationship between male sterility and seed setting in the progeny of BM-2.

TABLE 27 Seed setting of male fertile and male sterile plants which were segregated in F<sub>2</sub> populations of the cross between BM-2 and H-19

| Year | Phenotype of male sterility | Seed setting (%) |    |    |    |    |    |    |    |    |    |   | Total | Mean (%) |
|------|-----------------------------|------------------|----|----|----|----|----|----|----|----|----|---|-------|----------|
|      |                             | 100              | 90 | 80 | 70 | 60 | 50 | 40 | 30 | 20 | 10 | 0 |       |          |
| 1964 | Male fertile (N, S.S.a)     | 0                | 8  | 22 | 25 | 11 | 10 | 4  | 0  | 1  | 2  | 0 | 83    | 68.3     |
|      | Male sterile (MS-I-IV)      | 0                | 3  | 5  | 6  | 7  | 6  | 3  | 0  | 0  | 2  | 0 | 32    | 61.9     |
| 1965 | Male fertile (N, S.S.a)     | 14               | 44 | 67 | 37 | 20 | 5  | 5  | 3  | 2  | 0  | 0 | 197   | 76.6     |
|      | Male sterile (MS-I-IV)      | 2                | 11 | 15 | 11 | 8  | 5  | 2  | 3  | 0  | 0  | 0 | 57    | 71.2     |

creased significantly as compared with male fertile plants as shown in Figure 7. In the F<sub>2</sub> populations of the cross, BM-2×H-19, a similar relation was recognized for both observations made in 1964 and 1965 (Table 27). However, the percentage of seed setting is also affected considerably by the difference of year. Since the seed setting of male sterile plants shows a wide range of variation, there is a possibility that the seed setting of male sterile plants are improved by selection.

### C. Induced male sterility by gamma ray irradiation

#### 1. Purpose of the experiment

New sources of male sterility are expected to be obtained by interspecific crosses and induced mutations other than spontaneous mutations. In this experiment, gamma rays were used as the mutagen to induce male sterile characters which are not accompanied by seed sterility. There is a possibility of an appearance of induced male sterility due to point mutation and chromosomal aberration. However, the author has explored a new possibility in which the male sterility is produced by the alternation of cytoplasm or plasmagenic mutation in this gamma irradiation experiment.

#### 2. Materials and methods

Dry seeds of normal cytoplasm strain, H-19, were exposed to gamma rays of  $\text{Co}^{60}$ . The irradiation was carried out at the gamma room of the National Institute of Agricultural Science in Hiratsuka by courtesy of Dr. T. KAWAI in April, 1961 (The  $\text{Co}^{60}$  gamma ray source had a total activity of 100 curies). The seeds were treated for 20 days and the total exposures administered were 50, 100, 150 and 200 kR. The exposure rate and the distance from the source are as follows ;

| Total exposures | Exposure rate | Vertical distance<br>from the source | Horizontal distance<br>from the source |
|-----------------|---------------|--------------------------------------|----------------------------------------|
| 50 kR           | 1.75 R/M      | 42 cm                                | 87 cm                                  |
| 100 kR          | 3.45 R/M      | ditto                                | 56.5 cm                                |
| 150 kR          | 5.20 R/M      | ditto                                | 41 cm                                  |
| 200 kR          | 6.95 R/M      | ditto                                | 31 cm                                  |

The germination tests were carried out in a nursery bed in the greenhouse. Plants from the irradiated seeds were grown in the greenhouse during the summer of 1962. Flowering was induced in the spring of 1963 after applying photothermal induction to the roots.

#### 3. Germination and vegetative growth

The results of the germination tests are shown in Table 28. The germinated plants were counted at the outset of plants from the soil. Though the germination was not so different from the control even in high dosage treatments, in the majority of cases plants subjected to a dosage of over 100 kR died after the cotyledon stage. The immediate cause may be attributed to the failure of development of lateral roots and primary leaves. They are as shown in Plate III a. However, some of the plants exposed to 50 and 100 kR survived as shown in Exp. 2 and grew to maturity.

TABLE 28 Germination test of the seeds irradiated by gamma rays in a diploid strain, H-19

| Experiment | Radiation treatments | Germination rate (%) | Rate of survived plants (%) |
|------------|----------------------|----------------------|-----------------------------|
| 1          | Control              | 79                   | 79                          |
|            | 50 kR                | 64                   | 60                          |
|            | 100 "                | 62                   | 0                           |
|            | 150 "                | 86                   | 0                           |
|            | 200 "                | 71                   | 0                           |
| 2          | Control              | 42                   | 42                          |
|            | 50 kR                | 46                   | 19                          |
|            | 100 "                | 35                   | 7                           |

Abnormal shape and thickness of young leaves were observed in most of the plants from the irradiated seeds. The roots showed numerous solid tumor-like lump formation as shown in Plate III b. In  $M_1$  populations, abnormal plants with lax or dense setting were found in flower clusters (Plates IV a, b and c). A plant with a monogerm character was induced from the multi-germ strain, H-2002 after irradiation (Plate IV d).

#### 4. Occurrence of male sterility

41  $M_1$  plants grown in the greenhouse flowered during the spring of 1963. Male sterility in  $M_1$  plants were classified into the four types, N, S.S.a, S.S.b and C.S., according to the demarcation-standard applied to the cytoplasmic-genetic male sterility. Various degrees of partial male sterility appeared in the population while four plants of S.S.b type and three plants of C.S. type were obtained (Figure 8). The percentage of seed setting generally decreased below that of the average value of the control plants and showed a wide range of variation. The correlation coefficient between pollen fertility and percentage of seed setting was calculated as  $r=0.155$  (d.f.=37) in  $M_1$  plants, and  $r=0.073$  (d.f.=42) in the control plants. Thirty-two lines of  $M_2$  were produced from  $M_1$  mother plants under open pollination in an isolated field. The transmission of the male sterility from  $M_1$  to  $M_2$  generation are shown in Figures 8 and 9. The relatively high proportions (over 60%) of male sterile plants (S.S.b and C.S.) were transmitted into the progenies from three plants of C.S. type. The male sterile plants (S.S.b and C.S.) were obtained in the progenies of two plants of S.S.b type and a plant of N type in a smaller percentage (30-50%). In addition to these, a small number of male sterile plants also appeared in the progenies of six  $M_1$  plants which

showed N or S.S.a types. The average percentage of seed setting in  $M_2$  lines,  $\gamma$ -54,  $\gamma$ -20 and  $\gamma$ -27 were not so different from that of the control, H-19, while a wide range of variation was observed in the  $M_3$  lines. Inheritance of the male sterility from  $M_2$  plants to  $M_3$  lines under open pollination was examined in  $\gamma$ -54,  $\gamma$ -20 and  $\gamma$ -27 lines. As shown in Figure 10, the male sterility was inherited by  $M_3$  plants in a relatively high proportion even from

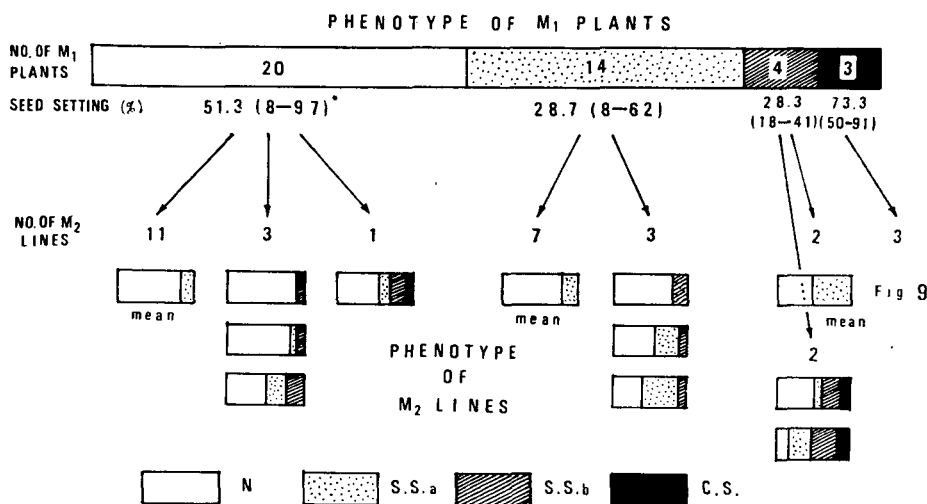


Figure 8. Inheritance of male sterility from  $M_1$  plants to  $M_2$  lines.

| LINE         | PH. OF $M_1$ PLANT | SEED SETTING ( $M_1$ ) % | PHENOTYPE OF $M_2$ LINE | OBS NO | SEED SETTING ( $M_2$ ) % |
|--------------|--------------------|--------------------------|-------------------------|--------|--------------------------|
| $\gamma$ -54 | C.S.               | 50.4                     | 38 53 9                 | 47     | 82.4 (54-97)*            |
| $\gamma$ -20 | C.S.               | 78.2                     | 17 19 50 14             | 64     | 74.8 (5-100)             |
| $\gamma$ -27 | C.S.               | 91.2                     | 8 11 66 15              | 82     | 81.7 (30-99)             |
| H-19 MS      | C.S.               | 87.8                     | 8 10 29 53              | 51     | 88.5 (54-98)             |
| H-19         | N                  | 85.2                     | 100                     | 49     | 84.6 (49-99)             |

N
  S.S.a
  S.S.b
  C.S.

Figure 9. Phenotypic ratios of male sterility in  $M_2$  lines.

mother plants of N type in  $M_2$  plants. In most of the  $M_3$  lines, the frequency of C.S. type was smaller than that of the cytoplasmic male sterile strain, H-19 MS-5. Similar results were obtained in the inheritance of the male sterility from  $M_3$  plants to  $M_4$  lines, as shown in Table 29. Thus, the male sterile character was maintained until the fourth generation ( $M_4$ ) through the mother

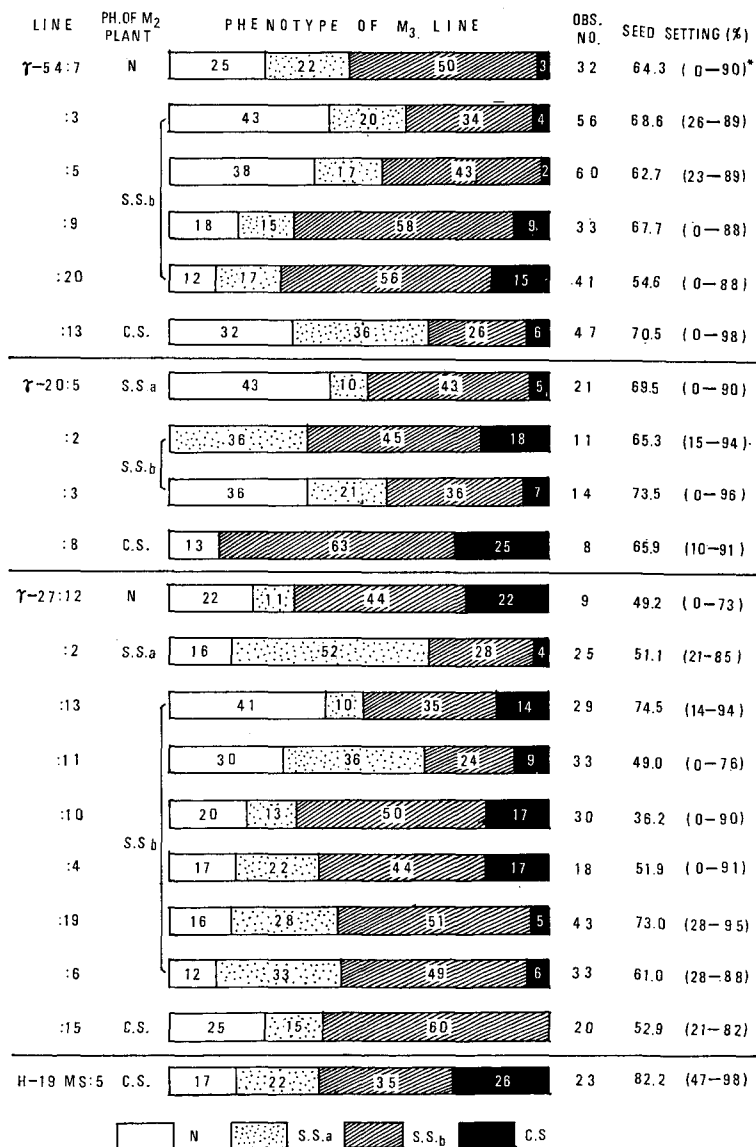


Figure 10. Phenotypic ratios of male sterility in  $M_3$  lines.

TABLE 29 Phenotypic ratios of male sterility in M<sub>4</sub> lines

| Line         | Phenotype of M <sub>3</sub> plant | Seed fert. of M <sub>3</sub> plants (%) | Phenotype of M <sub>4</sub> line (%) |       |       |      | Observed number | Seed setting (%) |               |
|--------------|-----------------------------------|-----------------------------------------|--------------------------------------|-------|-------|------|-----------------|------------------|---------------|
|              |                                   |                                         | N                                    | S.S.a | S.S.b | C.S. |                 | mean             | range of var. |
| 7-54-9 : 11  | S.S. b                            | 82                                      | 9                                    | 4     | 72    | 15   | 54              | 45.5             | 0-94          |
| : 1          | "                                 | 78                                      | 9                                    | 9     | 67    | 15   | 78              | 49.6             | 0-93          |
| : 3          | "                                 | 86                                      | 11                                   | 10    | 64    | 15   | 72              | 57.8             | 0-97          |
| : 13         | "                                 | 68                                      | 17                                   | 17    | 60    | 7    | 30              | —                | —             |
| : 14         | "                                 | 70                                      | 19                                   | 19    | 60    | 2    | 53              | 33.2             | 0-94          |
| : 10         | C.S.                              | 72                                      | 19                                   | 11    | 58    | 11   | 36              | 45.2             | 0-87          |
| 7-54-20 : 12 | S.S. b                            | 65                                      | 17                                   | 22    | 49    | 12   | 41              | —                | —             |
| : 30         | "                                 | 88                                      | 21                                   | 21    | 50    | 9    | 34              | —                | —             |
| : 42         | C.S.                              | 69                                      | 19                                   | 12    | 53    | 17   | 59              | —                | —             |
| 7-20-2 : 12  | S.S. b                            | 55                                      | 16                                   | 7     | 62    | 16   | 45              | 71.9             | 29-96         |
| : 1          | "                                 | 94                                      | 31                                   | 10    | 53    | 6    | 51              | 74.1             | 13-99         |
| : 5          | "                                 | 86                                      | 33                                   | 19    | 44    | 4    | 69              | 62.2             | 19-95         |
| 7-20-8 : 17  | "                                 | 90                                      | 3                                    | 2     | 48    | 47   | 60              | 56.3             | 26-90         |
| : 3          | "                                 | 82                                      | 5                                    | 7     | 58    | 30   | 92              | 44.3             | 0-90          |
| : 5          | "                                 | 91                                      | 15                                   | 9     | 43    | 34   | 47              | 58.6             | 0-93          |
| 7-27-19 : 9  | S.S. b                            | 73                                      | 5                                    | 8     | 83    | 5    | 40              | 66.8             | 12-96         |
| : 22         | "                                 | 84                                      | 10                                   | 22    | 67    | 0    | 9               | 70.4             | 56-90         |
| : 10         | C.S.                              | 75                                      | 5                                    | 8     | 44    | 44   | 39              | 48.5             | 0-86          |
| 7-27-15 : 3  | S.S. a                            | 82                                      | 29                                   | 24    | 48    | 0    | 21              | 72.1             | 15-93         |
| H-19-MS      | C.S.                              | 94                                      | 1                                    | 16    | 37    | 46   | 70              | 67.6             | 17-99         |

plants in the three lines, 7-54, 7-20 and 7-27.

##### 5. Mode of inheritance

First, a possibility of cytoplasmic inheritance was found in the male sterile character induced by the gamma irradiations. This may be said because the segregation mode of male sterile types in the progenies of the crosses between normal plants from the male sterile lines, 7-20 and 7-27, and the normal cytoplasm plants of H-19, differed remarkably from that in the progenies of the reciprocal crosses (Table 30). Hence, it is quite probable that the induced male sterility originated from a mutation of a cytoplasmic factor after the irradiation.

TABLE 30 Phenotype of male sterility in progenies of the reciprocal crosses between N type plants in M<sub>2</sub> lines and H-19 (N cytoplasm)

| Combination                                          | Phenotype of offspring |                   |                 |               | Total      |
|------------------------------------------------------|------------------------|-------------------|-----------------|---------------|------------|
|                                                      | N                      | S. S. a           | S. S. b         | C. S.         |            |
| <i>γ</i> -20-1 (N)×H-19-1<br>Reciprocal              | 30 (33)*<br>77 (95)    | 29 (32)<br>4 (5)  | 29 (32)<br>0    | 2 (2)<br>0    | 90<br>81   |
| <i>γ</i> -20-4 (N)×H-19-2<br>Reciprocal              | 7 (27)<br>8 (80)       | 7 (27)<br>2 (20)  | 9 (35)<br>0     | 3 (12)<br>0   | 26<br>10   |
| <i>γ</i> -20-28 (N)×H-19-1<br>Reciprocal             | 8 (30)<br>38 (86)      | 5 (19)<br>5 (11)  | 9 (33)<br>1 (2) | 5 (19)<br>0   | 27<br>44   |
| <i>γ</i> -20-28 (N)×H-19-1<br>Reciprocal             | 13 (22)<br>8 (89)      | 13 (22)<br>1 (11) | 25 (42)<br>0    | 9 (15)<br>0   | 60<br>9    |
| <i>γ</i> -20-30 (N)×H-19-4<br>Reciprocal             | 12 (52)<br>41 (100)    | 2 (9)<br>0        | 8 (35)<br>0     | 1 (4)<br>0    | 23<br>41   |
| <i>γ</i> -20-36 (N)×H-19-5<br>Reciprocal             | 10 (26)<br>62 (100)    | 3 (8)<br>0        | 24 (63)<br>0    | 1 (3)<br>0    | 38<br>62   |
| <i>γ</i> -20-36 (N)×H-19-6<br>Reciprocal             | 9 (39)<br>57 (90)      | 3 (13)<br>6 (10)  | 11 (48)<br>0    | 0<br>0        | 23<br>63   |
| <i>γ</i> -27-5 (N)×H-19-7<br>Reciprocal              | 11 (34)<br>24 (100)    | 7 (22)<br>0       | 10 (31)<br>0    | 4 (13)<br>0   | 32<br>24   |
| <i>γ</i> -27-7 (N)×H-19-8<br>Reciprocal              | 5 (16)<br>4 (100)      | 6 (19)<br>0       | 18 (56)<br>0    | 3 (9)<br>0    | 32<br>4    |
| <i>γ</i> -27-16 (N)×H-19-9<br>Reciprocal             | 12 (31)<br>46 (96)     | 8 (20)<br>2 (4)   | 9 (23)<br>0     | 10 (26)<br>0  | 39<br>48   |
| <i>γ</i> -27-21 (N)×H-19-9<br>Reciprocal             | 7 (26)<br>24 (89)      | 6 (22)<br>2 (7)   | 9 (33)<br>1 (4) | 5 (19)<br>0   | 27<br>27   |
| <i>γ</i> -lines (N)×H-19 (mean)<br>Reciprocal (mean) | (30.5)<br>(93.2)       | (19.4)<br>(6.2)   | (39.2)<br>(0.5) | (11.1)<br>(0) | 417<br>413 |

\* The percentage is given in parenthesis.

Thus, in order to examine the presence of a pollen restoring gene or genes for the new cytoplasmic factor, a completely male sterile plant in *γ*-27 line was crossed with a normal plant of H-2002. Two F<sub>1</sub> plants which showed a high pollen fertility (96 and 97%), were isolated to make a sib-mating and their seeds were planted as two F<sub>2</sub> populations, A and B. If the phenotypes were classified into two groups, viz. male fertile (N & S.S.a) and male sterile (S.S.b and C.S.), the mode of the segregation would be in close agreement with a monogenic ratio of 3 male fertile: 1 male sterile. The frequency of complete sterile plants was remarkably low. (Table 31.) Thus,

it is noted that only a single dominant gene is responsible for the pollen restoration interacting with the new cytoplasmic factor,  $S'$ , in contrast with the results obtained in  $S$  cytoplasm.

As mentioned in Section IV. A. 3 of this paper, the gene  $X$  for pollen restoration in  $S$  cytoplasm, is linked with the gene  $m$  for monogerm character in the intensity of 21.7% cross overs. In this experiment, the result of  $F_2$  segregation indicated a linkage relationship between the pollen restoring gene and the gene for monogerm character, with the recombination value,  $36.2 \pm 1.77\%$  or  $40.0 \pm 1.56\%$  (Table 32). Although the linkage intensities calculated in this cross, are weaker than that of the former experiment, it is probable that the pollen restoring gene for the new cytoplasmic factor is identical with the gene  $X$  or is closely linked with the gene  $X$ .

TABLE 31 Inheritance of male sterility induced by gamma irradiation

$F_2$ : Cross  $\gamma$ -27-7 (C.S.) $\times$ H-2002

| Phenotype | Normal<br>N | Semi-sterile<br>a<br>S.S. a | Semi-sterile<br>b<br>S.S. b | Complete<br>sterile<br>C.S. | Total |
|-----------|-------------|-----------------------------|-----------------------------|-----------------------------|-------|
| Pop. A    | 317         | 110                         | 143                         | 2                           | 572   |
| Pop. B    | 376         | 233                         | 211                         | 5                           | 825   |

N & S.S.a : S.S.b & C.S. = 3 : 1

Pop. A,  $\chi^2 = 0.037$ ,  $P = 0.80-0.90$ .

Pop. B,  $\chi^2 = 0.615$ ,  $P = 0.30-0.50$ .

TABLE 32 Linkage relation between the gene for monogerm and the gene for pollen restoration

$F$ : Cross  $\gamma$ -27-7 (C.S.) $\times$ H-2002

| Germ type      | Multigerm               |                            | Monogerm                |                            | Total  |
|----------------|-------------------------|----------------------------|-------------------------|----------------------------|--------|
| Male sterility | Male fert.<br>N & S.S.a | Male ster.<br>S.S.b & C.S. | Male fert.<br>N & S.S.a | Male ster.<br>S.S.b & C.S. |        |
| Pop. A         |                         |                            |                         |                            |        |
| Obs.           | 350                     | 90                         | 77                      | 55                         | 572    |
| Cal.           | 344.21                  | 84.79                      | 84.79                   | 58.21                      | 572.00 |
| Pop. B         |                         |                            |                         |                            |        |
| Obs.           | 495                     | 146                        | 114                     | 70                         | 825    |
| Cal.           | 486.75                  | 132.00                     | 132.00                  | 74.25                      | 825.00 |

Recombination value: Pop. A,  $36.2 \pm 1.77\%$ ,  $\chi^2 = 1.31$ ,  $P = 0.70-0.80$ .

Pop. B,  $40.0 \pm 1.56\%$ ,  $\chi^2 = 4.32$ ,  $P = 0.20-0.30$ .

### 6. Reconfirmation of the induced male sterility by gamma irradiation

The gamma irradiation was repeated for the seeds of H-19 and a multi-germ strain, H-2002 by using the same facilities at Hiratsuka in the spring of 1967. Total exposures applied to the seeds were 50 kR and other conditions were the same as in the former experiment.  $M_1$  plants were classified into the four phenotypes as shown in Table 33. It is noted that male sterile types (S.S.b and C.S.) occurred again at a relatively high frequency (13 or 18%). The percentage of seed setting decreased in  $M_1$  plants, when compared with that of normal plants.

A significant correlation was obtained between the pollen fertility and the percentage of seed setting in  $M_1$  plants of H-2002 ( $r=0.427$ , d.f.=184,  $P<0.001$ ), while no correlation existed in the control plants of H-2002 ( $r=0.111$ , d.f.=173,  $P>0.10$ ).

A preliminary test demonstrated that the male sterility is inherited from  $M_1$  plants to  $M_2$  progenies under open pollination (Table 34). A relatively high percentage (84%) of male sterile plants was transmitted to the  $M_2$  gen-

TABLE 33 Male sterility induced by gamma irradiation

| Strain | Radiation treatments | Phenotype of male sterility |                 |                |                 | Total        |
|--------|----------------------|-----------------------------|-----------------|----------------|-----------------|--------------|
|        |                      | N                           | S.S.a           | S.S.b          | C.S.            |              |
| H-19   | Obs. No.<br>%        | 7<br>(32)                   | 11<br>(50)      | 4<br>(18)      | 0<br>(0)        | 22<br>(100)  |
|        | Seed setting<br>(%)  | 35.8<br>(22-50)*            | 28.3<br>(12-58) | 18.5<br>(3-61) |                 |              |
| H-2002 | Obs. No.<br>%        | 78<br>(40)                  | 91<br>(47)      | 24<br>(12)     | 2<br>(1)        | 195<br>(100) |
|        | Seed setting<br>(%)  | 70.8<br>(2-100)             | 56.9<br>(0-97)  | 39.2<br>(5-98) | 43.5<br>(30-57) |              |
| H-19   | Obs. No.<br>%        | 89<br>(96)                  | 4<br>(4)        | 0              | 0               | 93<br>(100)  |
|        | Seed setting<br>(%)  | 93.4<br>(37-100)            | 80.6<br>(33-99) |                |                 |              |
| H-2002 | Obs. No.<br>%        | 173<br>(99)                 | 2<br>(1)        | 0              | 0               | 175<br>(100) |
|        | Seed setting<br>(%)  | 96.8<br>(81-100)            | 91.5<br>(85-98) |                |                 |              |

\* Range of variation.

TABLE 34 Inheritance of male sterility from  $M_1$  plants to  $M_2$  lines

| $M_1$ plant |           |                  | Phenotype of $M_2$ line. (No. & %) |            |            |            |             |
|-------------|-----------|------------------|------------------------------------|------------|------------|------------|-------------|
| Plant No.   | Phenotype | Pollen ster. (%) | N                                  | S.S. a     | S.S. b     | C. S.      | Total       |
| 51          | S.S. b    | 83               | 22<br>(49)                         | 20<br>(44) | 3<br>(7)   | 0<br>(0)   | 45<br>(100) |
| 115         | S.S. b    | 83               | 24<br>(73)                         | 6<br>(18)  | 3<br>(9)   | 0<br>(0)   | 33<br>(100) |
| 165         | S.S. b    | 100              | 13<br>(27)                         | 13<br>(27) | 18<br>(38) | 4<br>(8)   | 48<br>(100) |
| 60          | S.S. b    | 100              | 0<br>(0)                           | 8<br>(16)  | 25<br>(49) | 18<br>(35) | 51<br>(100) |

TABLE 35 Phenotype of male sterility in progenies of the reciprocal crosses between N type plants in a  $M_1$  line and H-2002 (N cytoplasm)

| Combination |                                       | Phenotype of offspring (No. & %) |            |            |           | Total        |
|-------------|---------------------------------------|----------------------------------|------------|------------|-----------|--------------|
|             |                                       | N                                | S.S. a     | S.S. b     | C. S.     |              |
| cross A     | No. 165 ( $M_1$ )-1 (N)<br>× H-2002-1 | 22<br>(16)                       | 33<br>(24) | 72<br>(53) | 10<br>(7) | 137<br>(100) |
|             | Reciprocal                            | 116<br>(83)                      | 22<br>(16) | 1<br>(1)   | 0<br>(0)  | 139<br>(100) |
| cross B     | No. 165 ( $M_1$ )-2 (N)<br>× H-2002-2 | 5<br>(28)                        | 3<br>(17)  | 9<br>(50)  | 1<br>(6)  | 18<br>(101)  |
|             | Reciprocal                            | 29<br>(83)                       | 5<br>(14)  | 1<br>(3)   | 0<br>(0)  | 35<br>(100)  |
| Total       | No. 165 ( $M_1$ )<br>× H-2002         | 27<br>(17)                       | 36<br>(23) | 81<br>(52) | 11<br>(7) | 155<br>(99)  |
|             | Reciprocal                            | 145<br>(83)                      | 27<br>(16) | 2<br>(1)   | 0<br>(0)  | 174<br>(100) |

eration from a  $M_1$  plant, No. 60. The cytoplasmic inheritance was confirmed in the reciprocal crosses between plants with N type from No. 165 ( $M_1$ ) and H-2002, as shown in Table 35.

#### D. Influence of grafting on the phenotypic expression of male sterility

Graft transmission of male sterility was demonstrated in sugar beets as well as in the case of petunia (CURTIS 1967). In opposititon to this, the

transmission of the male sterility was reported to be unsuccessful by means of grafting and by infection with aphids or other artificial means (CLEY 1967, THEURER et al. 1968). Thus the author also investigated the influence of grafting on the phenotypic expression of the cytoplasmic male sterility.

### 1. Experiment A.

Grafting experiments were conducted by the present author in a growth chamber at the Canada Department of Agriculture in Ottawa. Plants with different male sterile types from a cytoplasmic male sterile strain, H-19 MS were used for the stock and a single plant from H-19 was used as the scion. A slight modification of JOHNSON's method (1956) was applied by inserting two leaved seedlings into the axil of a leaf of an approximately 10 cm tall young shoot (Plates Va and b). The grafted scions were secured to the plants with 'stericrepe' a commercial elastic adhesive tape and the grafted plants were kept in an environment of high humidity (over 90%) and moderate temperature (18°~21°C) for a few weeks. Twenty four plants were grafted on September 6, 1962 and grafting succeeded in twelve plants, indicating a 50% success rate. After the photothermal induction, bolting began from the both scion and stock in six plants and from scion or stalk alone in four plants, at the beginning of February 1963. Pollen sterility was examined at the initiation of flowering (from February 25 to March 1) and mid bloom (from March 4 to March 10). As shown in Table 36, flowers from scions

TABLE 36 Pollen sterility in the grafted plants

Strains used: H-19 MS (*S* cytoplasm) as stock, H-19  
(*N* cytoplasm) as scion.

| Plant No. | Combination of grafting |       | Pollen sterility (%) |       |            |       |
|-----------|-------------------------|-------|----------------------|-------|------------|-------|
|           |                         |       | Stock                |       | Scion      |       |
|           | Stock                   | Scion | Initiation           | Bloom | Initiation | Bloom |
| 1         | N ( <i>S</i> )*         | N     | 23.4                 | 3.9   |            |       |
| 2         | S.S.a                   |       | 64.0                 | 41.2  | 1.3        | 2.3   |
| 3         | S.S.b                   |       | 96.0                 | 100   | 3.0        | 4.2   |
| 4         | "                       |       | 100                  | 100   | 1.7        | 2.9   |
| 5         | "                       |       | 100                  | 100   | 4.4        | 4.6   |
| 6         | C.S.                    |       | C.S.                 | C.S.  | 4.3        | 2.8   |
| 7         | "                       |       | "                    | "     | 5.2        | —     |
| 8         | "                       |       | "                    | "     | —          | —     |

\* Type of cytoplasm.

TABLE 37 Phenotype of male sterility in the progenies of the grafted plants

| Plant No. | Phenotype |       | Phenotype of offsprings |       |       |      |       |            |       |       |      |       |
|-----------|-----------|-------|-------------------------|-------|-------|------|-------|------------|-------|-------|------|-------|
|           |           |       | From stock              |       |       |      |       | From scion |       |       |      |       |
|           | Stock     | Scion | N                       | S.S.a | S.S.b | C.S. | Total | N          | S.S.a | S.S.b | C.S. | Total |
| 1         | N (S)     |       | 0                       | 1     | 3     | 8    | 12    |            |       |       |      |       |
| 3         | S.S.a     |       | 2                       | 8     | 23    | 33   | 66    |            |       |       |      |       |
| 5         | S.S.b     | N     | 1                       | 3     | 9     | 11   | 24    | 10         | 2     | 0     | 0    | 12    |
| 6         | C.S.      |       | 1                       | 3     | 18    | 32   | 54    | 6          | 0     | 0     | 0    | 6     |
| 8         | C.S.      |       | 0                       | 1     | 15    | 6    | 22    |            |       |       |      |       |

in all grafted plants indicated normal type regardless of the phenotype of the stock. No influence of grafting on pollen sterility of the scions was seen. Seeds were produced from the stock in five plants and from the scion in two plants. As shown in Table 37, there were no transmission of the male sterile phenotypes (S.S.b and C.S.) from the stock to the scion until the second generation of grafting.

## 2. *Experiment B.*

In this experiment, a different type of grafting was conducted. Plants immediately prior to flowering were used as the scion and stock. The young shoots with a differentiation of buds were cleft-grafted into the axil of a leaf of the flowering plant. It is possible to graft several scions onto a single plant. The grafted shoots began to grow in a week after grafting and flowering lasted for about a month (Plates Vc and d). Experiments were conducted over four different years. Male sterile and normal plants were reciprocally grafted. Pollen sterility of the scion was compared with that of the control plant, from which the scion was taken (Table 38). In the combination of grafting between N and C.S. types, the phenotype of the scion invariably retained C.S. type, while in the reciprocal combination of grafting, the phenotype of the scion showed N type although the pollen sterility of the scion was slightly higher than that of the control in the results of 1966. In the combination of grafting between S.S.b and N types, the phenotype of the stock did not affect the pollen sterility of the scion. It was noted that the pollen sterility of the scion is slightly lower than that of the control in the two combinations between N type in diploid and S.S.a or S.S.b type in tetraploid plants. Similar results were obtained in a combination between N type and S.S.a type in diploid plants, as shown in Table 39.

TABLE 38 Comparison of pollen sterility between the scion of the grafted plants and the original plants

| Year | Combination of grafting |                |             |                | Pollen sterility (%)          |                  |
|------|-------------------------|----------------|-------------|----------------|-------------------------------|------------------|
|      | Stock                   |                | Scion       |                |                               |                  |
|      | Strain                  | Pheno-<br>type | Strain      | Pheno-<br>type | Scion of the<br>grafted plant | Control<br>plant |
| 1963 | H-19 : a                | N              | H-19 MS : b | C. S.          | C. S.                         | C. S.            |
|      | : b                     | "              | "           | "              | "                             | "                |
|      | : c                     | "              | "           | "              | "                             | "                |
|      | : d                     | "              | "           | "              | "                             | "                |
|      | : e                     | "              | "           | "              | "                             | "                |
|      | Hon-iku-192             | "              | 4 M 50 : a  | S. S. a        | 28.1 ( 5- 76)*                | 45.2             |
|      | "                       | "              | : b         | S. S. b        | 45.2 (38- 65)                 | 84.8 (58-100)    |
|      | "                       | "              | : c         | "              | 77.0 (40-100)                 | 87.8 (78- 94)    |
|      | H-19 MS : a             | C. S.          | H-19 : a    | N              | 2.2 ( 1- 4)                   | 1.9 ( 1- 3)      |
|      | "                       | "              | : b         | "              | 4.0 ( 3- 5)                   | 6.8 ( 2- 16)     |
| 1966 | H-19 : a                | N              | H-19 MS : a | C. S.          | C. S.                         | C. S.            |
|      | "                       | "              | : b         | "              | C. S. & S. S. b               | "                |
|      | H-19 : b                | "              | : c         | "              | C. S.                         | "                |
|      | H-19 MS : d             | S. S. b        | H-19 : c    | N              | 2.0                           | 5.0              |
|      | "                       | "              | : d         | "              | 1.5 ( 1- 2)                   | 8.0              |
|      | "                       | "              | H-19 MS : e | C. S.          | C. S.                         | C. S.            |
|      | "                       | "              | : f         | "              | C. S.                         | "                |
|      | H-19 MS : g             | C. S.          | H-19 : e    | N              | 11.4 ( 1- 25)                 | 4.0 ( 3- 5)      |
|      | "                       | "              | : f         | "              | 13.0 ( 1- 30)                 | 3.0              |
|      | 1968                    | H-19 : a       | N           | H-19 MS : a    | C. S.                         | C. S.            |
| : b  |                         | "              | : b         | "              | "                             | "                |
| 1969 | H-19 : a                | N              | H-19 MS : a | C. S.          | C. S.                         | C. S.            |
|      | "                       | "              | : b         | S. S. b        | 91.7 (75-100)                 | 90.0 (70-100)    |
|      | "                       | "              | : c         | S. S. a        | 35.3 (22- 50)                 | 34.6 (11- 59)    |
|      | H-19 : b                | "              | H-19 MS : d | C. S.          | C. S.                         | C. S.            |
|      | "                       | "              | : e         | S. S. b        | 80.0 (52- 98)                 | 100.0            |
|      | H-19 MS : c             | S. S. a        | H-19 : b    | N              | 5.8 ( 0- 33)                  | 13.3 ( 3- 29)    |
|      | : b                     | S. S. b        | "           | "              | 10.6 ( 0- 86)                 | "                |
|      | : a                     | C. S.          | "           | "              | 7.9 ( 0- 52)                  | "                |

\* Range of variation.

The average pollen sterility of the scion decreased to about 13% in comparison with that of control. However, the difference of pollen sterility between the scion and the control plants was not significant statistically for

TABLE 39 Comparison of pollen sterility between the scion of the grafted plant and the original plant

Plants used: S.S.a type in H-19 MS as scion, H-19  
(N cytoplasm) as stock.

| Date    | Scion |               | Control plant |               |
|---------|-------|---------------|---------------|---------------|
|         | Mean  | Range of var. | Mean          | Range of var. |
| Nov. 28 | 33.4% | 24-43         | 85.7%         | 76-95         |
| Dec. 3  | 69.2  | 57-81         | 30.9          | 28-33         |
| 6       | 14.8  | 10-19         | 35.0          | 27-49         |
| 10      | 8.4   | 3-14          | 33.8          |               |
| 18      | 8.5   | 4-23          | 33.9          | 5-50          |
| 21      | 25.7  | 11-41         | 38.3          | 7-100         |
| 26      | 30.2  | 17-42         | 17.5          | 4-51          |
| Jan. 2  | 10.6  | 3-25          | 32.7          | 10-75         |
| Mean    | 25.1  |               | 38.5          |               |

the transformed data to an angle of  $\arcsin \sqrt{\text{percent}}$  by repeated observations ( $t=1.547$ , d.f.=7,  $P=0.10-0.20$ ).

Through two experiments, asexual transmission of male sterility was not demonstrated in the grafted plants. However it seems probable that the pollen sterility of a scion is more, variable when compared with that of the control plant.

#### E. Effect of a selective gametocide

Since the gametocidal effect of sodium 2, 3-dichloroisobutylate (FW-450) was shown in cotton, its effectiveness as a selective gametocide has been tested on sugar beets. Promising results have been obtained by many investigators. In this experiment, the author examined the phenotypic alternation of anther dehiscing and pollen sterility by this chemical agent in normal diploid and tetraploid sugar beets selected as the basis of utilization of the chemicals for the seed production of triploid sugar beets.

A field experiment was conducted in 1964 to determine the effects of FW-450 on a diploid strain, H-2002, and its tetraploids, H-4002, which possess a normal cytoplasm. The chemical was diluted to 0.1%, 0.3% and 1% aqueous solution and sprayed repeatedly on the plants at the stage of bud formation (June 18th) and at the stage prior to flowering (June 30th). Abnormality of anthers and pollens were classified into four types by standards

TABLE 40 Phenotypic ratios of male sterility induced by FW-450 in diploid and tetraploid plants

| Ploidy | Concentration (%) | July 7                          |       |       |      |                  | July 14                         |       |       |      |                  |
|--------|-------------------|---------------------------------|-------|-------|------|------------------|---------------------------------|-------|-------|------|------------------|
|        |                   | Phenotype of male sterility (%) |       |       |      | Number of plants | Phenotype of male sterility (%) |       |       |      | Number of plants |
|        |                   | N                               | S.S.a | S.S.b | C.S. |                  | N                               | S.S.a | S.S.b | C.S. |                  |
| 2X     | Control           | 100                             | 0     | 0     | 0    | 12               | 100                             | 0     | 0     | 0    | 46               |
|        | 0.1               | 57                              | 17    | 23    | 3    | 35               | 54                              | 20    | 20    | 7    | 61               |
|        | 0.3               | 28                              | 31    | 31    | 9    | 32               | 27                              | 26    | 21    | 26   | 62               |
|        | 1.0               | 40                              | 40    | 20    | 0    | 10               | 13                              | 26    | 20    | 41   | 39               |
| 4X     | Control           | 86                              | 14    | 0     | 0    | 14               | 74                              | 26    | 0     | 0    | 35               |
|        | 0.1               | 20                              | 16    | 36    | 28   | 25               | 35                              | 29    | 20    | 16   | 51               |
|        | 0.3               | 21                              | 34    | 34    | 10   | 29               | 24                              | 17    | 26    | 33   | 42               |
|        | 1.0               | 14                              | 29    | 38    | 19   | 21               | 15                              | 27    | 19    | 40   | 48               |

| Ploidy | Concentration (%) | July 21                         |       |       |      |                  | July 28                         |       |       |      |                  |
|--------|-------------------|---------------------------------|-------|-------|------|------------------|---------------------------------|-------|-------|------|------------------|
|        |                   | Phenotype of male sterility (%) |       |       |      | Number of plants | Phenotype of male sterility (%) |       |       |      | Number of plants |
|        |                   | N                               | S.S.a | S.S.b | C.S. |                  | N                               | S.S.a | S.S.b | C.S. |                  |
| 2X     | Control           | 92                              | 8     | 0     | 0    | 52               | 86                              | 14    | 0     | 0    | 49               |
|        | 0.1               | 22                              | 21    | 29    | 28   | 58               | 19                              | 63    | 15    | 3    | 68               |
|        | 0.3               | 2                               | 4     | 22    | 73   | 55               | 5                               | 24    | 51    | 20   | 55               |
|        | 1.0               | 0                               | 0     | 0     | 100  | 28               | 2                               | 20    | 35    | 43   | 51               |
| 4X     | Control           | 85                              | 15    | 0     | 0    | 33               | 83                              | 17    | 0     | 0    | 46               |
|        | 0.1               | 9                               | 7     | 44    | 40   | 43               | 2                               | 47    | 40    | 11   | 53               |
|        | 0.3               | 0                               | 5     | 27    | 68   | 41               | 0                               | 10    | 50    | 40   | 42               |
|        | 1.0               | 0                               | 0     | 0     | 100  | 29               | 0                               | 0     | 55    | 45   | 44               |

| Ploidy | Concentration (%) | Aug. 4                          |       |       |      |                  | Aug. 11                         |       |       |      |                  |
|--------|-------------------|---------------------------------|-------|-------|------|------------------|---------------------------------|-------|-------|------|------------------|
|        |                   | Phenotype of male sterility (%) |       |       |      | Number of plants | Phenotype of male sterility (%) |       |       |      | Number of plants |
|        |                   | N                               | S.S.a | S.S.b | C.S. |                  | N                               | S.S.a | S.S.b | C.S. |                  |
| 2X     | Control           | 81                              | 19    | 0     | 0    | 48               | —                               | —     | —     | —    | —                |
|        | 0.1               | 89                              | 7     | 2     | 2    | 57               | 86                              | 14    | 0     | 0    | 7                |
|        | 0.3               | 41                              | 47    | 10    | 2    | 58               | 61                              | 17    | 22    | 0    | 23               |
|        | 1.0               | 12                              | 46    | 32    | 10   | 59               | 52                              | 12    | 21    | 15   | 33               |
| 4X     | Control           | 73                              | 25    | 2     | 0    | 48               | —                               | —     | —     | —    | —                |
|        | 0.1               | 58                              | 36    | 6     | 0    | 36               | 43                              | 57    | 0     | 0    | 14               |
|        | 0.3               | 36                              | 38    | 19    | 7    | 42               | 45                              | 26    | 26    | 3    | 31               |
|        | 1.0               | 22                              | 24    | 37    | 17   | 46               | 45                              | 17    | 24    | 14   | 29               |

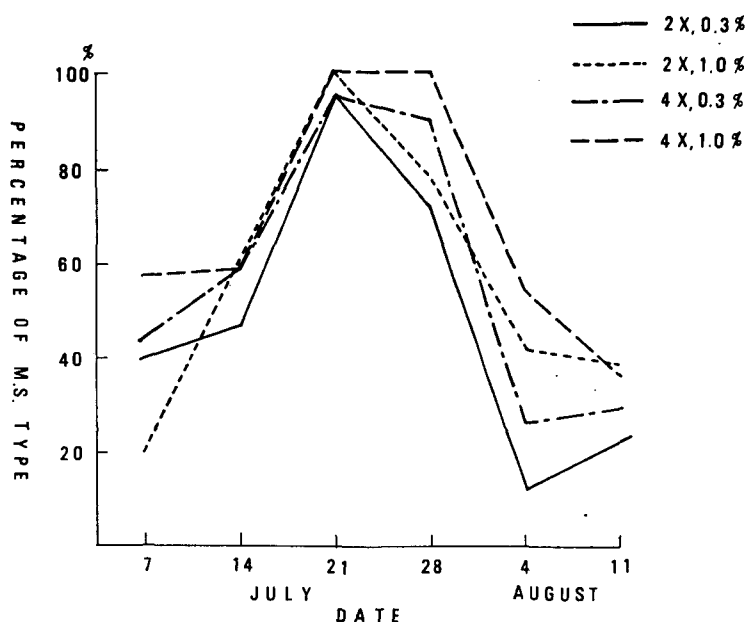


Figure 11. Male sterility induced by gamatocide FW-450.

TABLE 41 Degree of injury by FW-450 in diploid and tetraploid plants

| Ploidy | Concentration (%) |           |           |
|--------|-------------------|-----------|-----------|
|        | 0.1               | 0.3       | 1.0       |
| 2X     | 0.4 (0-1)         | 3.3 (0-7) | 4.5 (2-9) |
| 4X     | 1.4 (0-5)         | 2.4 (0-5) | 5.0 (2-8) |

applied to the cytoplasmic-genetic male sterility. A survey on the effect of the chemical was conducted from the onset of flowering every 7 days. As shown in Table 40 and Figure 11, a 0.1% concentration was not sufficient to produce male sterile plants (S.S.b and C.S.) throughout the flowering period both at diploid and tetraploid levels. A considerable percentage (over 70%) of male sterile plants which lasted for one or two weeks was produced in the drug concentration over 0.3%. There were no significant difference of the effect by the chemical between diploid and tetraploid plants. The injury of leaves and branches was prominent in a high concentration (0.3 and 1%) of the chemical. The degree of the injury was recorded by the following standard of visual observation at the end of flowering, 0... no injury, 1...

indication of injury, 3... leaf damage, 5... half of the leaves died and flower buds were injured, 7... branches were injured, 10... plants died. The percentage of seed setting was also affected when the damage exceeded 5 of the above standard. As shown in Table 41, severe injury appeared when a 1% solution was applied. Through this experiment it was inferred that the induction of male sterility by FW-450 did not show a higher effectivity or reliability as compared with the male sterility produced by genetical causes. More information is required to solve problematic points prior to the practical application of FW-450.

## F. Cytological studies on the male sterile characters

### 1. Chromosome number in male sterile strains

The chromosome number was counted at the mitosis of young root tips in diploid and tetraploid male sterile plants, together with normal plants. As shown in Table 42 and Plates VI a, b, a cytoplasmic male sterile strain, H-19 MS contained two aneuploid plants within 100 plants, while another strain of *S* cytoplasm, K-3-11 and a normal cytoplasm strain H-19 consisted of diploids only. The progeny of the male sterile mutant 'BM-2' which contains male sterile plants and normal plants at about an equal frequency, is also composed of pure diploids. No triploid plant was observed in all strains, in contrast to the data by ELLERTON and HENDRIKSEN (1959).

In tetraploid strains, the frequency of eu-tetraploid was 80% in a normal cytoplasm strain, H-4002, while the frequency of eu-tetraploid decreased down to 51% in a tetraploid male sterile strain, 4M-50. Six tetraploid plants were chosen from a population of 4M-50 which was planted in an isolated field. The frequency of eu-tetraploid in the progenies of six tetraploid plants indicated a considerable range of variation (44-84%), showing a significant heterogeneity among the maternal lines (Table 43, Plates VI c, d). It is uncertain

TABLE 42 Survey of chromosome numbers in diploid normal and male sterile lines

| Type of cytoplasm | Lines   | Chromosome numbers |    |       | Number of observed plants |
|-------------------|---------|--------------------|----|-------|---------------------------|
|                   |         | 18                 | 19 | 19+1f |                           |
| <i>N</i>          | H-19    | 100                |    |       | 100                       |
| <i>S</i>          | H-19 MS | 98                 | 1  | 1     | 100                       |
| <i>S</i>          | K-3-11  | 100                |    |       | 100                       |
| <i>N</i>          | BM-2*   | 100                |    |       | 100                       |

\* A progeny of the male sterile mutant in *Beta maritima* L.

whether the chromosomal instability in tetraploid male sterile plants are related to the male sterile character, because a disturbance of chromosome numbers was observed in normal tetraploid varieties (ROMMEL 1965, MITSUISHI 1965, TAKAHASHI and KINOSHITA 1965).

The somatic chromosome numbers were examined in  $M_2$  lines and  $F_2$  populations which inherited the male sterility induced by gamma irradiation. Though a small number of trisomic, triploid and tetraploid plants were

TABLE 43 Survey of chromosome numbers in tetraploid normal and male sterile lines

| Lines        | Chromosome number |     |      |         |         |     |     |      |     |     |     |         | Total |
|--------------|-------------------|-----|------|---------|---------|-----|-----|------|-----|-----|-----|---------|-------|
|              | 18                | 26  | 27   | 28...30 | 31...33 | 34  | 35  | 36   | 37  | 38  | 39  | 40...45 |       |
| H-4002       |                   |     |      |         | 2       |     | 5   | 86   | 5   | 2   |     |         | 100   |
| 4 M-50 A     | 6                 |     | 3    | 1       |         | 4   | 15  | 51   | 17  | 3   |     |         | 100   |
| B            |                   |     | 22   | 5       |         |     | 4   | 103  | 6   | 8   | 1   | 1       | 150   |
| (%)          |                   |     | (15) | (3)     |         |     | (3) | (69) | (4) | (5) | (1) | (1)     |       |
| H-19 MS (4X) |                   | 1   | 4    | 1       | 1       | 2   | 2   | 32   | 4   |     |     | 1       | 48    |
| (%)          |                   | (2) | (8)  | (2)     | (2)     | (4) | (4) | (67) | (8) |     |     | (2)     |       |

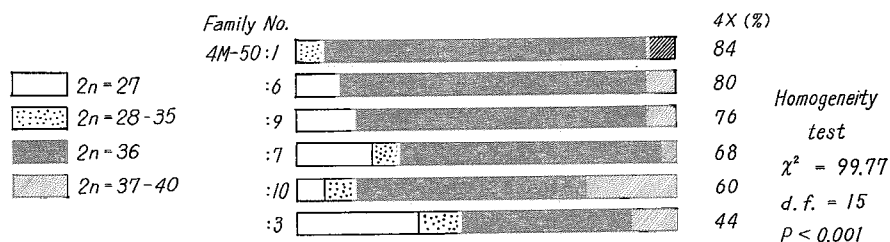


TABLE 44 Survey of chromosome numbers in  $M_2$  lines and  $F_2$  populations

| Strains or populations  | Chromosome numbers |    |    |    | Total |
|-------------------------|--------------------|----|----|----|-------|
|                         | 18                 | 19 | 27 | 36 |       |
| r-20 ( $M_2$ )          | 25                 | 0  | 0  | 0  | 25    |
| r-27 ( $M_2$ )          | 31                 | 1  | 0  | 0  | 32    |
| r-54 ( $M_2$ )          | 25                 | 0  | 1  | 1  | 27    |
| r-27 × H-2002 ( $F_2$ ) |                    |    |    |    |       |
| Pop. A                  | 36                 | 0  | 0  | 0  | 36    |
| Pop. B                  | 49                 | 0  | 0  | 1  | 50    |

TABLE 45 Average chromosome associations of first metaphase

| Plant      | Type of cytoplasm | Somatic chromosome numbers | Average association of chromosomes |              |            |            |            | Observed numbers |
|------------|-------------------|----------------------------|------------------------------------|--------------|------------|------------|------------|------------------|
|            |                   |                            | I                                  | II           | III        | IV         | VI         |                  |
| H-19: 1    | N                 | 18                         | 0                                  | 9.0          | 0          | 0          | 0          | 122              |
| H-19 MS: 1 | S                 | 18                         | 0.01 (0-1)                         | 8.91 (6-9)   | 0.03 (0-2) | 0.02 (0-1) | 0          | 103              |
| 4 M-50: 1  | S                 | 36                         | 0.02 (0-1)                         | 16.13 (8-18) | 0.02 (0-1) | 0.92 (1-5) | 0          | 61               |
| 4 M-50: 2  | S                 | 34                         | 0.58 (0-3)                         | 8.81 (1-17)  | 0.67 (0-4) | 3.44 (0-8) | 0.02 (0-1) | 48               |

\* range of variation

TABLE 46 Chromosomal distribution at first anaphase

| Plant      | Type of cytoplasm | Somatic chromosome numbers | Distribution of chromosomes |                  | Observed numbers |
|------------|-------------------|----------------------------|-----------------------------|------------------|------------------|
|            |                   |                            | normal                      | abnormal         |                  |
| H-19: 1    | N                 | 18                         | (9+9) 100%                  |                  | 105              |
| H-19 MS: 1 | S                 | 18                         | (9+9) 92.5                  | (9+8+1 etc) 7.5% | 67               |
| 4192: 1    | S                 | 36                         | (18+18) 75.0                | (19+17 etc) 25.0 | 40               |
| 4 M-50: 3  | S                 | 36                         | (18+18) 77.8                | (19+17 etc) 22.2 | 45               |
| 4 M-50: 4  | S                 | 37                         | (18+19) 70.5*               | (20+17 etc) 29.5 | 44               |
| 4 M-50: 2  | S                 | 34                         | (18+16) 41.9*               | (19+15 etc) 58.1 | 74               |

\* Normal gametes ( $n=18$ ) are produced in a half of the percentage.

involved in some of the  $M_2$  lines and  $F_2$  populations, most of the plants were diploids. Therefore, the induced male sterility may not be due to aneuploidy and polyploidy (Table 44).

## 2. *Meiotic behavior*

Meiosis was observed in male sterile plants and normal plants both at diploid and tetraploid levels. The results on chromosome associations at the first metaphase are shown in Table 45. A slight irregularity was observed in chromosome pairing of a male sterile plant, H-19 MS-1, while regular nine bivalents were produced in a normal plant of H-19 (Plates VII a and b). Chromosome association of tetraploids varied among individual plants regardless of male sterility; 4M-50-6 showed a relatively low frequency of quadrivalents, while a hypotetraploid plant ( $2n=34$ ) showed the highest frequency of quadrivalents ranging from 0 to eight (Table 45, Plates VII c, d). Abnormal distribution of chromosomes at anaphase I corresponded fairly well with the irregularities of chromosome pairing which are shown in Table 46 and Plates VIII a to d. There is a possibility that microspores of  $n=17$  and 19 developed a viability as well as those of 18 in tetraploid plants (TAKAHASHI et al. 1968). Therefore, it is not conceivable that the male sterility in C.S. and S.S.b types of plants resulted from an abnormality of meiosis at diploid and tetraploid levels.

## 3. *Development of microspores*

Growth of buds and anthers were examined in relation to the development of microspores. In all individual plants, the size of buds at the tetrad stage was determined by microscopical observation and marked by black paint. The four buds with a black mark were sampled at an interval of two days until the initiation of flowering. Thereafter, the sampled buds were fixed in FARMER's fixative solution and stored in a refrigerator. Microspores were stained with cotton blue in a lacto-phenol solution. The length of bud and anther and diameter of the microspore were measured under a microscope. In the first experiment, six plants from a cytoplasmic male sterile strain, H-19 MS and three plants from a tetraploid male sterile strain, 4M-50 were planted in the greenhouse together with a control plant from a normal cytoplasm strain, H-19. As shown in Table 47, the buds attained flowering within 11 days after the tetrad stage. No definite difference was observed in the size of bud and anther among the four types of male sterile plants and normal plants at each ploidy level throughout all stages of growth. The floral organs and microspores in tetraploid plants were larger than those in diploid plants. In the development of the microspores, there were con-

TABLE 47 Growth of bud, anther and pollen grain from tetrad stage to flowering

| Ploidy | Strain    | Type of cytoplasm | Pheno-type of M.S. | Pollen sterility (%) | Average date of flowering <sup>1)</sup> | Length of bud (mm) <sup>2)</sup> |     |     |     |
|--------|-----------|-------------------|--------------------|----------------------|-----------------------------------------|----------------------------------|-----|-----|-----|
|        |           |                   |                    |                      |                                         | Days from tetrad stage           |     |     |     |
|        |           |                   |                    |                      |                                         | 2                                | 4   | 6   | 8   |
| 2X     | H-19-1    | N                 | N                  | 11                   | 11                                      | 1.7                              | 2.1 | 2.1 | 2.3 |
|        | H-19 MS-1 |                   | N                  | 6                    | 11                                      | 1.8                              | 2.0 | 2.0 | 2.1 |
|        | 2         |                   | S.S.b              | 100                  | 10                                      | 1.4                              | 2.0 | 2.1 | 2.3 |
|        | 3         | S                 | S.S.b              | 100                  | 10                                      | —                                | 1.9 | 2.0 | 2.1 |
|        | 4         |                   | C.S.               | C.S.                 | 10                                      | 2.1                              | 2.2 | 2.3 | 2.4 |
|        | 5         |                   | C.S.               | C.S.                 | 11                                      | 1.8                              | 2.0 | 2.1 | 2.2 |
| 4X     | 4M-50-1   |                   | N                  | 48                   | 10                                      | 1.8                              | 2.2 | 2.2 | 2.2 |
|        | 2         | S                 | S.S.a              | 61                   | 10                                      | 2.0                              | 2.3 | 2.3 | 2.6 |
|        | 3         |                   | S.S.b              | 97                   | 10                                      | 2.0                              | 2.0 | 2.0 | 2.2 |

| Strain    | Pheno-type of M.S. | Length of anther (mm) <sup>3)</sup> |     |     |     |     | Diameter of pollen grain ( $\mu$ ) <sup>4)</sup> |      |      |      |      |
|-----------|--------------------|-------------------------------------|-----|-----|-----|-----|--------------------------------------------------|------|------|------|------|
|           |                    | Days from tetrad stage              |     |     |     |     | Days from tetrad stage                           |      |      |      |      |
|           |                    | 2                                   | 4   | 6   | 8   | 10  | 2                                                | 4    | 6    | 8    | 10   |
| H-19-1    | N                  | 0.6                                 | 0.9 | 0.9 | 0.9 | 0.9 | 14.6                                             | 16.7 | 17.0 | 18.3 | 21.1 |
| H-19 MS-1 | N                  | 0.7                                 | 1.0 | 1.0 | 1.0 | 1.0 | 15.4                                             | 16.6 | 17.1 | 17.9 | 20.8 |
| 2         | S.S.b              | 0.5                                 | 0.7 | 0.7 | 0.7 | 0.8 | 12.8                                             | 14.0 | 14.3 |      | 14.6 |
| 3         | S.S.b              | —                                   | 0.7 | 0.7 | 0.7 | 0.8 | Tet.                                             | 14.3 | 14.4 | 14.6 | 15.1 |
| 4         | C.S.               | 0.7                                 | 0.8 | 0.8 | 0.8 | 0.8 | 10.7                                             | 10.8 | 9.8  | —    | —    |
| 5         | C.S.               | 0.7                                 | 0.8 | 0.8 | 0.9 | 0.9 | 11.3                                             | 12.7 | 12.5 | —    | —    |
| 4M 50-1   | N                  | 0.8                                 | 1.2 | 1.2 | 1.2 | 1.3 | 17.5                                             | 18.9 | 19.7 | 19.8 | 24.0 |
| 2         | S.S.a              | 1.2                                 | 1.3 | 1.3 | 1.4 | 1.4 | 17.0                                             | 17.9 | 18.3 | 19.5 | 25.2 |
| 3         | S.S.b              | 1.0                                 | 1.3 | 1.3 | 1.3 | 1.3 | 16.8                                             | 17.2 | 17.3 | 17.9 | 17.9 |

1) Days from tetrad stage.

2) Average of four buds.

3) Average of eight anthers.

4) Average of 16 pollen grains. In N and S.S.a types, sound pollen grains were sampled for measurement of diameter.

TABLE 48 Growth of pollen grain from tetrad stage to flowering unit =  $\mu$ 

| Ploidy | Strain         | Type of cyt. | Pheno. of M.S. | Pollen st. (%) | Date of flower. | Days from tetrad stage |      |      |      |      |      |      |
|--------|----------------|--------------|----------------|----------------|-----------------|------------------------|------|------|------|------|------|------|
|        |                |              |                |                |                 | 2                      | 4    | 6    | 8    | 10   | 12   | 14   |
| 2X     | H-19-1         | N            | N              | 1              | 12              | 13.5                   | 16.2 | 17.1 | 17.5 | 20.2 | 21.6 |      |
|        | 2              |              |                | 3              | 12              | 12.2                   | 16.9 | 17.2 | 17.5 | 21.2 | 21.6 |      |
|        | 3              |              |                | 6              | 12              | 12.6                   | 13.5 | 17.0 | 17.9 | 19.3 | 21.0 |      |
|        | 4              |              |                | 6              | 13              | 11.6                   | 16.2 | 17.5 | 18.1 | 19.2 | 21.6 | 21.9 |
|        | 5              |              |                | 12             | 13              | 10.1                   | 14.4 | 18.3 | 18.2 | 21.6 | 21.6 | 22.3 |
|        | H-19 MS-1      | S            | N              | 3              | 13              | 14.8                   | 17.2 | 18.3 | 18.9 | 21.5 | 21.3 | 22.6 |
|        | 2              |              |                | 5              | 13              | 10.4                   | 15.3 | 16.6 | 17.5 | 19.5 | 21.5 | 22.3 |
|        | 3              |              |                |                |                 |                        |      |      |      |      |      |      |
|        | 4              |              | S.S.a          | 43             | 13              | 9.7                    | 12.5 | 16.5 | 17.1 | 18.2 | 18.8 | 21.7 |
|        | 5              |              |                | 51             | 13              | 15.9                   | 16.4 | 17.2 | 18.8 | 19.0 | 21.3 | 21.9 |
|        | 6              |              |                | 51             | 13              | 13.2                   | 14.8 | 15.9 | 15.9 | 17.0 | 20.1 | 20.7 |
|        | 7              |              |                | 66             | 13              | Tet.                   | 12.4 | 17.5 | 18.2 | 21.2 | 19.0 | 21.8 |
|        | 8              |              | S.S.b          | 100            | 14              | 13.3                   | 16.4 | 16.1 | 15.6 | 16.4 | 15.5 | 15.2 |
|        | 9              |              |                | 100            | 14              | 12.9                   | 15.4 | 16.0 | 15.9 | 16.5 | 14.8 | 15.4 |
|        | 10             |              |                | 100            | 13              | Tet.                   | 14.9 | 16.1 | 16.5 | 16.5 | 15.9 | 16.0 |
|        | 11             |              |                | 100            | 14              | Tet.                   | 11.6 | 15.2 | 16.1 | 15.4 | 14.8 | 15.3 |
|        |                |              | C.S.           | —              | 12              | 9.7                    | 9.9  | 11.0 | 10.9 | 12.1 | —    | —    |
| 4X     | H-19-1 (4X)    | N            | N              | 5              | 14              | 11.6                   | 18.3 | 18.7 | 19.4 | 20.7 | 22.2 | 24.5 |
|        | 2              |              |                | 6              | 14              | 13.1                   | 19.3 | 20.1 | 21.3 | 21.7 | 23.2 | 26.1 |
|        | H-19 MS-1 (4X) | S            | S.S.a          | 57             | 14              | 13.6                   | 19.9 | 20.7 | 21.3 | 21.6 | 22.4 | 22.1 |
|        | -2             |              | S.S.b          | 100            | 14              | Tet.                   | 16.1 | 18.6 | 19.2 | 18.7 | 19.0 | 21.1 |
| 2X*    | BM-2-1         | N            | N              | 4              | 14              | Tet.                   | 11.9 | 15.2 | 16.2 | 16.5 | 16.9 | 19.4 |
|        | 2              |              |                | 27             | 13              | 10.6                   | 14.9 | 16.0 | 16.1 | 16.5 | 18.9 | 19.5 |
|        | 3              |              | S.S.b          | 79             | 13              | Tet.                   | 12.6 | 14.6 | 15.0 | 14.4 | 14.7 | 14.6 |
|        | 4              |              | S.S.b or C.S.  | 100            | 14              | 12.1                   | 12.6 | 14.2 | 14.9 | 14.7 | 14.4 | 15.0 |

\* *Beta maritima* L.

spicuous differences among the male sterile types both at diploid and tetraploid levels. Microspores of complete sterile plants ceased their development soon after the tetrad stage and microspores were completely destroyed within eight days or thereabouts after the tetrad stage, while microspores of S.S.b type

TABLE 49 Diameter of fertile and sterile pollens in various stages of pollen development

| Strain     | Pheno-<br>type<br>of<br>M.S. | Days from tetrad stages |       |       |       |       |       |       |       |       |       |
|------------|------------------------------|-------------------------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
|            |                              | 6                       |       | 8     |       | 10    |       | 12    |       | 14    |       |
|            |                              | Fert.                   | Ster. | Fert. | Ster. | Fert. | Ster. | Fert. | Ster. | Fert. | Ster. |
| H-19: a    | N                            | 16.4                    | 10.1  | 17.0  | 13.9  | 19.2  | 16.4  | 21.7  | 16.6  | 22.2  | 16.9  |
| H-19 MS: a | S.S.a                        | 16.4                    | *     | 17.3  | 16.4  | 19.3  | 16.6  | 20.2  | 17.2  | 22.2  | 17.3  |
| H-19 MS: b | S.S.a                        | 14.4                    | *     | 14.8  | 14.6  | 17.3  | 15.0  | 20.2  | 14.6  | 21.1  | 14.9  |

\* Indistinguishable from fertile pollens.

developed until four days after the tetrad stage and remained in abortive pollens. A similar relation was observed in the male sterile types at tetraploid level. The development of sound pollen grains in S.S.a type proceeded normally while abortive pollens appeared around 8 days after the tetrad stage.

A second experiment was conducted under field conditions in July, 1964. Twenty four plants were chosen from H-19, H-19 MS, H-19 (4X), H-19 MS (4X) and BM-2, and the buds at tetrad stage which were determined under a microscope were marked by black paint in every sound plant. As shown in Table 48, the average date from the tetrad to flowering was 12 or 13 days in N, S.S.a and C.S. types of diploids and 13 or 14 days in S.S.b type of diploids and all tetraploid plants and the wild beet. The pollen grains of tetraploid plants were larger than those of diploid plants, while the pollens of wild beet were rather smaller than those of diploid sugar beet. Cessation of development occurred at approximately two days after the tetrad stage in C.S. type and six days in S.S.b type. In S.S.a and N types, abortive pollen became discernible from eight days after the tetrad stage. There was no difference in the growth of sound microspores between S.S.a and N types (Table 49). The results indicate that differentiation between the male fertile types (N and S.S.a) and the male sterile types (S.S.b and C.S.) is possible approximately eight days after the tetrad stage or five days before flowering by observing young pollen grains. Throughout the three experiments, the environmental conditions showed a positive effect on the growth of microspores bringing about a difference in the duration from the tetrad stage to flowering.

Development of microspores in N type of tetraploids were examined as shown in Plates IX a to c. In C.S. type of tetraploid male sterile plants, the microspores released from their quartets showed weaker stainability as

compared with the microspores of N type (Plate IX d). Subsequently microspores were completely destroyed within a few days. At times small distorted abortive pollens with a thin exine were found adhered to the anther wall (Plate IX e). In S.S.b type microspores failed to grow beyond the stage of the exine development as shown in Plate IX f. Abnormal small pollens with a thick exine were observed in the anther at flowering. In S.S.a type, various percentages of microspores developed normally forming perfect pollen grains (Plate IX g). There were two sizes in abortive pollens of tetraploids. Since they are contained both in male sterile and normal plants, the smaller size of abortive pollen is possibly caused by chromosomal aberrations at meiosis in tetraploid plants.

Development of microspores in the genetic male sterility caused by the gene *ms* are shown in Plates IX h and i, both at diploid and tetraploid levels. Abnormality of microspores in the MS-I type (equivalent of C.S. type) appeared in a later stage than in C.S. type of the cytoplasmic-genetic male sterility. Abnormal development of microspores in the MS-II type (equivalent of S.S.b) resembled the results obtained in S.S.b type of sugar beet, except that the pollen grains of wild beet are smaller than those in sugar beet.

#### 4. *Anther development in the cytoplasmic-genetic male sterility*

It has been recognized that the pollen abortion of cytoplasmically inherited male sterility is associated with the abnormal development of anther tapetum (ARTSCHWAGER 1947, HOSOKAWA et al. 1954, OHTA and MATSUMURA 1960, NAGAO and KINOSHITA 1962). ROHRBACH (1965 a) argued that the increased growth of the tapetum in male sterile plants is not the cause, but one of the results of the inhibition which also affects the conductive tissue and thereby the nutritions. In the present experiment, the author observed microsporogenesis of the male sterility, paying special attention to comparative observations between diploid and tetraploid levels.

##### a. *Observation at diploid level*

Prior to meiosis, the multicellular archesporium is differentiated into the tapetum, two middle layers adjacent to the tapetum and the endothecium lying beneath the epidermis. During meiosis, the tapetal cells become binucleate and are characterized by increasingly dense staining cytoplasm than those of the surrounding tissue. The development beyond this stage shows a distinct difference between normal and male sterile plants. First the anther development of the complete sterile type (C.S.) was compared with that of the normal type (N). In complete sterile anthers, tapetal cells changed into irregular shapes in certain locules during meiosis. At the tetrad stage, tapetal

cells of normal plants usually become elongated tangentially and show an intense stainability (Plate X a). In the corresponding stage, tapetal cells began to form plasmodium and showed some variability among the locules. No abnormality was observed in the microspores and the tissues connecting locules such as ground parenchyma and vascular bundles in male sterile plants (Plate X b).

Tapetal differences between normal and complete sterile anthers were more evident in the young microspores released from their quartets. In normal anthers, the tapetal cells continued to retain their intense stainability and a loss of continuity was apparent. The microspores here were at the stage of second constriction (Plate X c). In the complete sterile anthers, the plasmodial jacket increased in width and bulged prominently into the anther cavity (Plate X d). In an exceptional case, the tapetal plasmodium was seen enlarged prominently enclosing the quartets (Plate XI a). The plasmodium shown here has attained a maximum development containing multi-nuclei and large vacuoles (Plate XI b). Microspores failed to develop and were crowded into the narrowing lumen of the anther. In contrast to this, normal anthers had dark staining young pollens and a degenerated tapetum at the corresponding stage (Plate XI c). After the tapetal plasmodium of complete sterile anthers had reached its maximum development, it degenerated with microspores (Plate XI d). The partition between the two microsporangia in the same lobe were broken in certain anthers. In normal anthers, the tapetum has completely broken leaving a pale narrow band lining attached to the endothecium. Subsequently, the epidermis was stretched and the endothecium indicated a characteristic banded structure.

Later in complete sterile anthers, the microsporangia were collapsed and the anther cavity became empty except for a strand of blackened matter and shrunken microspores. All that remained of the endothecium was a thin line of densely stained tissue (Plate XII a). In the normal anthers ready to dehisce, the tapetum showed a complete disappearance and the partition between two microsporangia in the same lobe was destroyed producing one large cavity in each lobe. Normal anthers dehisced when the thin thread of the cells between the lip cells ruptured and the mature microspores or pollen grains were ready to be released (Plate XII b).

In semi-sterile anthers (S.S.a or S.S.b), the development of the tapetum and microspores proceeded normally until the stage when the microspores were released from their quartets. In a more advanced stage of the development, the tapetal cells enlarged slightly and contained vacuoles. The degeneration of the tapetum was prolonged at the time when an exine and germination

pores of microspores were developed. This delayed degeneration may account for the partial sterility of the pollen grains, since nutrients from the tapetum necessary for proper development of pollen grains may not be available at the appropriate time. The degenerating tapetum adhered to the anther wall producing a dark stained band lining the anther cavity (Plate XII c). A lack of uniformity was observed in the abnormality of the tapetum and microspores among the different locules in a single flower (Plate XII d). A very feeble development of fibrous thickening of the endothecium and the lack of the differentiation in the lip cells resulted in the non-dehiscence of the anthers. In the S.S.a type, dehiscent and non dehiscent anthers were produced in various ratios even in a single flower.

From histological observation, it was shown that the first symptom of abnormal anther development occurs in the tapetum and the degeneration of microspores follows in all types of male sterility. It is probable that a disturbance in the transfer of nutrients from the tapetum to microspores causes a variation in the degree of male sterility.

#### **b. Observation at tetraploid level**

As mentioned in Section, IV. A. 7 of this paper, male sterile types at the tetraploid level may be classified into four types utilizing a standard similar to that in the sterile types at the diploid level. Thus, the development and differentiation of anthers were observed in the representative plants of complete sterile (C.S.), semi-sterile type-b (S.S.b), semi-sterile type-a (S.S.a) and normal (N) types. The anther wall and sporogenous cells differentiated into epidermis, endothecium, two or three middle layers and tapetum prior to meiosis, both in normal and male sterile plants (Plate XIII a). Tapetal cells became vertically elongated and binucleate during the prophase of meiosis (Plate XIII b).

In normal anthers, the tapetum layer was intensely stainable throughout the stages of meiosis and tetrad stage. The tapetal cells were arranged regularly adhering to the parietal layers at the tetrad stage (Plate XIII c). When the microspores have separated, the tapetal cells lost their contact with one another and later began to degenerate (Plate XIII d). When microspores developed to dark staining young pollens, the tapetum disintegrated leaving a thin remnant layer. The endothecium attained its maximum development with thickened cell walls and a characteristic banded appearance (Plate XIV a and b). The lip cells at the stomium were well developed and the partition between the two microsporangia in the same lobe was destroyed producing one large cavity in each lobe (Plate XIV c). By the time that the anther dehisces, the tapetum layer completely disappeared.

In the anthers of the complete sterile type (C.S.), the tapetal cells were irregular shaped and stained lighter than that of normal plants during meiosis (Plate XV a). At the tetrad stage, a first sign of plasmodium formation was observed in the tapetum tissue, while the quartets developed normally (Plate XV b). Tapetal cells formed periplasmodium at the stage of liberation of the microspores from the quartets (Plate XV c). The plasmodium bulged prominently into the anther cavity containing multi-nuclei and large vacuoles. In other parts of the anther tapetal plasmodium enclosed microspores (Plate XV d). Although microspores developed thin exine and germ pores, the contents were lost. The partition between the microsporangia in each lobe was broken down following the growth of plasmodium and later tapetal plasmodium degenerated together with microspores (Plate XVI a). The anther cavity became empty except for a strand of blackened matter and degenerated microspores (Plate XVI b). The development of conductive tissue in C.S. type was compared with that of N type. No conspicuous difference was observed until the microspore stage subsequent to the tetrad (Plates XVII a and b). Following the abnormal behavior of tapetum and microspores, the differentiation of vascular bundles was prominently inhibited and the surrounding parenchyma tissue showed signs of degeneration (Plates XVII c and d).

In semi-sterile anthers of S.S.a and S.S.b types, the development of the tapetum and microspores were not different from those in normal anthers until the stage in which microspores are liberated from the quartets. In most cases of S.S.b type, abnormality of microsporogenesis was associated with a slight enlargement of tapetal cells (Plate XVIII a). The degeneration of the tapetum was prolonged and a thick layer of the remnant was seen adhered to the anther wall (Plates XVIII b and c). Although the microspores reached a fairly large size, they gradually lost their contents. This delayed degeneration can account for the partial sterility of pollen grains since nutrients from the tapetum necessary for proper development of microspores may not be available at the appropriate time. The development of the endothecium was also inhibited causing a feeble development of fibrous thickening. Although the degeneration of the tapetal layer was complete by the time the anther reached maturity, dehiscence of anthers was inhibited by a poor development of lip cells and endothecium (Plate XVIII d). Anther cavity contained only empty pollen grains. In S.S.a type, development of dehiscent anthers was similar to that in normal anthers, while development of non-dehiscent anthers resembled the process seen in S.S.b type. The persistence of the tapetum was associated with the formation of sterile pollen grains.

A remarkable lack of uniformity was observed in a plant of S.S.a type.

In this plant pollen sterility of anthers varied from 59% to 100%. Some anther lobes showed an abnormality at an earlier stage of the meiosis, while others showed a typical periplasmodium at the later stage whereas the remaining anther lobes showed partial sterility or close to a normal condition within the single plant (Plates XIX a and b). Degeneration of tapetal cells and sporogenous cells occurred before the initiation of meiosis in this plant (Plate XIX c). Complete degeneration of the tapetum at the tetrad stage was observed in different locules (Plate XIX d). In other plants, a disturbance of regular timing in meiosis was observed in some cases. Sometimes, different division stages were found between a pair of locules.

Throughout the observation at the tetraploid level, the abnormality of the tapetum always preceded the breakdown of microspores in the male sterile plants. Though a considerable variation of tapetal abnormality was observed in the plant of S.S.a type, the abnormality of the tapetum in tetraploid plants generally coincided with that in diploid plants.

##### 5. *Anther development in the genetic male sterility*

As mentioned in Section IV, B. 2 of this paper, the male sterility which is caused by the gene 'ms' were classified into six phenotypes. The development of the tapetum and microspores in a typical plant of MS-I type was compared with those of a normal type in the progeny of a male sterile mutant, BM-2.

In normal anthers, a degenerating tapetum and dark staining young pollen grains were observed at the stage one week before anthesis (Plate XX a). In the corresponding stage of MS-I type (equivalent of C.S. type) anthers, tapetal cells were enlarged and partially formed a pronounced plasmodium, while microspores ceased their development (Plate XX b). However a typical periplasmodium with pseudopodium-like incursions was not observed in contrast to the C.S. type in cytoplasmic-genetic male sterility. Later the tapetal cells were highly vacuolated and the development of endothecium was inhibited (Plate XX c). Degeneration of tapetum and breakdown of microspores proceeded rapidly and shrunken sterile pollens with a black strand were contained in the anther cavity prior to anthesis (Plate XX d).

As mentioned in Section IV, B. 6 of this paper, small and underdeveloped flowers were contained in the upper parts of inflorescence of MS-I and MS-II types. In such flowers opening of the calyx is inhibited. A transverse section of the flower indicated that anther walls degenerated leaving a thin line of remaining endothecium (Plate XXI a). Destroyed microspores and degenerated tapetum composed of a strand of blackened matter in the lobes were seen. Degeneration of tapetal cells enclosing the quartet pollens was

observed in the anther cavity of underdeveloped flowers (Plate XXI b).

In other types of the male sterility (MS-II, III and IV), similar abnormalities of tapetum and microspores were observed both at the diploid and tetraploid levels. A lack of uniformity was frequently observed in the abnormality of anthers within a single flower (Plates XXI c and d).

Although an association between the pollen abortion and the abnormal development of tapetal cells existed in this male sterility, the growth of the tapetal plasmodium was not so pronounced when compared with the tapetal abnormality in the C.S. type which was brought about by a genotype, *S xx zz*.

6. *Anther development in the male sterile plants induced by gamma irradiation*

Three plants of complete sterile type were found in the plants produced from the irradiated seeds of a normal cytoplasm strain, H-19 by gamma rays.

These plants were named as  $\gamma$ -20,  $\gamma$ -27 and  $\gamma$ -54. Anther development was observed in the three plants. The sign of abnormality in the tapetum appeared around the end of meiosis. The tapetal plasmodium bulged prominently into the anther cavity at the stage of liberation of the microspores from the tetrads (Plates XXII a and b). Swollen tapetum enclosed microspores in other locules. The tapetal plasmodium degenerated with microspores after its maximum development (Plate XXII c). In the mature locules of the anthers, microspores were destroyed completely and an endothelial layer and empty locules with black strands were seen remaining (Plate XXII d). These characteristics in the tapetum coincided with those in the C.S. type of the cytoplasmic-genetic male sterility.

Various deformities of floral organs were observed in the male sterile plants. In  $\gamma$ -27 plant, a sepal was united with an anther lobe by failure of development (Plate XXII c). In  $\gamma$ -20 plant, two sepals joined each other and the flower consisted of four sepals (Plate XXII d). These deformities may be related to the irradiation effect. The association between tapetal abnormality and collapse of microspores corresponded with those of the C.S. type in the cytoplasmic-genetic male sterility.

7. *Anther development in the male sterility affected by the treatment of low temperature*

It is known that phenotypic expression of male sterility is modified by environmental conditions such as low temperature (Section IV, A. 3). Pollen sterility and dehiscence of anthers were affected to some extent following low temperature treatment in most of the plants (Table 7). In the experiments

of 1963, four plants with altered phenotypes after treatment, were used for cytological observations. The small buds were sampled two times in each plant one day prior to the treatment and at two days after the completion of the treatment. Anther development was compared between the materials which were sampled at both times.

The plant No. 6 of H-19 (a normal cytoplasm strain) showed an S.S.a type after the treatment. Anther development was normal in the material which was taken before the treatment, whereas the tapetal cells were slightly enlarged and were adhered to the anther wall longer than normal type in the material sampled after the treatment (Plate XXIII a).

The plant No. 3 of H-19 MS (a cytoplasmic male sterile strain) indicated a most drastic change of the phenotype, from N to C.S. In the material before the treatment, development of tapetum and microspores proceeded normally, while a typical development of tapetal plasmodium was observed in the material after the treatment.

A timing unbalance in meiosis within a flower was observed in the plant No. 4 of H-19 MS which showed an altered phenotype from S.S.b to C.S. type after the treatment. Anthers of an earlier stage of meiosis and tetrad stage were found mixed within the same flower (Plate XXIII b).

A new type of abnormality of anthers was found in the material after the treatment in the plant No. 1 of 4M-50 (a tetraploid male sterile strain). Anther walls thickened prominently and the growth of the anther cavity was inhibited at the tetrad stage (Plate XXIII c). The number of pollen mother cells were fairly decreased. This may be due to a certain low temperature effect.

In the plant No. 12 of 4M-50 a characteristic of the tapetal plasmodium and complete breakdown of microspores were observed in the materials after the treatment, while the tapetal enlargement was not so pronounced in the material before the treatment.

The observations showed that a tapetal abnormality is associated with a breakdown of microspores in the induced male sterility by low temperature. The degree and timing of abnormality in the tapetum are also modified by low temperature in the cytoplasmic-genetic male sterility.

#### 8. *Anther development in the male sterility induced by the gametocide 'FW-450'*

The selective gametocide 'FW-450' induced a partial and complete male sterility in the normal cytoplasm plants as mentioned in Section IV, F. Anther development was observed in the materials sampled one week after the first treatment by the chemical.

Abnormal development of anther tapetum was observed in the plant which

showed an alteration of its phenotype to C.S. type. Tapetal cells enlarged radially containing large vacuoles at the tetrad stage and grew prominently enclosing microspores released from the quartets. However, a typical growth in a part of tapetal periplasmodium was not observed in the materials of C.S. type. Later the hypertrophied tapetal cells deteriorated sharply and left crushed microspores in the center of the anther cavity (Plate XXIII d).

It is indicated that a close relationship exists between the development of tapetal cells and the breakdown of microsporogenesis in the male sterility induced by the gametocide as well as in the male sterility due to the genetical causes and low temperature.

## V. Discussion

### A. Nature and inheritance of cytoplasmic-genetic male sterility

Recently, cytoplasmic-genetic male sterility is extensively utilized for the seed production of commercial hybrid varieties and triploid varieties (McFARLANE et al. 1961, BANDLOW 1964 a, b). The nature and inheritance of this character are important as the basis of the effective use of the character in the production of hybrid seeds. Abnormality of anther and pollen grains were classified into four classes, considering the variations in this character. Although HOGABOAM (1957) and OLDEMEYER (1957) set out 8 or 10 classes, most of investigators use a four type classification (OWEN 1945, KNAPF 1955, BANDLOW 1964 a, ROHRBACH 1965 a). Variation of pollen sterility during the flowering periods and in different flowering seasons, were examined with reference to the phenotypic stability of this character. It is indicated that the semi-sterile type-a fluctuated considerably, while the complete sterile and the semi-sterile type-b were quite stable. Frequently, S.S.a changed into N or S.S.b and a reverse change was recognized during the flowering period and between different seasons within the individual plants. HOGABOAM (1957) also observed that the partial sterile type varied considerably between dates for several seasons of the individual plants. In addition to this, the stability of pollen sterility within a single flower was examined in representative plants from each phenotype. A remarkable difference of pollen sterility was observed between different anthers and between different lobes even within a single flower especially in S.S.a type, whereas C.S. type and most of S.S.b type were quite stable throughout the entirety of the plant. It seems that pollen sterility and dehiscence of anthers are affected prominently depending on some internal factors which interact with environmental conditions during the developmental process of the plant. STEIN et al. (1959) recognized

the reversion of fertile pollens from the typical complete male sterile plants. They presented the assumption that the reversion is due to an accumulation of "fertility substance" or to exhaustion of a "sterility substance" which are associated with the relationship between vegetative and generative development.

Modification of male sterility was investigated in two extreme conditions, i.e. low temperature and starvation culture. The results showed that low temperature is effective in the inducement of pollen sterility in *N* cytoplasm and changes the degree of the male sterility in *S* cytoplasm. In an extreme case, a *N* type plant with *S* cytoplasm showed an alteration to the C.S. type after the treatment. The effect of starvation culture was not so strong as compared with the effect of low temperature. However, several plants significantly increased their pollen sterility under cultivation in the vermiculite without fertilizer application. This result depends on the fact that a halved root stores sufficient nutrients to induce a normal generative development. According to CORTESSI (1967), various environmental conditions affect the expression of male sterility. The phenotypic expression of male sterility was influenced to some extent by different combinations of day length and temperatures, light intensity and pretreatment of stecklings and seeds. ROHRBACH (1965c) denoted "modifiability" for the changes in the phenotype in different habitats and in different years, distinguishing from "variability" in the same habitat during the flowering period. "Modifiability" are affected by starvation culture, gene mutation and solar radiation in different latitudes. A genetical influence is assumed to be shown in common for modifiability and variability in the same way. The stability of a male sterile character for the environmental conditions is a fundamental problem in the use of male sterility. In onions, male sterility is not influenced even under long and normal photoperiods and temperatures below 70 degree F (BARHAM and MUNGER, 1950). However, in corn, cool and humid conditions at flowering times and omission of N fertilizer cause a higher percentage of pollen fertility (DUVICK 1960, 1965).

According to the author's studies on the mode of inheritance, it was ascertained that two chromogenes interacted with the sterility-inducing cytoplasm. As shown in the genic assumption by OWEN (1945), the two types of cytoplasm which are indicated by the symbols *N* and *S* respectively, exist and the male sterility is caused by *S* cytoplasm. The plants with *N* cytoplasm produce normal pollens irrespective of the presence or absence of a modifying gene or genes. A complementary action of two dominant genes or "restorer" genes, possibly *X* and *Z* by OWEN (1945), are responsible for

the pollen restoration in the sterility-inducing cytoplasm, *S*.

The digenic scheme on pollen restoring genes by OWEN (1945) are supported entirely or partly by several investigators, while monofactorial and a poly-hybrid mode of inheritance are reported on the pollen restoration of the plants having *S* cytoplasm. OWEN (1950) recognized at a later date that a difficulty existed in distinguishing *S xx zz* and *S xx Zz* phenotypically under certain environmental conditions because of the minor effect of the gene *Z*. HOGABOAM (1957) reported a new gene *Sh* which enhances the pollen producing ability of the genotype, *S Xx* in such a way that they appear to be male fertile. BLISS and GABELMAN (1965) reported that complete pollen restoration of the plants having *S* cytoplasm can be explained by the presence of a dominant allele (*X*). Another single gene (*Z*) is responsible for the partial male fertility and appears to be independent of and hypostatic to the dominant gene (*X*). SAVITSKY (1963) also observed monofactorial inheritance in the study of tetraploid inheritance. ROHRBACH (1965 b) assumed that the inheritance of pollen sterility occurs in a more complicated way than in the dihybrid scheme. He also presented an argument on the monohybrid inheritance at the tetraploid level because polygenic inheritance of the character was shown in the same way, both at diploid and tetraploid levels.

According to the author's experimental results, the pollen restoration in *S* cytoplasm is due to the complementary effect of *X* and *Z*, irrespective of the dose of dominant genes and heterozygous conditions. The degree of pollen producing ability, namely N and S.S.a types are conditioned by another enhancing gene or genes. The expressivity of the enhancing gene or genes is fluctuable or modifiable by environmental conditions. The complementary action of *X* and *Z* genes are confirmed in the crosses between the male sterile sugar beet and a normal table beet. The digenic scheme are also ascertained in the crosses between the complete sterile sugar beet and a normal wild beet at tetraploid level. The segregation ratio of the tetrasomic inheritance indicates an intermediate ratio between the two theoretical ratios based on the parameters  $\alpha=0$  and  $\alpha=1/7$ . Therefore, it is appropriate to assume the complementary action of the two major genes *X* and *Z*, basically for the pollen restoration of *S* cytoplasm. However, if the semi-sterile type-b is used as a pollen sterile type, an inheritance on a monogenic basis is possible for the pollen restoration as shown by BOLZ (1968). It is possible that the minor modifying gene or genes and environmental factors also appear in part to play a role in the inheritance of the male sterile character. A similar digenic scheme was established for the pollen restoration in the cytoplasmic-genetic male sterility in corn (DUVICK 1965, CHASE 1968), wheat

(LIVERS 1964) and carrots (BANGA et al. 1964).

A linkage relation was indicated between the gene *X* and the gene *m* for monogerm, with a recombination value of 21.7 or 24.5%. HOGABOAM (1957) estimated a weaker recombination value, with 37.5%. However, he classified the male sterility in a different manner, in which white anthers were dominant over the yellow anthers, and the recombination value was calculated in a repulsion phase\*. A correlation was recognized between the red pigmentation of anthers and the types of male sterility. Although the mode of inheritance in the pigmentation of anthers is not clarified as yet, there is a possibility that a genetical correlation exists between the gene or genes for anther pigmentation and the pollen restoring genes. STEIN and GABELMAN (1959) also reported a similar relation between the anther coloration and pollen sterility. Independent association was recognized between the genes for pollen restoration, *X* and *Z*, and the gene *G* for the coloration of root.

From a practical utilization of the male sterility, the pollen parent with a special genotype, *N xx zz* is required for the reproduction and the maintenance of the complete sterile plant with the genotype *S xx zz*. This pollinator is designated as a type-0 plant (OWEN 1948). Although OWEN (1950) estimated that about 50% of plants is the type-0 in some commercial varieties, the author obtained results which showed that a relatively small number of the type-0 are observed in a population from Hon-iku-192. The frequency of type-0 plants varied from 0 to 30%, in different varieties or populations; namely 4.3 or 15.5% by PETERSON (1952), 0-20% by KNAPP (1955) and 1-30% by IMANISHI (1968).

The present author states that even if plants from a random sampling of Hon-iku-192 are used as the pollinators, a relatively high percentage (71 or 78%) of practical male sterility (C.S. and S.S.b) is maintained in the progeny of S.S.b type plants. A larger percentage (81-96%) of male sterile plants are produced in the progenies of tetraploid male sterile plants which are crossed with pollinators from random sampling in tetraploid varieties. As to the maintenance of the male sterility, the present author tentatively made inter-crossings between "male sterile group (C.S. and S.S.b)" and "male fertile group (S.S.a and N)" within the same strains. Since the frequencies of the male sterile group in the original populations were maintained at a similar frequency in the progenies of the inter-crossings, this type of cross would serve to maintain the male sterility within the strains.

OWEN (1948) proposed a scheme on the utilization of male sterility in

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\* In the original report, it was referred mistakenly as the coupling phase.

breeding of hybrid varieties to produce pure triploids. The author proposed a scheme on the utilization of male sterility both at diploid and tetraploid levels, for triploid seed production from the present experimental results. A trial seed production of triploids was produced by the use of diploid and tetraploid male sterile plants which were produced without the use of type-0 plants. Almost pure triploids were obtained from the crossings between tetraploid male sterile strains and diploid pollinators, whereas the use of diploid male sterile strains which were produced without the use of type-0 plants and contained partially male fertile plants, was not effective for the production of pure triploids. It is considered that complete male sterile plants which are produced by the use of type-0 plants, are indispensable for the triploid seed production between diploid male sterile plants and tetraploid pollinators. The use of tetraploid male sterile plants which was produced without the use of type-0 plants and contained a small number of male fertile plants is promising for a sufficient efficiency to obtain pure triploids.

Yield tests in gross sugar production covering two years showed that the triploid hybrids from the crosses between tetraploid male sterile strains and diploid pollinators were superior to parental diploid and tetraploid varieties and triploid hybrids from reciprocal crosses. STEWART and GASKILL (1952) reported the results of yield tests with three triploid hybrids, one of which was obtained by a crossing between a diploid male sterile strain and a tetraploid variety. The triploid hybrid indicated equivalent productivity in yield of gross sugar to that of hybrids which can be produced from the crossings of related lines on diploid levels. In 1958, a substantially true triploid variety was produced by crossing a diploid male sterile type with a tetraploid pollinator and was released on the market under the name of 'Trirave' or 'Triplex' (ELLERTON and HENDRIKSEN 1959). According to the results by BANDLOW (1964 b), some of the triploid hybrids which were produced with diploid male sterile plants are superior to the diploid control variety. However, the combinations with the highest proportion of triploids were not always the best ones but in some cases they were the ones with good combining ability. Preliminary tests showed that the yields of almost pure triploid hybrids which were produced with the tetraploid male sterile plants were significantly better than the diploid standard variety. The author's experimental results also indicated that the use of male sterile tetraploids has a potential value for an efficient seed production of triploids.

### B. Genetic male sterility

Genetic male sterility depending on one or more recessive genes has been found in many different crop species. In sugar beets, OWEN (1952) described two different genes,  $a_1$  and  $a_2$  for the Mendelianly inherited male sterility. The gene  $a_1$  was inherited independently from eight other genetic markers (THEURER 1968).

A male sterile mutant, BM-2 was found by the present author in a strain of wild beet, *Beta maritima* L. The type of male sterility in the progeny of BM-2 was classified into six types because of the appearance of the modified types from semi-sterile type-b and complete sterile type. The male sterility was basically due to a single recessive chromogene, which has nothing to do with the cytoplasmic factor. A progeny test indicated that the variation in the male sterility is mainly influenced by environmental conditions. The symbol of the causal gene is designated as *ms*. This character was transferred to sugar beets by successive back crossings. Since the segregation ratio of trihybrids is recognized for pollen restoration in the  $F_2$  of the cross between H-19 MS (cytoplasmic male sterility) and BM-2-13 (a heterozygous normal plant of the genotype *Ms ms*), the pollen restoring genes *X* and *Z*, and the gene *ms* for the genetic male sterility have an independent relationship. According to the genic identification between the genic male sterility in sugar beet and wild beet, the gene *ms* was non-allelic with the gene  $a_1$  or  $a_2$ , and an independent relationship existed between the genes, *ms* and  $a_1$  or  $a_2$ . Therefore, a digenic segregation ratio 9:7 was brought out in the  $F_2$  of the cross between CT-5 and BM-2-1. The average seed setting in male sterile plants was decreased significantly from that of normal plants. That is caused by mixing of abnormal flowers in the male sterile plants. Backcross and  $F_2$  populations indicated a wide range of variation in the seed setting of male sterile plants.

As to the utilization of the genetic male sterility, OWEN (1952, 1954) suggested that the Mendelian male sterility is useful for emasculation in the genetic and breeding investigations where extensive roguing has not been necessary. He also proposed the scheme of a double cross, in which the cytoplasmic male sterility and the genetic male sterility are used for the female parents of the first cross.

Because 50 percent male sterile plants are at the maximum in back cross populations, pollen producers must be rogued out during the bud stage (OWEN 1954). If the gene for male sterility possesses a pleiotropic effect or are tightly linked with suitable phenotypic markers, pollen producing plants

can be rogued out when plants are small (DUVICK 1968). WIEBE (1960) and RAMAGE (1965) suggested a system of using phytocides in barley, if the linked markers present resistance to such chemicals as DDT. More information is required for the genetic male sterility of beets along this line. DUVICK (1968) suggested the use of "pseudo-MS" genotypes for hybrid seed production. Since the genetic male sterility due to the gene *ms*, exhibits a considerable variation by environmental conditions, it might be possible that pure-breeding pseudo-MS lines are produced by positive control of the environment. In addition, the use of a balanced tertiary trisomic system with recessive male sterile genes can provide a successful commercial production of hybrid seeds (RAMAGE 1965, WIEBE 1968). Since the trisomic plants are produced in sugar beets (BUTTERFASS 1964, KALTSIKES and EVANS 1967), a similar system may well be established.

It is known that *Beta maritima* L. contains numerous distinctive characters such as resistance to leaf spot disease which are utilized extensively in the sugar beet breeding (COONS 1954). Since it has been proven that *Beta maritima* L. produces successfully viable hybrids with the species in the sections, *Vulgares*, *Patellares*, and *Corollinae*, the desirable traits of wild species can be transferred to sugar beets through bridge hybrids (OLDEMEYER and BREWBAKER 1956). In addition to this, a grafting technique has been developed to grow unthrifty  $F_1$  seedlings from the interspecific crosses to maturity (COE 1954). Thus genetic male sterile plants which originated from *Beta maritima* L. can be used for the breeding of interspecific hybrids in the genus *Beta*.

### C. Induced male sterility by gamma irradiation

The different expressions of male sterility in *Beta vulgaris* L. are caused by different genes which influence the cytoplasm, as well as by the different types of male-sterile cytoplasm (SAVITSKY 1958). New sources of male sterile cytoplasm are found in wild species of the genus *Beta* (OLDEMEYER 1957) and a Mendelian male sterility is obtained from the X-rayed materials (OWEN 1952).

Single-gene recessive male sterile types have been induced and studied in a number of crops, e.g. in peas, jute and pepper by X-rays (GOTTSCHALK and JAHN 1964, RAKSHIT 1967, DASKALOFF 1968), in tomatoes by  $P^{32}$  (M. M. LESLEY and J. W. LESLEY 1958) and in barley by the chemical mutagen, ethyl methanesulfonate (EMS) (GAUL et al. 1966, SATO and GAUL 1967). In the testplant *Arabidopsis*, monogenic recessive male sterile mutants were induced after EMS-treatment, while an attempt to find a cytoplasmic male

sterile mutant was unsuccessful (VAN DER VEEN and WIRTZ 1968).

In the author's experiment, the seeds of a diploid monogerm strain, H-19, were irradiated with gamma rays at doses over 50 kR to induce a new source of male sterility in sugar beets. The lethal rate for gamma irradiation was estimated to be around 100 kR. Deformity of leaves and tumored roots were characteristic of plants from the treated seeds. Some of these characters might be caused by chromosomal aberrations and/or physiological causes arising from the treatment. DONÀ DALLE ROSE (1961) observed chromosomal aberrations during mitosis and meiosis in sugar beets arising from the gamma irradiated seeds.

Various degrees of male sterility were observed in plants grown from the irradiated seeds of H-19. The male sterility was inherited by the next generation ( $M_2$ ) through the mother plants at a relatively high frequency. In the three lines from H-19, the male sterility was maintained until the fourth generation ( $M_4$ ), through the mother plants at high frequencies exceeding 50%. It is noted that the maternal inheritance is recognized in the male sterility induced by the gamma irradiation. A single gene is responsible for the pollen restoration in the new cytoplasm. Since the linkage relation is established between the pollen restoring gene and the gene *m* for the monogerm character, the locus of the pollen restoring gene is possibly identical with the gene *X*, or closely linked with *X*. Cytological observations showed that an abnormality of anther tapetum was associated with the pollen abortion. Thus, it is probable that the induced male sterility originated from a mutation of the cytoplasmic factor following gamma irradiation.

ERICHSEN and ROSS (1963 a) reported that the colchichine-induced male sterility in sorghum was caused by a mutation of the cytoplasmic factor. Although the mutagen is different, the possibility of plasmagenic mutation is well in accordance with the results obtained in the author's experiment.

As to the cytoplasmic alternation or changes, LINDSTROM (1933) reported that a cytoplasmic change, associated with a genic change was observed both caused by radium treatment of tomato. RHOADES (1943, 1950) described that the *iojap* gene (*ij*) induces both cytoplasmic male sterility and plastid mutations in striped sectors, but the sectors did not coincide, indicating that different elements were involved both in male sterility and plastid. Recently, MICHAELIS (1968) showed that the induced plastid and plasmon mutations could be analyzed by the use of the certain nuclear gene *mp* which increases the rate of mutability in *Epilobium*. MURRAY and CRAIG (1968) presented evidence in which they reported that a "forward and reversed" mutation of curled-leaf character in *Chenopodium rubrum* is based on the alternation of

an extranuclear factor or factors. In sugar beets, CLEÿ (1967) obtained the following results in which *S* cytoplasm changed into *N* cytoplasm after thermal shock up to 55°C. The heritable change of *S* cytoplasm into *N* cytoplasm also occurred spontaneously during vegetative propagation of a sterile clone of *Epilobium* (JONES and GABELMAN 1965).

In the author's experiment, three complete male sterile plants were found in 41 plants of the  $M_1$  generation and the progenies of three plants showed a maternal inheritance of the male sterility. Although the nature of a cytoplasmic factor or factors for male sterility are scarcely known, a high mutation frequency might be the criteria for cytoplasmic inheritance (JINKS 1964, SAGER and RAMANIS 1966, MURRAY and CRAIG 1968).

RHOADES (1950) in *iojap* maize, suggested that mutated mitochondria might induce the male sterile condition. The site of the cytoplasmic factor determining the male sterility might be expanded to include plastids, ribosomes and other nucleic acid organelles (EDWARDSON 1968). HESLOP-HARRISON (1963 a, 1967) stated that the male sterility can be accounted for at least formally in the system of a regulator-gene/operon controls, but there remain phenomena associated with the organelles which are not so readily explained. The induction of the plasmagenic mutation by gamma irradiation may provide an effective means to elucidate the nature of the cytoplasmic factor or factors for male sterile characters.

If the cytoplasmic male sterility is induced repeatedly by the irradiation, it may become possible to breed male sterile strains without introducing alien sterile cytoplasm. Such a possibility is promised as shown in the repeated experiments by gamma irradiation using the diploid strsin, H-2002.

#### D. The nature of the cytoplasmic factor in male sterility

Experimental evidences indicate that a virus or a virus-like entity may control cytoplasmic male sterility in corn, petunia and sugar beet based on grafting experiments and electron microscopical observations (FRANKEL 1956, 1962, EDWARDSON and CORBETT 1961, EDWARDSON 1962, 1968, CURTIS 1967). ATANASOFF (1964) proposed that all cases of cytoplasmic inheritance should be re-examined from a stand point of virus infections. MICHAELIS (1964) argued with this extreme proposition and pointed out experimental evidences in conflict with hypothesis of the participation of viruses. However he stated that some individual examples of cytoplasmic inheritance may eventually be shown to result from virus infections. Asexual transmission of male sterility by grafting has failed in corn (RHOADES 1933, SHUMWAY and BAUMAN 1966), wheat (ZEVEN 1967), tobacco (SAND 1960), pepper (OHTA

1961, DASKALOFF 1968), *Crotalaria* (EDWARDSON 1967) and sugar beet (CLEY 1967, THEURER et al. 1968).

The author's experimental results also indicated that the autonomy of male sterile types in the scions and the stocks are maintained until the second generation after grafting. Since there is an indication that the pollen sterility of the scion is slightly affected by the different phenotype of the stocks, the transmission of some nutrient to promote pollen fertility through the graft union might be possible in some combinations of grafting. STEIN et al. (1959) suggested that a diffusible substance for sterility or fertility might affect the expression of male sterility to some extent.

As already mentioned in Section IV, A, 2, pollen sterility in some male sterile plants shows a considerable variation within a single plant. An intra-individual variation of pollen sterility at the blooming is representatively shown in Table 50. Small or large groups of lobes appear in partial fertility,

TABLE 50 Intra-individual variation of pollen sterility in the semi-sterile type-b plant, H-19 MS-6

| Branch      | Flower rank | anther 1 |        | anther 2 |        | anther 3 |        | Mean sterility in a flower (%) |
|-------------|-------------|----------|--------|----------|--------|----------|--------|--------------------------------|
|             |             | lobe a   | lobe b | lobe a   | lobe b | lobe a   | lobe b |                                |
| 1<br>(Base) | a           | (23)*    | 98     | 100      | 100    | 97       | 100    | 86.3                           |
|             | b           | 98       | 100    | 98       | 98     | 91       | 97     | 97.0                           |
|             | c           | (50)     | 100    | 68       | 86     | (49)     | 86     | 73.2                           |
| 2           | a           | (47)     | 73     | 92       | 100    | (20)     | (28)   | 60.0                           |
|             | b           | 81       | 84     | (19)     | 51     | (45)     | 58     | 56.3                           |
|             | c           | 51       | 99     | 100      | 100    | (42)     | 91     | 80.5                           |
| 3           | a           | 100      | 100    | 95       | 100    | 100      | 100    | 99.2                           |
|             | b           | 97       | 100    | 94       | 95     | 97       | 98     | 96.8                           |
|             | c           | 100      | 100    | 99       | 100    | 92       | 97     | 98.0                           |
| 4           | a           | (24)     | (26)   | (22)     | (44)   | (16)     | (20)   | 25.3                           |
|             | b           | (36)     | 53     | (24)     | (26)   | (16)     | (42)   | 32.8                           |
|             | c           | 51       | 67     | (18)     | (41)   | 54       | 59     | 48.3                           |
| 5<br>(Top)  | a           | 92       | 96     | 95       | 97     | 88       | 91     | 93.2                           |
|             | b           | 100      | 100    | 100      | 100    | 97       | 99     | 99.3                           |
|             | c           | 93       | 96     | 100      | 100    | 97       | 99     | 97.5                           |

\* Lobes with less than 50% sterile pollen grains are enclosed in parentheses to emphasize "islands of fertility".

TABLE 51. Intra-individual variation of pollen sterility in the tetraploid plant with normal cytoplasm, H-19 (4X)-2

| Branch      | Flower rank | anther 1 |        | anther 2 |        | anther 3 |        | Mean sterility in a flower (%) |
|-------------|-------------|----------|--------|----------|--------|----------|--------|--------------------------------|
|             |             | lobe a   | lobe b | lobe a   | lobe b | lobe a   | lobe b |                                |
| 1<br>(Base) | a           | 8        | 8      | 10       | 14     | 6        | 13     | 9.8                            |
|             | b           | (86)*    | (99)   | 23       | 31     | (65)     | (80)   | 64.0                           |
|             | c           | 7        | 11     | 8        | 11     | 14       | 21     | 12.0                           |
| 2           | a           | 4        | 11     | 4        | 21     | 6        | 10     | 9.3                            |
|             | b           | 5        | 6      | 4        | 5      | 5        | 5      | 5.0                            |
|             | c           | 3        | 12     | 4        | 15     | 6        | 16     | 9.3                            |
| 3           | a           | (97)     | (98)   | 40       | (53)   | 13       | 33     | 55.7                           |
|             | b           | 4        | 8      | 6        | 8      | 4        | 7      | 6.2                            |
|             | c           | 4        | 6      | 5        | 6      | 10       | 10     | 6.8                            |
| 4           | a           | 11       | 12     | 4        | 6      | 5        | 12     | 8.3                            |
|             | b           | 9        | 9      | 4        | 10     | 3        | 10     | 7.5                            |
|             | c           | 5        | 7      | 5        | 8      | 4        | 9      | 6.3                            |
| 5<br>(Top)  | a           | (99)     | (99)   | (100)    | (100)  | (100)    | (100)  | 99.7                           |
|             | b           | (100)    | (100)  | (100)    | (100)  | (100)    | (100)  | 100.0                          |
|             | c           | (100)    | (100)  | (100)    | (100)  | (100)    | (100)  | 100.0                          |

\* Lobes with more than 50% sterile pollen grains are enclosed in parentheses to emphasize "islands of sterility".

while most of the lobes remain in the S.S.b type. In other cases, "islands of sterility" (Duvick 1965) appear in plants which are quite highly fertile. The degree of similarity between lobes, increases with their proximity to each other. However, a similar phenomenon is observed also in an intra-individual variation of pollen sterility in some plants with normal cytoplasm. A tetraploid normal plant, H-19 (4X)-2, showed a typical mixing of highly sterile lobes or anthers within an individual plant at the late blooming stage (Table 51). Therefore, the variable expression of male sterility within an individual plant of semi-sterile types is not necessarily due to the direct effect of somatic segregation or sorting out of cytoplasmic determinants. It seems to depend on the physiological nature in partially sterile plants.

Similar results were obtained in partially fertile plants both in Texas and normal cytoplasm in corn (Duvick 1965). Thus, Gabelman's hypothesis on the particulate male sterile factor is not supported from the present day

knowledge of the genetic and environmental interaction with the male sterility (DUVICK 1965). However, a satisfactory model of somatic segregation of an extrachromosomal determinant or a plasmagene is demonstrated in the plastid segregations in *Epilobium* (MICHAELIS 1958). In addition to this, a number of hypothesis have been developed to define the cytoplasmic units in a testable manner (SAGER and RYAN 1963, JINKS 1964, GRANICK 1965, OHTA 1966, HESLOP-HARRISON 1967, EDWARDSON 1968). WATSON and CASPARI (1960) described a mathematic model for the behavior of nuclear genes and cytoplasm in an example of male sterility in *Linum*.

#### **E. Application of a selective gametocide for chemical emasculation**

Male sterility can be induced artificially by use of chemicals such as 2, 4-dichlorophenoxyacetic acid, maleic hydrazide, gibberellin, tri-iodobenzoic acid and sodium 2, 3-dichlorisobutyrate (REHM 1952, WARREN and DIMMOCK 1954, WIT 1960). In his preliminary experiments, the author used 2, 4-D, MH-30 and FW-450, and recognized that the FW-450 (sodium 2, 3-dichlorisobutyrate) is most promising among the three chemicals. Optimum concentrations and application times have been examined by several investigators. Two applications of a 0.2% or 0.3% solutions gave desirable results by WIT (1960), DUDLEY (1960), BUTTERFASS (1960), SALMON (1963) and FÜRSTE (1964). A higher concentration (0.5–1.5%) was recommended by RUEBENBAUER and SCHULTIS (1960) and ISÁK (1963). Tetraploid normal plants were used for the experiments, and the proportion of triploids was two to three times higher in the treated plots than in the control plots, when pollinated with diploid plants (ISÁK 1963, FÜRSTE 1964).

The experimental results by the author showed that a considerable percentage (over 80%) of practical male sterile types are produced and lasted for one or two weeks in the two applications of a 0.3% or 1% solution. There were no remarkable differences on the effects of the chemicals between diploid and tetraploid plants. In fact severe injury by the chemical appeared in the application of a 1% solution. From a standpoint of the triploid seed production, it seems that the use of the chemicals to tetraploid plants is more promising than its use to diploid plants in consideration of the certiation between haploid and diploid pollens.

#### **F. Cytological nature of male sterile characters**

##### *1. Cytoplasmic-genetic male sterility*

Normal meiosis followed by degeneration of pollen tetrads or microspores

is characteristic for cases of cytoplasmic-genetic male sterility (EDWARDSON 1968). The male sterility in sugar beets is also associated with tapetal abnormalities (ARTSCHWAGER 1947, HOSOKAWA et al. 1954, OHTA and MATSUMURA 1960, NAGAO and KINOSHITA 1962, ROHRBACH 1965 a). An occurrence of unreduced chromosome numbers in egg cells of diploid male sterile plants was reported by ELLERTON and HENDRIKSEN (1959). In carrots with male sterile cytoplasm, ZENKTELER (1962) observed meiotic irregularities which are due to a reciprocal translocation.

The author has found two trisomic plants in a diploid strain with male sterile cytoplasm and a considerable range of variation in somatic chromosome numbers in a tetraploid male sterile strain. However it is uncertain whether the chromosomal aberrations are due to the type of cytoplasm associated with male sterility or not. Through the observation on chromosome association at the first metaphase and distribution of chromosome numbers in the first anaphase, it seems improbable to assume meiotic irregularities as the causation of complete and semi-sterile type-b of male sterility, at both diploid and tetraploid levels.

In normal conditions, fertile or normal plants do not make tapetal plasmodium during the microsporogenesis and exhibit a glandular tapetum. In complete sterile plants both at diploid and tetraploid levels, the tapetal plasmodium is formed at the tetrad stage or as soon as the microspores are released from the quartets. The periplasmodium protrude or bulge prominently into the locule, containing multinuclei and large vacuoles. Sometimes the tapetal cells expand toward the center of the locule in such a way that the microspores become closely packed in the lumen prior to breakdown. The tapetal plasmodium of complete male sterile plants degenerate with the microspores after its maximum development. Thereafter the development of endothecium and connective between the anther lobes which include the differentiation of vascular bundles are inhibited or are found collapsed. Through the observations, abnormal symptoms first appear in the tapetum around the tetrad stage in complete sterile plants. No remarkable difference is found in regard to the tapetal abnormality between diploid and tetraploid complete sterile plants.

According to MAHESHWARI (1950) and VASIL (1965, 1967), the tapetum has a considerable physiological significance, for all food materials, growth substances, water and other vital supplies entering into the sporogenous cells must pass through the tapetal cells. JUEL (1915) reviewed the behavior of tapetal cells during the course of microsporogenesis in angiosperms. Formation of tapetal plasmodium was noted as an effective route to supply the

nutrition of the spores in some species (HANNIG 1911, TISCHLER 1915). The plasmodial aggregations resulting in pseudopodium-like incursion (JUEL 1915) or wandering amoeboid cells (PICKETT 1916) are to be regarded as normal in these species. CLAUSSEN (1927) classified this kind of tapetum, generally referred to as the "amoeboid" tapetum in contrast with the glandular tapetum, into four subtypes. The periplasmodium formation in the complete sterile type of sugar beets usually takes place after the release of microspores from the quartets, so that it resembles the *Sagittaria* type.

WHYTE (1929) reported a case of multi-nucleate plasmodium in one abnormal plant of *Ranunculus acris*. He assumed that the tapetal cells in the form of a plasmodium are no longer functional as a source of supply of nutritive materials. KOSTOFF (1930) recognized the parallelism drawn between the morphology of tapetal cells and morphology of somatic cells attacked by foreign agents (wounds, tumors, galls, chemicals, graft-callus. etc.), and postulated that the toxic substances form pollen mother cells or around them cause the abnormal appearance of the tapetum. The large vacuoles which are a notable feature of the tapetal plasmodium may be places of storage for excess waste products resulting from unusual metabolic activities (ARTSCHWAGER 1947). Although it is not possible to determine the cause and effect relationship between the tapetum and the microspores in the author's study, the abnormal behaviour of the tapetum has a close correlation with pollen abortion.

The formation of a pronounced tapetal plasmodium are observed in the cytoplasmic-genetic male sterility in the many crops, such as onion (MONOSMITH 1928, TATEBE 1952), carrots (ZENKTELER 1962), corn (CHANG 1954), flax (DUBEY and SINGH 1965), sorghum (BROOKS et al. 1966), wheat (CHAUHAN and SINGH 1966) and rice (SHIBUYA 1966).

PAINTER (1943) and COOPER (1952) reported a biochemical relationship between the tapetum and the pollen formation. TAYLOR (1959) clarified the deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) synthesis in sporogenous and tapetal cells of lily anthers preceding and during meiosis by autoradiographic studies. Protein synthesis occurs in the tapetal cells during meiosis but it is especially rapid during the maturation of microspores. This is of significance, for the tapetal cells secrete wall material for the microspores. TAKATS (1962) demonstrated that the label in tapetal nuclei by  $H^3$ -thymidine was lost shortly before DNA synthesis in the microspores and is distinctly incorporated into the inner part of the microspore wall. Since the inner wall material is laid down by the microspore protoplast, the break down products from tapetal DNA may be utilized by the microspores.

Observations by electron microscopy of *Tradescantia* have shown that the exine is covered by materials that may be derived from the tapetum (ROWLEY 1959). There is a structural continuity from the tapetal cytoplasm through submicroscopic channels in the exine to the voids in the pollen wall (ROWLEY et al. 1959, CHAMBERS and GODWIN 1961). TAYLOR (1959) found that  $S^{35}$  is bound simultaneously by young microspore walls and the tapetal droplets. Thus, the primary function of the tapetum appears to be the secretion of materials for wall formation on the microspores. The tapetum are also presumed to be the source of the large amount of RNA which accumulates in the cytoplasm of the pollen grains (PAINTER 1943). In addition to this, the tapetum also produces some fatty and resinous substances which are more commonly known as the cement substances or Pollenkitt (PANKOW 1958).

In the complete sterile type, the exine fails to thicken and all cell contents disappear in pollen grains. Exine material is synthesized by tapetal mitochondria and is later transported and deposited on the cellulose walls of young microspores (HESLOP-HARRISON 1926, 1963 b, VASIL 1967). Therefore, it is probable that the tapetum failed to supply the necessary nutrients for the development of microspores, resulting in a failure to mature into pollen grains. However, the complex phenomena such as meiosis and pollen development cannot be entirely dependent on the main function of the tapetum alone. The influence exerted by the vegetative parts and organs of a plant or by the flower cannot be overlooked (VASIL 1967). JOPPA et al. (1966) reported that the male sterility of wheat are caused by vascular deficiencies in the stamen. Although ROHRBACH (1965 a) intensified the inhibition of the conductive tissue in the male sterile type of sugar beet, the collapse of endothecium, parenchyma and vascular bundles were observed following the abnormality of tapetum and microspores in the complete sterile type. Pollen abortion associated with a cellular tapetum (ARTSCHWAGER 1947) was not observed in the complete sterile type.

The association between the pollen abortion and the abnormal development of tapetal cells were recognized likewise in the semi-sterile type-b, though the growth of the tapetal plasmodium was not observed. The prolonged adherence of hypertrophied tapetum can account for the pollen abortion, since substances from the tapetum necessary for proper development of pollen grains may not be available at the appropriate time. The endothecium in the indehiscent anthers shows a feeble development of the striations. In some anthers, the stomium was not produced.

The hypertrophied tapetal cells were associated with the genetic or cytoplasmic-genetic male sterility in some kinds of crops, such as alfalfa

(CHILDERS 1952, CHILDERS and McLENNAN 1960), grain sorghum (SINGH and HADLEY 1961), Sudan grass (ALAM and SANDAL 1967), orchard grass (FILION and CHRISTIE 1966), barley (KAUL and SINGH 1966), *Pelargonium* (HARNEY and KUNG 1967) and *Brassica campestris* L. (CHOWDHURY and DAS 1968).

A timing unbalance in meiosis leads to male sterility in sweet pea (UPCOTT 1937). A chimerical arrangement of division stage within a locule was observed in the male sterility in *Hebe townsoni* (FRANKEL 1940). NISHI and HIRAOKA (1958) considered that the unbalance relationship between the development of microspores and the process of disintegration of tapetum in the male sterility of some vegetable crops could be referred to a kind of "timing unbalance".

A mixture of different division stages in meiosis was observed in the locules within a single flower of the semi-sterile type in the author's materials. In addition to this, a remarkable lack of uniformity was observed in the abnormality of microsporocytes or microspores in the semi-sterile type-a. In an extreme case of a tetraploid semi-sterile plant, the breakdown of tapetal and sporogenous cells at premeiotic stage, the formation of tapetal plasmodium around the tetrad stage and the prolonged adherence of tapetum during the development of microspores were observed together within a single plant.

Three types of abnormal behaviour of the tapetum leads to abortion of microspores in the male sterile plants of hexaploid wheat (CHAUHAN and SINGH 1966). As suggested by NISHI and HIRAOKA (1958), different nutritive requirements should exist for various stages of the phasic development. The degeneration of microsporocytes and microspores appears to be caused by the abnormality of the tapetum at each of the corresponding stages. Thus, it might be possible that an irregular distribution of the limited substances through the tapetum in each stage gives rise to such a marked variation among the locules within a flower.

According to the histochemical studies by HOSOKAWA et al. (1963, 1965), the increase or decrease of starch and sugar in the anther tissue showed a remarkable difference between normal and male sterile types of sugar beets. Relatively large quantities of alanine were present during meiosis and in the microspore stages in male sterile anthers, while a definite lack of proline was noted in sterile anthers during the formation of microspores. Similar relationships of free amino acids in the anther tissues were studied by paper chromatography in wheat, corn (FUKASAWA 1954, KHOO and STINSON 1957), sorghum (BROOKS 1962) and vegetable crops (FUJISHITA 1964, 1965). LINSKENS (1966) demonstrated that each cytological stage shows a specific protein

and enzyme pattern for normal meiosis and subsequent microspore development. EDWARDSON (1968) states that "the mechanism of pollen abortion will undoubtedly be elucidated through the studies on the metabolic differences between androecia of sterile and normal inflorescences".

Through the author's observation, it is probable that a disturbance in the transport of essential nutrients from the tapetum to microspores at different stages causes a variation in the degree of male sterility both at diploid and tetraploid levels.

## 2. *Genetic male sterility*

The present observations on genetic male sterility revealed an association between the abnormal behavior of the tapetum and pollen abortion. Hyper-trophied tapetum appeared at the time when microspores were liberated from the quartets, although the growth of the tapetal plasmodium was not so pronounced when compared with the abnormality due to the cytoplasmic-genetic male sterility brought about by a genotype, *S xx zz*. The prolonged adherence of the tapetum may indicate that it is not serving to supply the nutrition to the developing microspores at a proper time and may lead to the premature degeneration of the microspores. Degeneration of microspores proceeds rapidly and collapses of microsporangia are observed. Sometimes, swollen tapetum encloses the quartet microspores and disintegrates earlier than the development of microspores. The growth of other floral organs is also inhibited remarkably in such a case. Thus underdeveloped flowers are frequently contained at the upper part of the inflorescence in the male sterile plants.

ARTSCHWAGER (1947) also observed the abnormality in tapetum and microspores in Mendalian male sterility which is caused by the gene,  $a_1$  or  $a_2$ . Though the tapetum behaviour resembles genetic male sterility caused by the gene, *ms*, fully developed endothecium was seldom observed in the author's materials at the onset of the tapetal abnormality.

In tomato mutants, RICK (1948) described that six different genes for male-sterility cause the break down of male gametophytes at six different times, from pre-meiosis to late microspore stage, respectively. It is suggested that the different genes for male sterility such as  $a_1$ ,  $a_2$  and *ms* may insert their effects for pollen abortion at different times of anther development in beets.

## 3. *Induced male sterility by gamma irradiation*

In general the observation made on the gamma-induced male sterile plants corresponds to those on the cytoplasmic-genetic male sterility. The

plasmodial aggregation making pseudopodium-like incursions are observed in the anther tapetum of the induced male sterile plants as well as in those of complete sterile plants, having *S* cytoplasm and which differs from those of the genetic male sterility. Thus cytological evidence substantiates the fact that the induced male sterility originated from a mutation of the cytoplasmic factor, after gamma-irradiation.

ERICHSEN and ROSS (1963 b) studied the microsporogenesis in the colchicine-induced male sterile mutants of sorghum, which were assumed to be caused by a mutation of the cytoplasmic factor. Cytological and histological studies indicated that the colchicine-induced male sterility appears to have the same or a similar mechanism causing the cytoplasmic-genetic male sterility in sorghum.

#### 4. *Male sterility caused by low temperature treatment*

The anther and the component tissues, especially the sporogenous and the tapetal tissues, are extremely susceptible to even mild environmental and internal changes (VASIL 1965). SAX (1937) observed many irregularities in meiosis and microspore development when the plants subjected to low and high temperature. SAKAI (1949) found that hypertrophied tapetum has a close relation with pollen abortion when rice plants are subjected to low temperature under 14°C during the meiosis and microspore stages.

In this experiment, normal and male sterile plants were placed under low temperature (5°C) for 3 days. Tapetal periplasmodium making pseudopodium-like incursions into the anther cavity was observed in the plants which altered their phenotypes from N or S.S.b type to C.S. type following the low temperature treatment. The results indicate that the formation of plasmodium is not always a feature appropriate to the genotype, *S xx zz*, but may also be brought by physiological causes. Tapetal abnormalities such as the prolonged adherence of the swollen tapetum are also associated with the pollen abortion induced by low temperature in the normal cytoplasm plants. In addition to this, abnormal thickness of anther walls and smaller microsporangia were observed in a tetraploid semi-sterile plant after low temperature treatment.

OHTA and MATSUMURA (1960) reported that the relation between the tapetal tissue and pollen grains is thought to be the most important cause of male sterility both in physiological and genetical causes. It seems highly probable that the mechanism of pollen abortion by low temperature resembles that of male sterility caused by interaction with the nuclear genes and the sterility-inducing cytoplasm.

### 5. Male sterility induced by the gametocidal effect

Complete male sterile plants have been induced by application of sodium 2,3-dichloroisobutyrate both in normal diploid and tetraploid sugar beets. Cytological observations showed that the tapetal cells were considerably hypertrophied and protruded into the anther cavity to such an extent that the microspores were crushed. A similar phenomenon was observed in the anther development of the semi-sterile type (ARTSCHWAGER 1947) and the male sterile plants induced by the gamma irradiation.

KAUL and SINGH (1967) observed a similar abnormality of tapetum and microspores in chemically induced male sterility of fenu-greek (*Trigonella foenum-graecum* L.). The similarity in the mode of pollen abortion in chemically induced and genetical male-sterile plants suggests that the effects of the chemical may be the same as those genetically controlled. The mechanism of gametocidal effect was studied by applying radiocarbon-labeled FW-450 in cotton (SCOTT 1961). Pantothenic acid alone or in combination with D-ribose enhanced the translocation of the radiocarbon to reproductive tissues.

OHTA and MATSUMURA (1962) observed that the tapetal cells were hypertrophied homogenously and remained for an unusually longer duration than those of normal plants in the chemically induced male sterility of sugar beets.

In many respects the abnormality of tapetum and microspores induced by gametocidal effects are similar to those induced by low temperature and those of the male sterility by genetical causes.

## VI. Conclusion

Recently cytoplasmic-genetic male sterility is extensively utilized for the production of commercial hybrid seeds. Several triploid varieties are produced by the use of diploid male sterile plants.

As a basis for the practical use of male sterility, the nature and the mode of inheritance were studied on the cytoplasmic-genetic male sterility. Environmental modification and the intra-individual variation indicated that the complete sterile type and semi-sterile type-b plants can be used as practical male sterile plants, exhibiting relatively stable phenotypic expressions.

The mode of inheritance are based on the interaction between the sterility-inducing cytoplasm and the complementary genes, X and Z, which are responsible for the various degrees of pollen restoration. Possibly, another chromogene or genes may exert their enhancing effect on pollen fertility—in other words may turn S.S.a into N. In addition to this, minor modifying

genes and environmental variations may partly have a role in the expression of male sterile types. Therefore, the monofactorial inheritance and polyhybrid mode of inheritance which have been reported by several investigators, may be possible in different materials under diverse environmental conditions. A linkage relation between the gene *X* and the gene *m* for monogerm character, is rather profitable for the breeding of the monogerm male sterile lines.

It is noted that almost pure triploids were obtained from the crossings between the tetraploid male sterile strains and diploid pollinators. It is believed that a high frequency of practical male sterile plants in the tetraploid male sterile strains which were crossed with the pollinators randomly chosen from normal varieties and a faster growth of haploid pollen tubes from diploid pollinators than that of diploid pollens from normal tetraploids which are contained in the male sterile tetraploid strains, have contributed to the production of the pure triploids. Therefore, the use of type-O plants can be omitted for the practical use of tetraploid male sterility in triploid seed production and maintenance of tetraploid male sterile strains. Through the yield tests covering two years, the triploid hybrids with the use of tetraploid male sterile strains showed a superiority in comparison with the parental diploids, tetraploids and the triploids with the use of diploid male sterile strains. Thus the use of tetraploid male sterile strains are promising for an efficient seed production of pure triploids.

In the present work, a genetic male sterile plant was found in the progeny of the wild beet, *Beta maritima* L. This particular mutant behaved as a single Mendelian male sterile plant. Its causal gene was designated as *ms* and is different from the gene *a*<sub>1</sub> or *a*<sub>2</sub> which was reported in sugar beets. This character was transferred to sugar beets by successive back crossings.

Male sterile mutants were induced by gamma irradiation in a diploid monogerm strain with *N* cytoplasm. Cytoplasmic inheritance was demonstrated in the induced male sterility. A single gene which has a linkage relation with the gene *m* for the monogerm character, was responsible for the pollen restoration interacting with a new cytoplasmic factor. The cytological evidence indicated that the abnormality of anther tapetum and microspores are in accordance with those in the cytoplasmic-genetic male sterility. It is possible that the induced male sterility originated from a mutation of the cytoplasmic factor after gamma irradiation. Similar results on the occurrence of cytoplasmic-genetic male sterility were obtained in the repeated experiments by using another multigerm strain, H-2002. Thus a new and potent source of male sterility might be produced by ionizing radiation and chemical mutagens.

Abnormal anther tapetum was associated with the pollen abortion both in cytoplasmic-genetic and genetic male sterility at diploid and tetraploid levels. The diverse forms of abnormal tapetum corresponded to the constitution of the pollen restoring genes in *S* cytoplasm. It is probable that a disturbance in the transport of the essential nutrients from the tapetum to microspores at different developmental stages causes a variation in the degree of male sterility. In spite of the phenotypic resemblance, the growth of the tapetal plasmodium in the genetic male sterility was not so pronounced when compared with the tapetal abnormality due to the cytoplasmic-genetic male sterility brought about by a genotype, *S xx zz*. The abnormalities of tapetum and microspores caused by the effects of the low temperature and the gametocide, are similar to those of the male sterility arising from genetical causes.

Though more information is required for the clarification of the above mentioned problems, further investigations on the nature of male sterile characters will be in the direction of a study of fine structure of the cytoplasm and elucidation of the metabolic difference between male sterile and normal inflorescences, as suggested by FUKASAWA (1962), DUVICK (1965) and EDWARDSON (1968).

As a consideration to the pollen parents which are used in the hybrid seed production, the female sterile character can be utilized efficiently because of an abundance of pollen production but with a complete failure of seed production (CHILDERS 1960 b). The female sterile mutants are found in alfalfa (CHILDERS 1960 a), tomato (HONMA and PHATAK 1964), cotton (PATE and DUNCUN 1963), tobacco (WAN and MANN 1967) and sugar beets (ZAHARIEV et al. 1963). The combined utilization of male sterility and female sterility may be promising in the facilitation of controlled pollination in the production of hybrid seeds.

## VII. Summary

1. The present investigation deals with the nature and inheritance of male sterile characters both at diploid and tetraploid levels together with some information on the application of this character to triploid breeding.
2. In cytoplasmic-genetic male sterility, the types of abnormality in the anthers and pollen grains in the progenies of male sterile strains or populations are classified into following four classes; complete sterile (C.S.), semi-sterile type-b (S.S.b) equivalent of type-1 by OWEN (1945), semi-sterile type-a (S.S.a) equivalent of type-2 and normal (N), on the basis of repeated observations throughout the blooming period.

3. A considerable variation of pollen sterility was observed during the flowering duration or between different seasons, in most of the S.S.a type plants and some plants of the N and S.S.b types.
4. A remarkable difference of pollen sterility was observed among different parts within a single plant, especially in the S.S.a type plants. It was demonstrated that the pollen sterility in partial sterile plants is unstable under different environmental and internal conditions.
5. The character expression of the male sterility was significantly affected by low temperature and starvatin culture. In an extreme case, the N type changed into C.S. type after a 3 day treatment under low temperature (5°C).
6. It was ascertained that the complementary action of two chromogenes, *X* and *Z* is responsible for the pollen restoration under the co-existence of the sterility-inducing cytoplasm. Namely, the genotypes of normal (N) or semi-sterile type-a (S.S.a), semi-sterile type-b (S.S.b) and complete sterile (C.S.) are governed by the double dominant, single dominant and double recessive condition of the said genes. In addition to this, there remains a possibility of the existence of another gene or genes which may exert an enhancing effect on pollen fertility.
7. A linkage relation was established between the gene for pollen restoration (*X*) and the gene for monogerm character (*m*). The recombination values calculated in the coupling phase were 21.7% and 24.5%. A genetic association exists between the phenotypic expression of male sterility and the pigmentation of the anthers.
8. Tetrasomic inheritance of two basic genes (*X* and *Z*) were confirmed for the pollen restoration in tetraploid crosses. The segregation ratio was the intermediate of the two theoretical ratios based on parameters  $\alpha=0$  (minimum value) and  $\alpha=1/7$  (maximum value).
9. Under the genic assumption of the existence of *X* and *Z*, the genotype *xx zz* with *N* cytoplasm (designated as type-O) is desirable for the pollinator crossed with complete sterile plants (*S xx zz*). A relatively small number of the homozygous plants of the said genes, including type-O plants, were observed in a population of Hon-iku-192, in comparison with the frequency of the heterozygous plants.
10. The results also showed that even if plants from random sampling in a variety, Hon-iku-192 are used as the pollinators, a relatively high percentage (71 or 77%) of practical male sterile plants (C.S. and S.S.b) are maintained in the progeny. A large percentage (81-96%) of the male sterile plants were produced in the progenies of tetraploid male sterile

plants when they were crossed with pollinators from random sampling in tetraploid varieties.

11. Trial seeds of triploid hybrids were produced by the use of diploid and tetraploid male sterile strains which were maintained without the use of type-O plants. Almost pure triploids were obtained from the crossings between tetraploid male sterile strains and diploid pollinators whereas the use of diploid male sterile strains which were produced without the use of type-O plants was not effective for the production of pure triploids.
12. Yield tests in gross sugar production covering two years showed that the triploid hybrids with the use of tetraploid male sterile strains are superior to parental diploid and tetraploid varieties, and the triploid hybrids with the use of diploid male sterile strains.
13. The author proposed a scheme on the utilization of male sterility both at diploid and tetraploid levels. In the use of tetraploid male sterility, the pollinators which were randomly chosen from the normal varieties can be used, instead of the use of type-O plants. Thus the use of tetraploid male sterile strains has a potential value for an efficient seed production of pure triploids.
14. A genetic male sterile mutant was found in a strain of wild beet, *Beta maritima* L. The male sterile types in the progeny of BM-2 were classified into six types because of the appearance of the modified type for semi-sterile type-b and complete sterile type. However, a progeny test showed that the variation of the male sterility is mainly influenced by environmental conditions. Through several crosses within *B. maritima* and between *B. maritima* and *B. vulgaris*, it was confirmed that this particular mutant behaved as a single-gene recessive to the normal fertile type. The symbol of its causal gene was designated as *ms*.
15. In the present studies it was indicated that the gene *ms* has an independent relationship with the pollen restoring genes, *X* and *Z* when they coexisted with a plasmagene, *S* for male sterility. In addition to this, an independent association was obtained between the gene, *ms* and the gene, *m* for monogerm character.
16. According to the genic identification between the genetic male sterility in sugar beet and wild beet, the gene *ms* was non-allelic with the gene, *a*<sub>1</sub> or *a*<sub>2</sub>, and an independent relation existed between the genes, *ms* and *a*<sub>1</sub> or *a*<sub>2</sub>. Therefore, a digenic ratio, 9:7 was brought out in the F<sub>2</sub> of the cross between CT-5 (a sugar beet with genetic male sterility) and BM-2-1 (a heterozygous normal plant of the genotype, *Ms ms*).
17. The seed setting of male sterile plants was decreased significantly from

that of normal plants because of the mixing of abnormal flowers in the upper part of inflorescence. However, there is a possibility that the seed setting of male sterile plants may be improved by selection. Thus, genetic male sterile plants which originated from *B. maritima* can be used for the breeding of interspecific hybrids in the genus, *Beta*.

18. The dry seeds of a normal diploid strain, H-19 were irradiated with gamma rays with doses of 50 and 100 kR. An occurrence of male sterile and female fertile plants was observed in the progeny of the plants grown from the irradiated seeds. The male sterility was inherited by the next generation ( $M_2$ ) through the mother plants in a relatively high frequency over 50%. In the three strains from  $M_1$  plants of H-19, the male sterility is maintained until the fourth generation ( $M_4$ ), through the mother plants.
19. Cytoplasmic inheritance was demonstrated in the male sterility induced by the gamma irradiation. Segregation of male sterile types in the progeny of the crosses between normal plants from the male sterile lines,  $\gamma$ -20 and  $\gamma$ -27 and normal cytoplasm plants H-19, differed remarkably from that in the progeny of the reciprocal crosses.
20. Inheritance of the male sterility was studied in the cross between a complete male sterile plant in  $\gamma$ -27 line and a normal plant of H-2002. A single dominant gene was responsible for the pollen restoration when it co-existed with a new plasmagene,  $S'$ . In addition to this, the pollen restoring gene for the new plasmagene is linked to the gene for the monogerm character with a recombination value of  $36.2 \pm 1.77\%$ . Therefore, it is probable that the pollen restoring gene for the new plasmagene is identical with the gene,  $X$ , or is closely linked to the gene,  $X$ .
21. The experiment was repeated by using another multigerm strain, H-2002. Similar results on the occurrence of male sterility were obtained in  $M_1$  and  $M_2$  generations. The cytoplasmic inheritance was confirmed again in the induced male sterility. Thus, it is probable that the induced male sterility originated from a mutation of the plasmagene or the cytoplasmic factor after gamma-irradiation.
22. The grafting experiments on the asexual transmission of male sterility indicated that the autonomy of male sterile type in the scions and the stocks are maintained until the second generation after grafting. Since there is an indication that the pollen sterility of the scion is slightly affected by the different phenotype of the stocks, the transmission of some nutrients through the graft union may promote the pollen fertility of the scion in some combinations of grafting.

23. A gametocide 'FW-450' was tested for the induction of male sterility using diploid and tetraploid strains. The chemical, sodium 2, 3-dichloroisobutyrate was diluted to 0.1% 0.3% and 1% aqueous solution and sprayed two times on the plants at the stages of bud formation and initiation of flowering. The experimental results showed that two applications of a 0.3% solution is most suitable for the plants both at diploid and tetraploid levels. However, perfect male sterility lasted only a week or ten days even with an 1% solution. In fact severe injury by the chemical appeared in the application of 1% solution. More information is required for the practical use of the chemical.
24. Cytological observations were made in the male sterile and normal beets at diploid and tetraploid levels. A cytoplasmic male sterile strain, H-19 MS, contained two trisomic plants within 100 plants, while another strain of *S* cytoplasm, K-3-11 and a normal cytoplasm strain, H-19 consisted of diploids only. The progeny of the male sterile mutant, BM-2 was also composed of pure diploids. A considerable range of variation in somatic chromosome numbers was observed in a tetraploid male sterile strain, 4 M-50. However, it is uncertain whether the chromosomal aberrations are due to the type of cytoplasm associated with the male sterility or not.
25. The somatic chromosome numbers were relatively stable in  $M_2$  lines and  $F_2$  populations which inherited the male sterility induced by the gamma irradiation. Though a small number of trisomic, triploid and tetraploid plants were involved in some of the  $M_2$  lines and  $F_2$  populations, most of the plants were diploids. Therefore, the induced male sterility may not be due to aneuploidy or polyploidy.
26. Meiotic behaviour in most diploid male sterile plants were fairly regular while the formation of quadrivalent chromosomes and abnormal distribution of chromosome numbers were observed regardless of the presence or absence of male sterile characters in their tetraploids.
27. Abnormal development of the tapetal plasmodium was observed in the anthers of complete sterile plants both at diploid and tetraploid levels. In semi-sterile type-b plants, the prolonged adherence of hypertrophied tapetum was associated with the pollen abortion. A feeble development of the striations of endothecium and lack of stomium resulted in the non-dehiscence of the anthers.
28. Semi-sterile type-a plants sometimes contained normal anthers and abnormal anthers within the same flower. A remarkable lack of uniformity was observed in the abnormality of microspores in a tetraploid semi-

sterile plant. Some anther lobes showed an abnormality at the premeiosis stage, others showed a typical formation of tapetal periplasmodium at the later stage, whereas the remaining anther lobes showed partial sterility or close to a normal condition within the single plant. It is probable that a disturbance in transport of the essential nutrients to microspores through the tapetum in different developing stages, causes a variation in the degree of male sterility.

29. Tapetum abnormality was also observed in the genetic male sterility caused by the gene *ms*, while the growth of the plasmodium was not so pronounced when compared with the tapetal abnormality due to the cytoplasmic-genetic male sterility brought about by a genotype, *S xx zz*. Sometimes, swollen tapetum enclosed the quartet microspores and disintegrated earlier than the development of microspores. In such a case, the endothecium degenerated to a thin line of tissue and the anther cavity contains only a strand of blackened matter consisting of destroyed microspores.
30. The abnormality of the anther tapetum was associated with pollen abortion in the gamma-induced male sterility. The plasmodial aggregation making pseudopodium-like incursions are observed in the anther tapetum of the induced male sterile plants as well as in those of the complete sterile plants, having *S* cytoplasm and which differs from those of genetic male sterility. Thus the gamma-induced male sterility appears to have the same or a similar mechanism causing the cytoplasmic-genetic male sterility, supporting the alternation or mutation in the plasmagene or the cytoplasmic factor.
31. The anther and component tissues, especially the sporogenous and the tapetal tissues are susceptible to the environmental changes, such as low temperature. Tapetal periplasmodium making pseudopodium-like incursions was observed in the plants which altered their phenotype from N or S.S.b type to C.S. type after low temperature treatment (5°C for 3 days). The results indicate that the formation of tapetal plasmodium is not always a feature appropriate to the genotype, *S xx zz*, but may also be brought about by physiological causes. In addition to this, prolonged adherence of the swollen tapetum was also associated with the pollen abortion induced by low temperature in the normal cytoplasm plants. Besides these phenomena, abnormal thickness of anther walls and smaller microsporangia were induced in a tetraploid semi-sterile plant after low temperature treatment.
32. Complete male sterile plants were induced by the application of sodium

2, 3-dichloroisobutyrate (FW-450) both in diploid and tetraploid normal sugar beets. Cytological observation showed that the tapetal cells were considerably hypertrophied and protruded into the anther cavity to such an extent that the microspores were crushed. In many respects, the abnormality of the tapetum and microspores induced by physiological causes such as low temperature and gametocide are similar to those induced by genetical causes.

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# PLATES

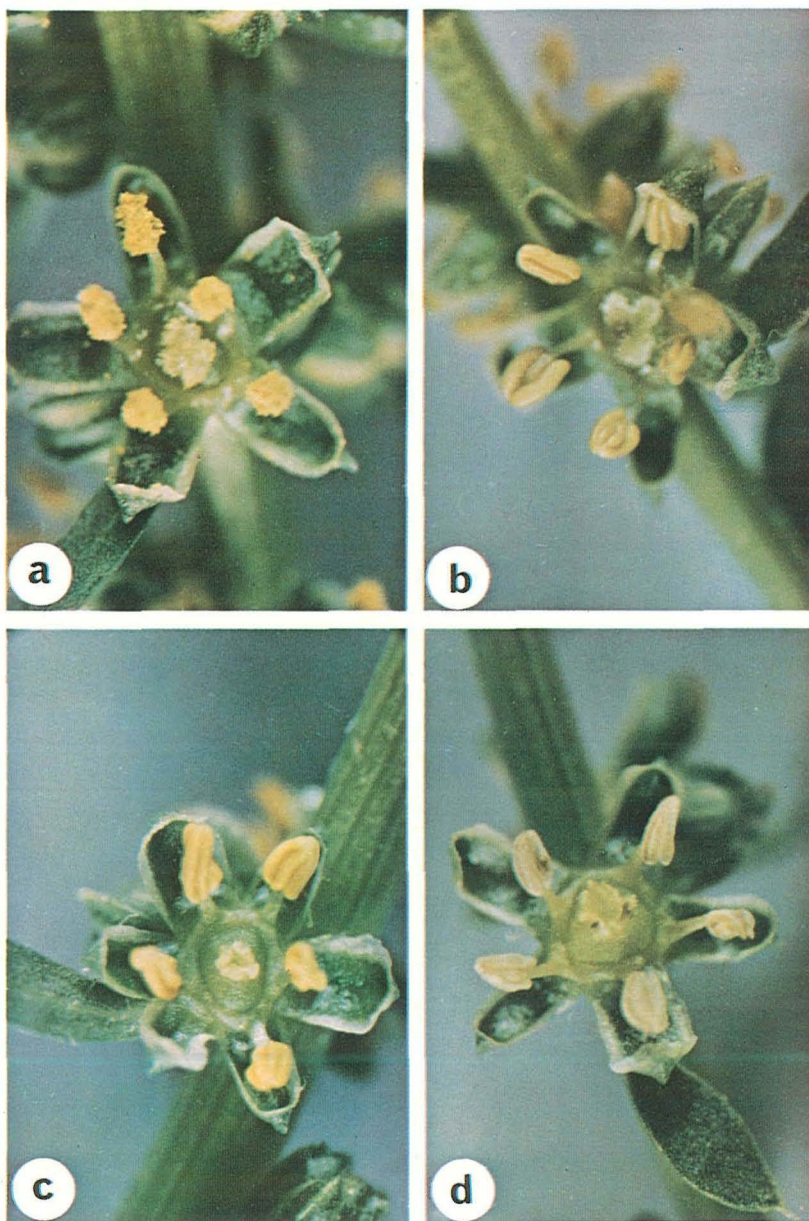
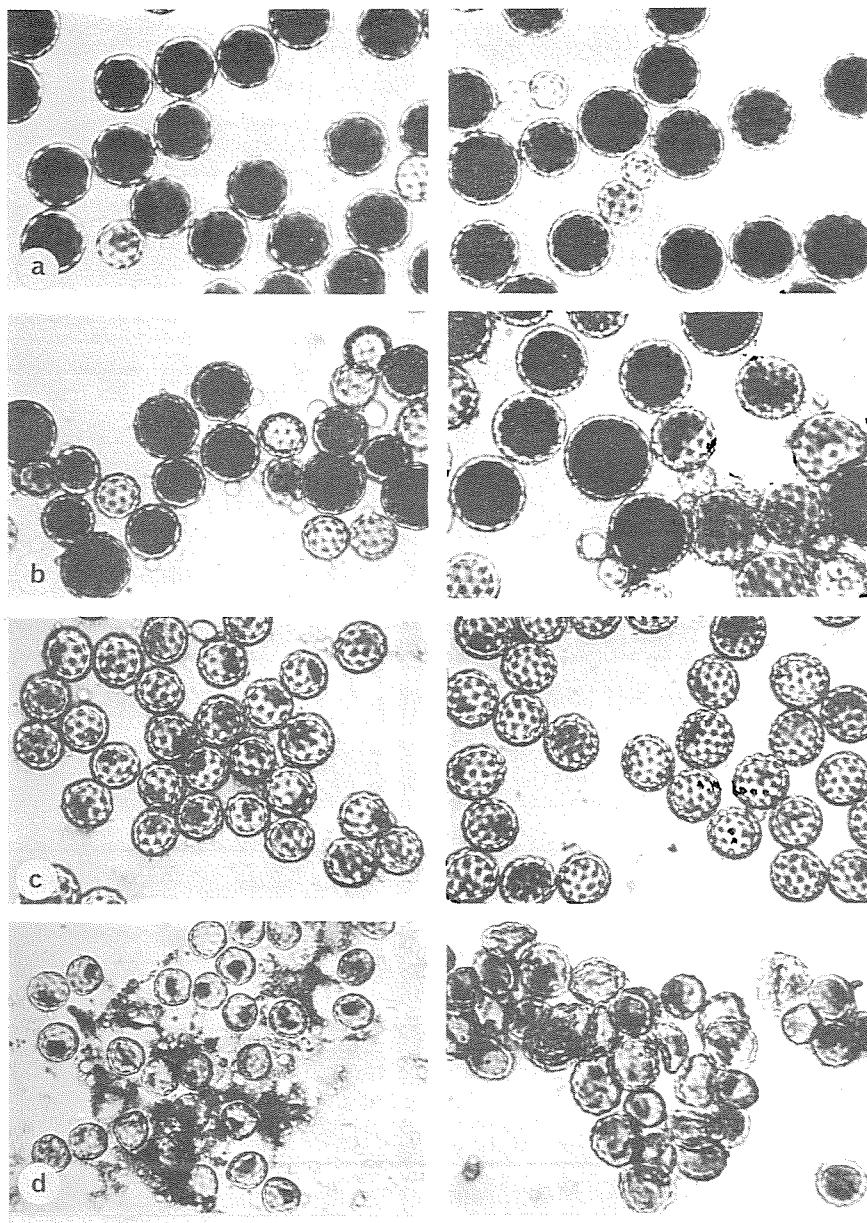


PLATE I

Flowers of normal and male sterile plants.  $\times 12$ .

- a. Normal type (N).
- b. Semi-sterile type-a (S.S.a).
- c. Semi-sterile type-b (S.S.b).
- d. Complete sterile type (C.S.).



## PLATE II

Pollen grains of normal and male sterile plants at diploid and tetraploid levels.  $\times 370$ .

- a. Normal type of diploid (left) and tetraploid (right).
- b. Semi-sterile type-a of diploid (left) and tetraploid (right).
- c. Semi-sterile type-b of diploid (left) and tetraploid (right).
- d. Complete sterile type of diploid (left) and tetraploid (right).

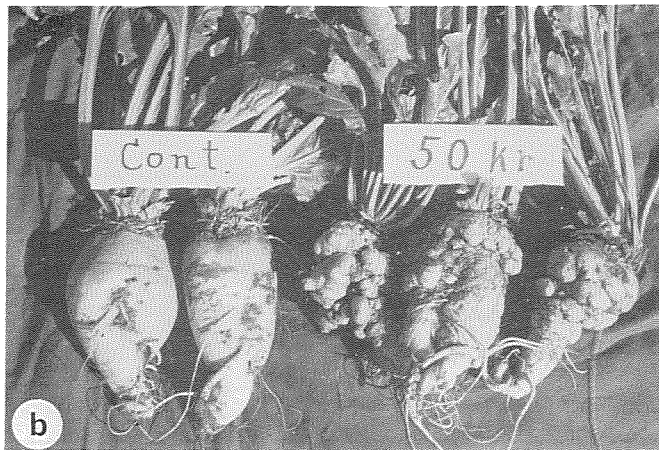
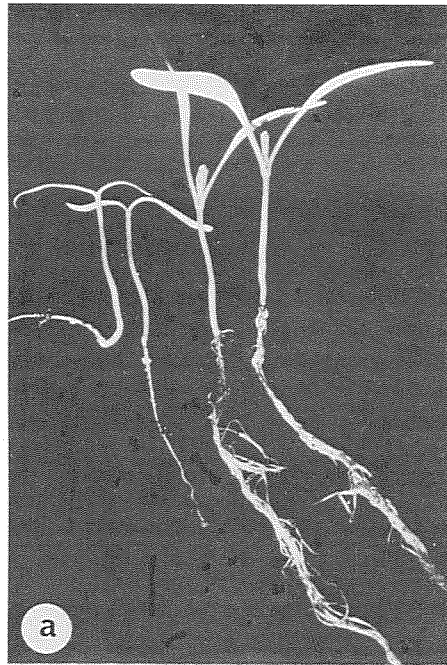


PLATE III

Abnormalities caused by gamma irradiation.

- a. Lethal seedlings (left).
- b. Tumor-like lump formation. (right).

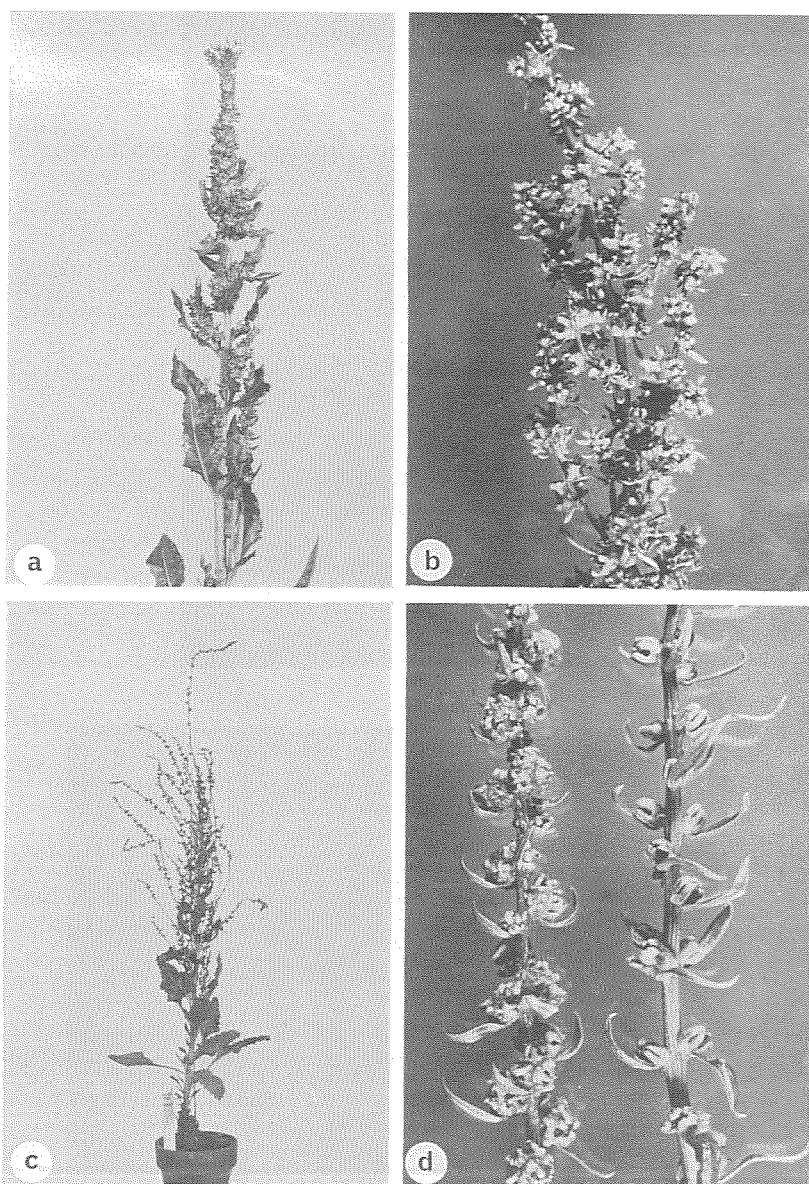


PLATE IV

Abnormal characters found in  $M_1$  plants.

- a. Dense flower-setting.
- b. Dense and homogeneous flower-setting.
- c. Lax flower-setting.
- d. Monogermity (left).

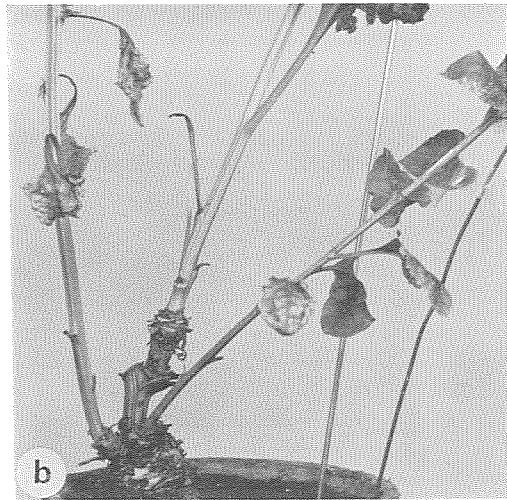
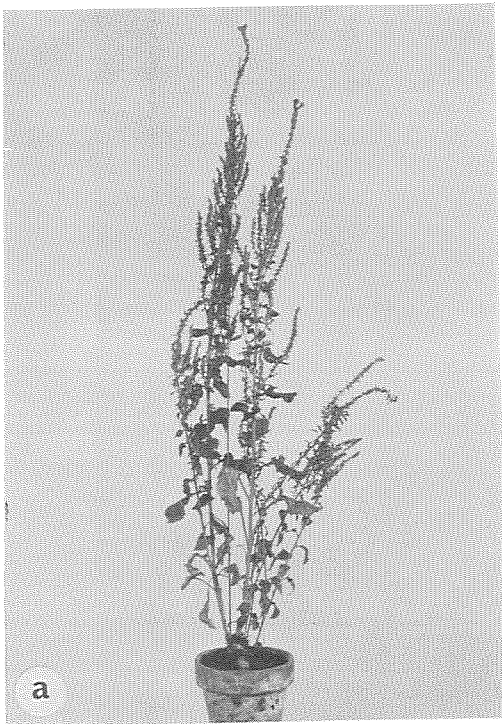


PLATE V

Grafting between male sterile and normal plants.

- a. A grafted plant; two shoots from a scion and a stock are flowering.
- b. Grafted part forming callus.
- c. Cleft-grafting between a diploid (stock) and a semi-sterile tetraploid (scion).

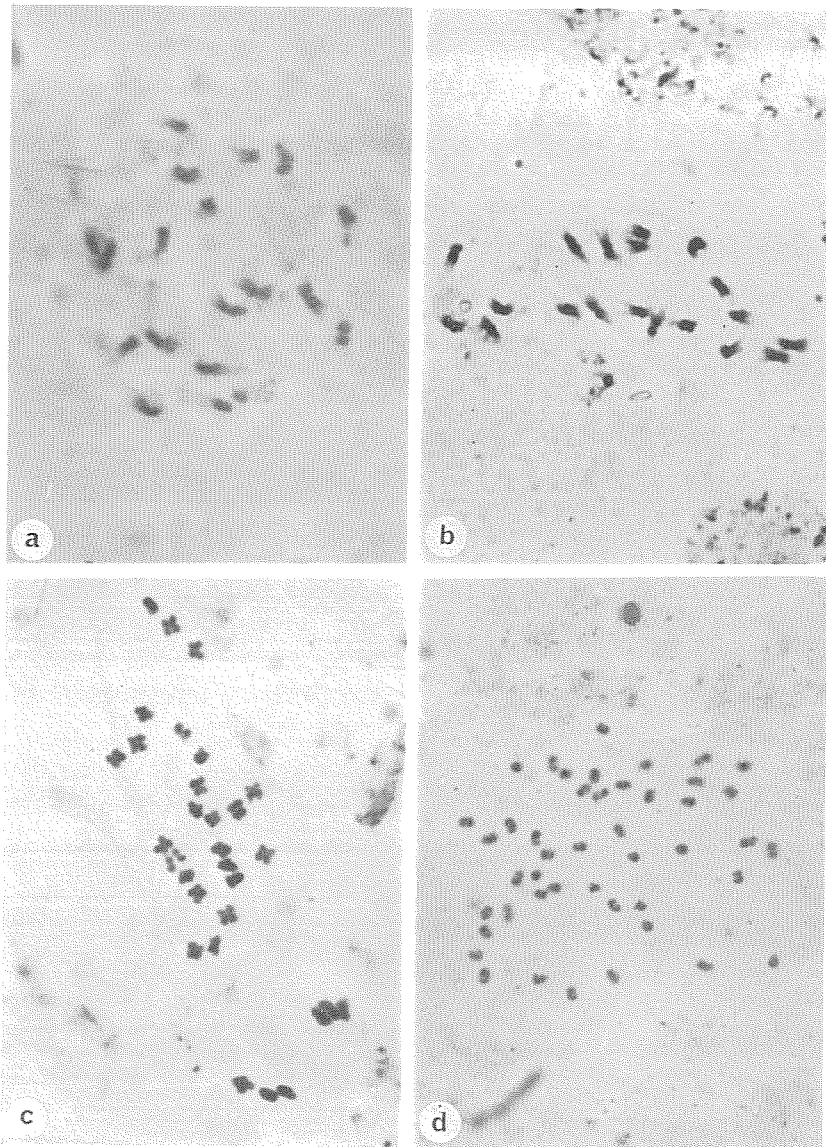
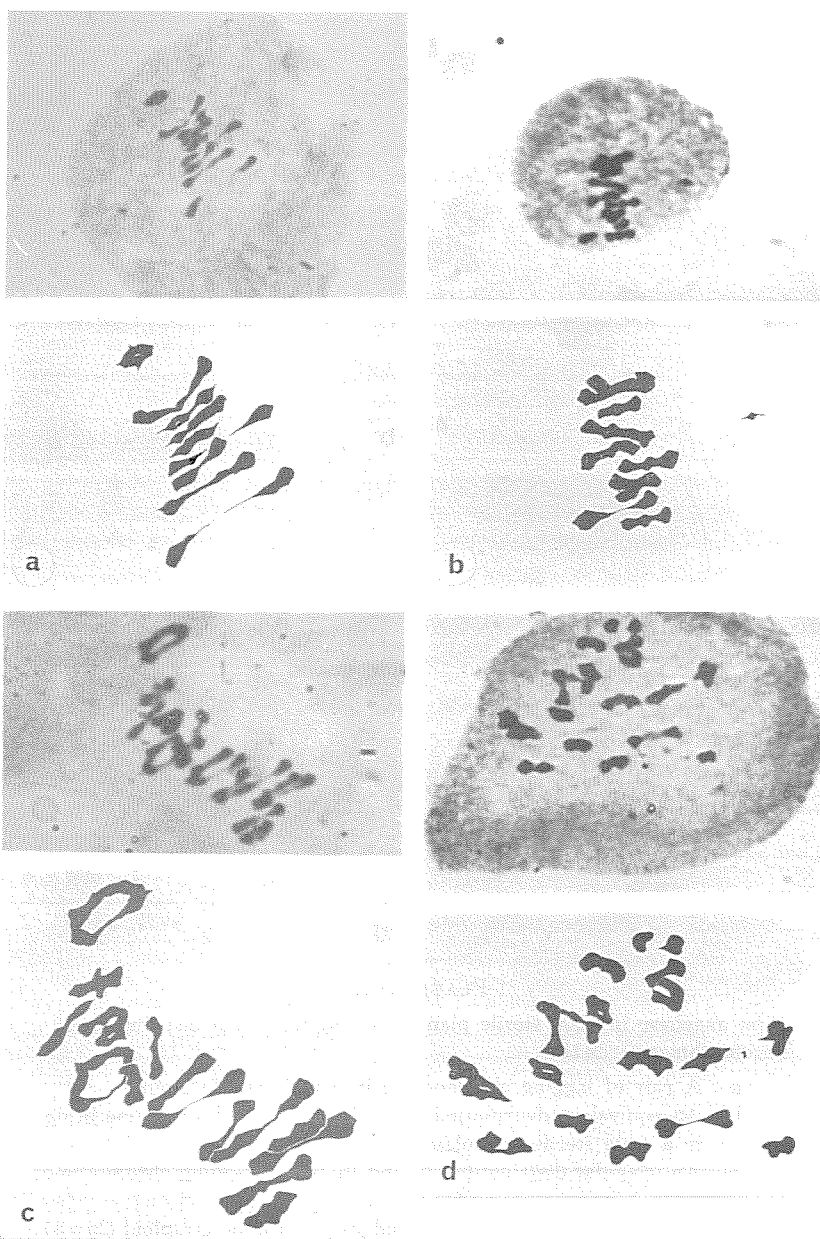


PLATE VI

Somatic chromosomes of aneuploids and a polyploid found in male sterile strains.  $\times 1300$ .

- a. Trisomic plant ( $2n=19$ ).
- b. Trisomic plant with one fragmentary chromosome ( $2n=19+1f$ ).
- c. Aneuploid ( $2n=30$ ).
- d. Pentaploid ( $2n=45$ ).



## PLATE VII

First metaphase in diploid and tetraploid male sterile plants.  $\times 1700$   
(microphotograph).

- a. 9 II in a male sterile diploid. microphotograph (upper)  
and drawing by camera lucida (lower).
- b. 6II+2 III+1f in a male sterile diploid. ditto.
- c. 3 IV+12 II in a male sterile tetraploid. ditto.
- d. 2 IV+1 III+11 II+1 I in a male sterile hypotetraploid  
( $2n=34$ ). ditto.

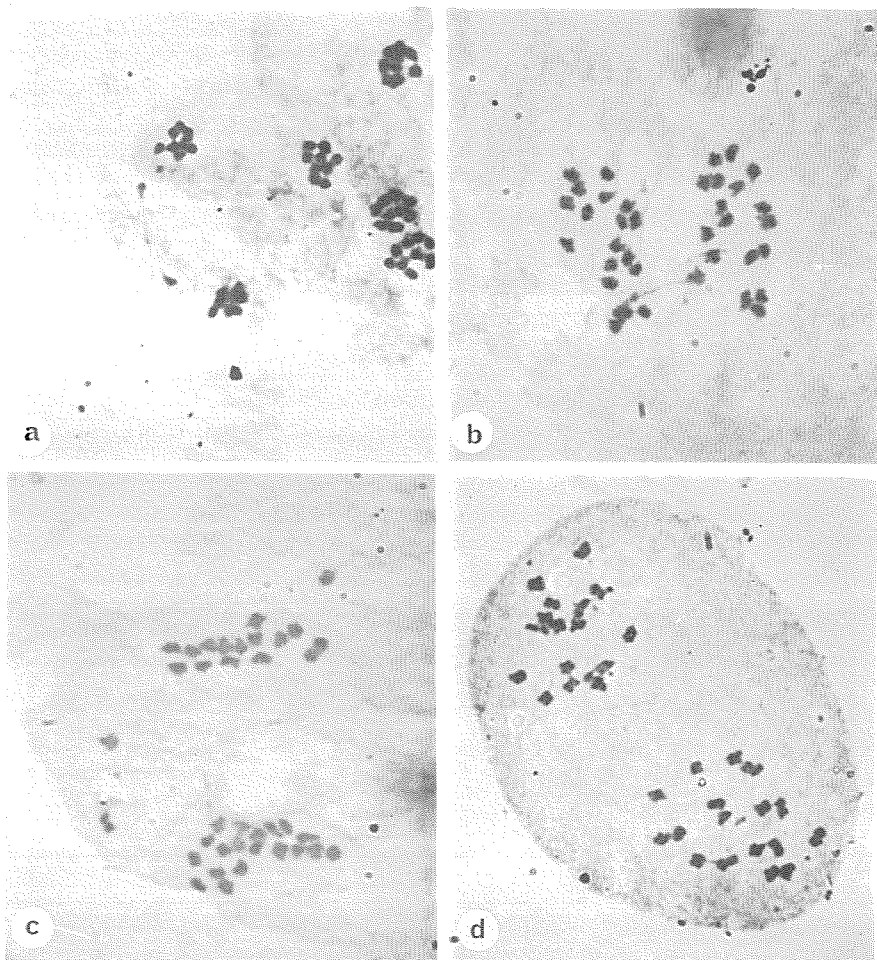


PLATE VIII

First anaphase in male sterile plants showing abnormal distribution of chromosomes.  $\times 1700$ .

- a. A pair of lagging chromosome in a male sterile diploid.
- b. An equivalent distribution (18 and 18) with a chromosome bridge in a male sterile tetraploid.
- c. An irregular distribution (19 and 16) with 2 lagging chromosomes in an hypertetraploid ( $2n=37$ ).
- d. An irregular distribution (18 and 16) in an hypotetraploid ( $2n=34$ ).

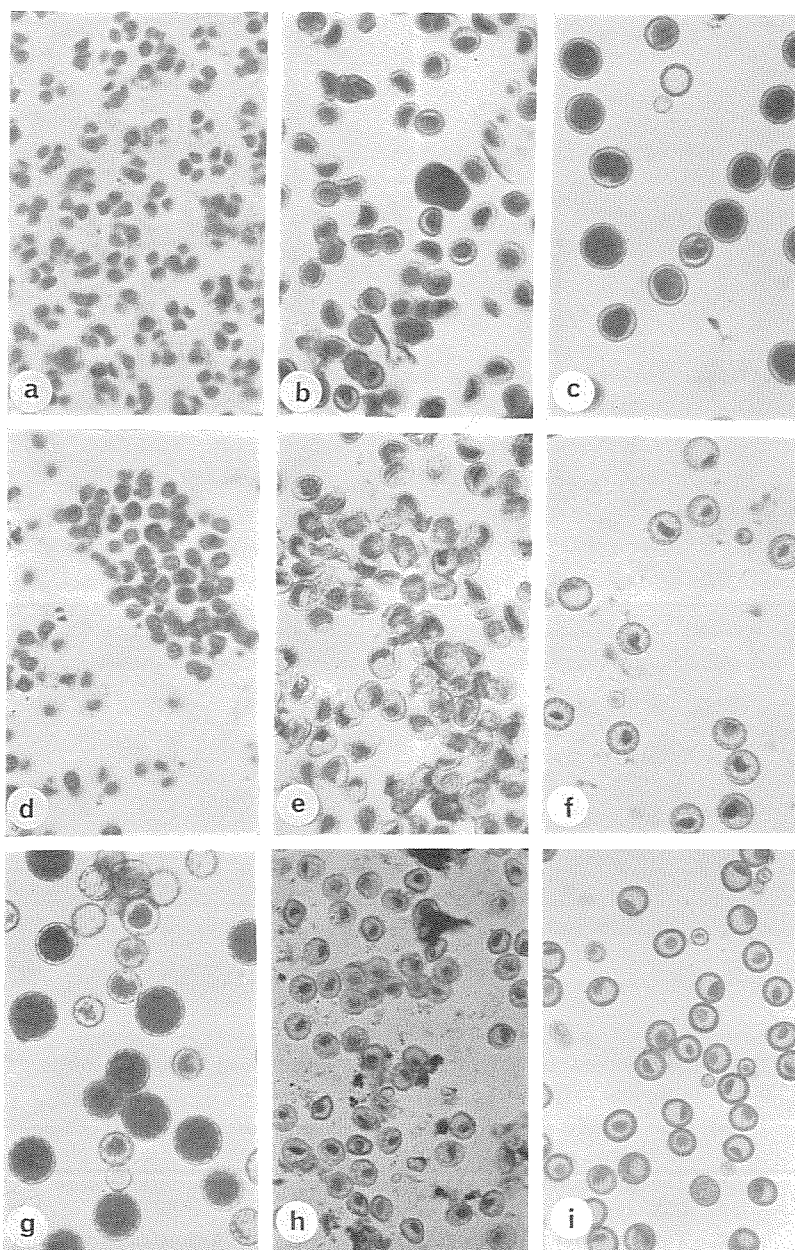


PLATE IX

Development of microspores in normal and male sterile plants in tetraploid cytoplasmic-genetic male sterility and in genetic male sterility.

- a. Tetrad stage. ×250.
- b. Microspores at the stage of second constriction in N type.
- c. Deposition of food materials in young pollen grains in N type.
- d. Microspores liberated from the quartets in young anthers of C. S. type.
- e. Degenerating microspores in young anthers of C. S. type.
- f. Microspores formed exine in young anthers of S. S. b type.
- g. Fertile and sterile pollen grains at the anthesis of S. S. a type.
- h. Abnormal microspores in young anthers of MS-I type (equivalent with C. S. type) in diploid genetic male sterility.
- i. Abnormal microspores in young anthers of MS-I type in tetraploid genetic male sterility.

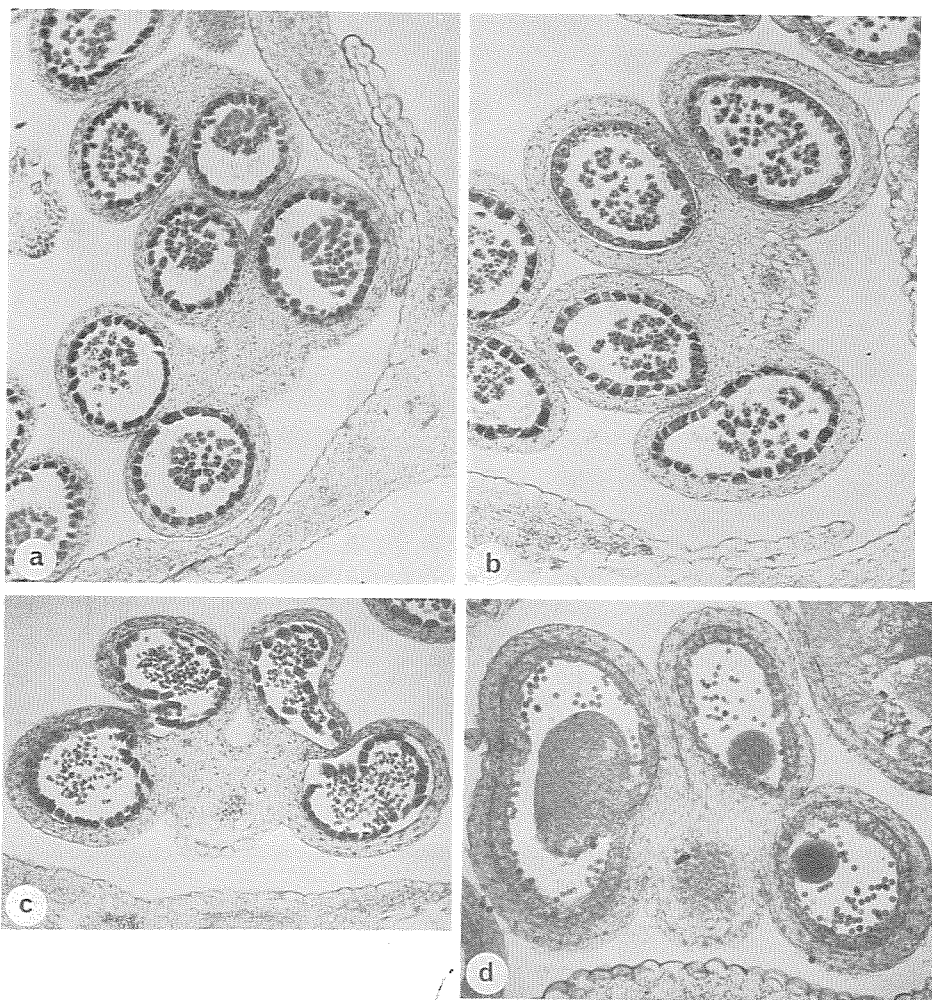
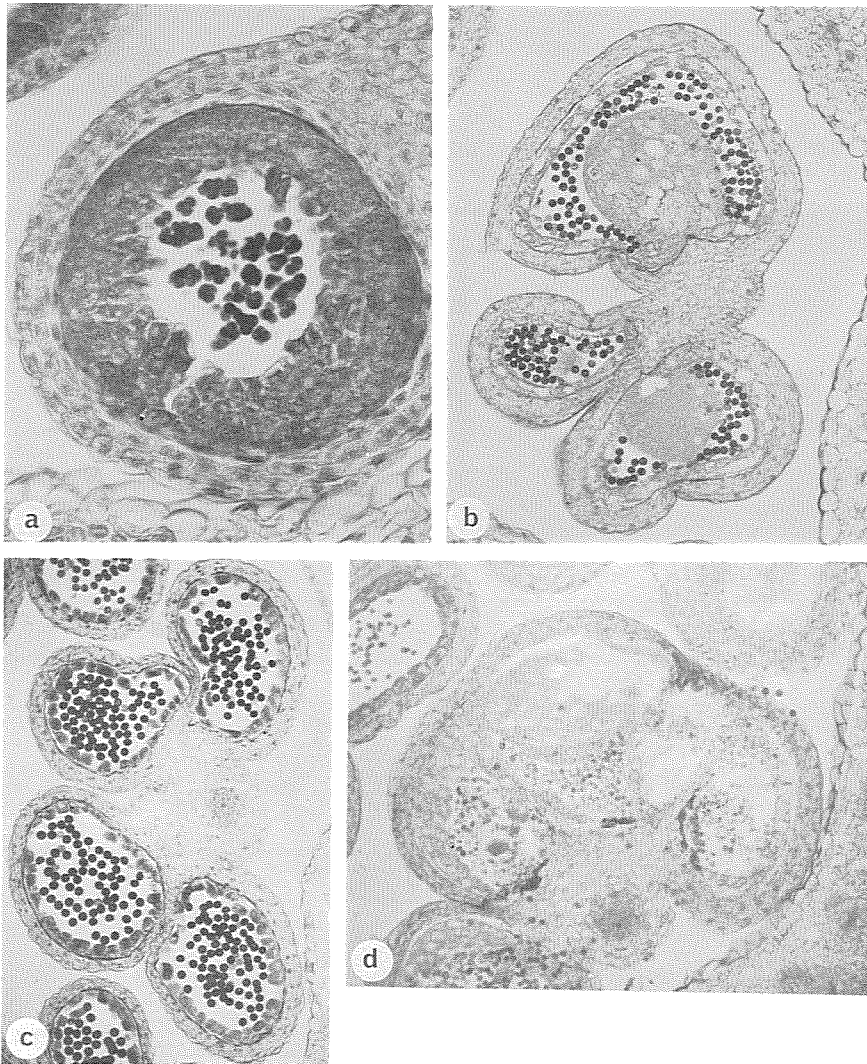


PLATE X

Transverse sections of young anthers of normal and complete sterile plants in diploid cytoplasmic-genetic male sterility.

- a. The densely stained tapetum and microspores in the tetrad stage of N type.  $\times 100$ .
- b. The formation of tapetal periplasmodium in the tetrad stage of C. S. type.  $\times 100$ .
- c. Tapetum and microspores at a stage of second constriction in the N type.  $\times 100$ .
- d. Tapetum showing pseudopodium-like incursions at the stage of exine-formation of microspores.  $\times 100$ .



## PLATE XI

Transverse sections of young anthers of normal and complete sterile plants in diploid cytoplasmic-genetic male sterility.

- a. Tapetal plasmodium enclosing the quartets.  $\times 200$ .
- b. Maximum development of tapetal periplasmodium showing multi-nuclei and large vacuoles in the C. S. type.  $\times 100$ .
- c. Dark strained young pollens and degenerating tapetum in the N type.  $\times 100$ .
- d. Degeneration of microspores and tapetal plasmodium in the C. S. type.  $\times 100$ .

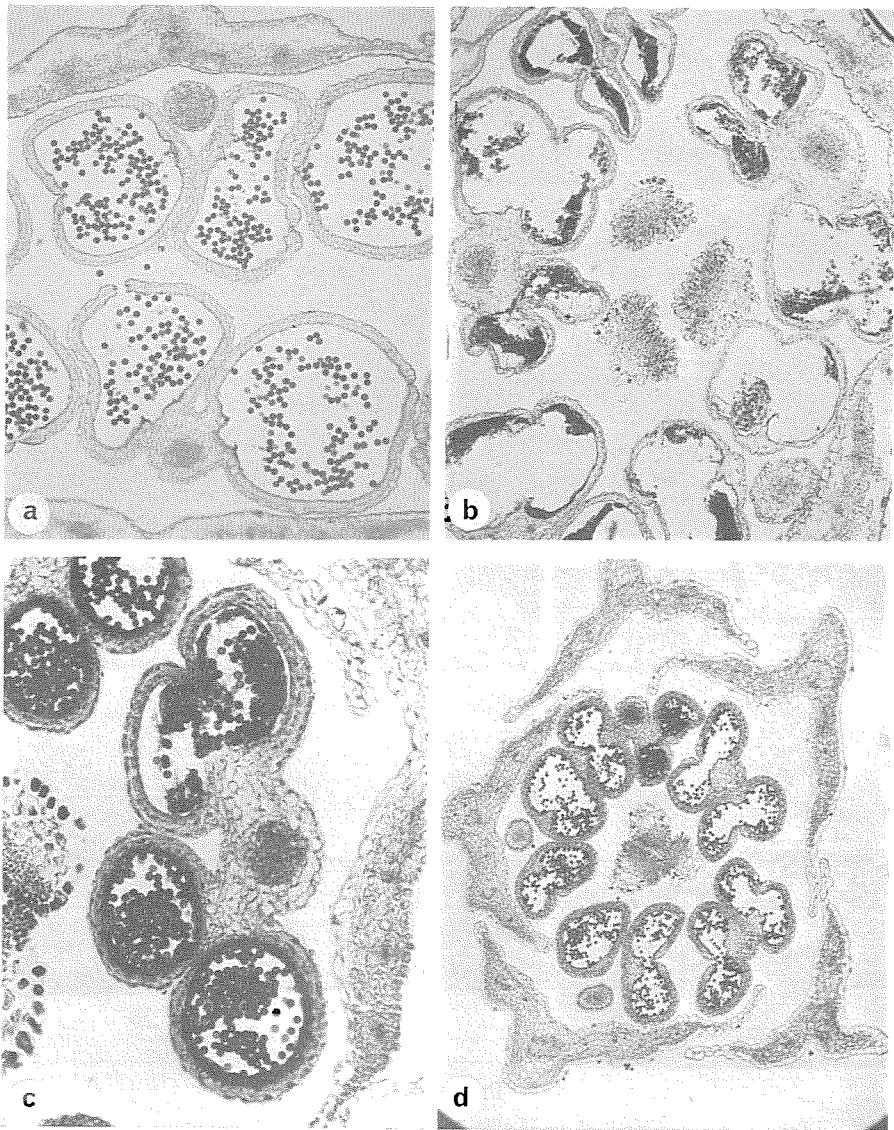


PLATE XII

Transverse sections of anthers of normal, semi- and complete sterile plants in diploid cytoplasmic-genetic male sterility.

- a. Mature pollen grains and dehiscence of anthers in the N type.  $\times 50$ .
- b. Collapsed microsporangium containing shrunken microspores and a strand of blackened matter, in the C. S. type.  $\times 50$ .
- c. Persistent tapetum producing a dark stained band lining the anther cavity in the S. S. b type.  $\times 100$ .
- d. Flower of S. S. a type at anthesis showing a lack of uniformity in anther development within a single flower.  $\times 50$ .

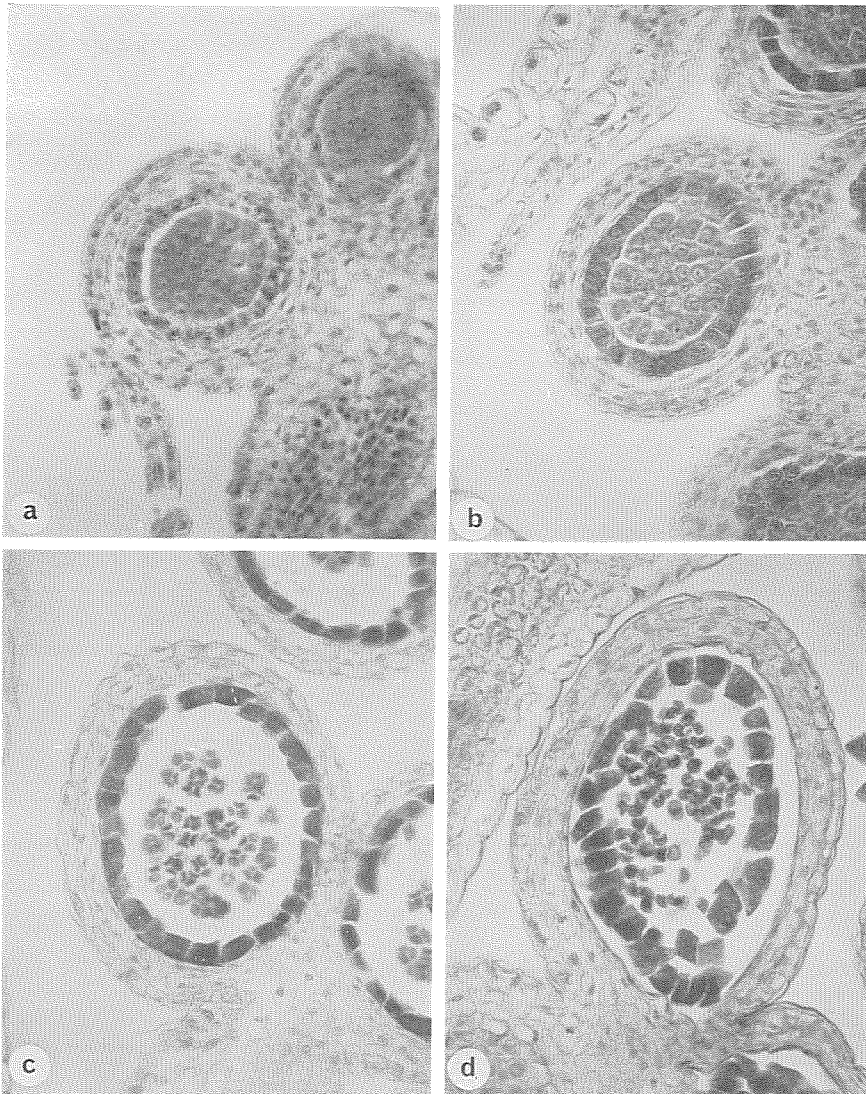


PLATE XIII

Transverse sections of anthers in the N type of tetraploid cytoplasmic-genetic male sterility.  $\times 200$ .

- a. Four wall layers surrounding the sporogenous cells.
- b. Binucleate condition of tapetal cells during the prophase of microspore mother cells.
- c. Dark stained tapetal cells regularly adhering to the parietal layers at the tetrad stage.
- d. Tapetum separated from the anther wall at the young microspore stage.

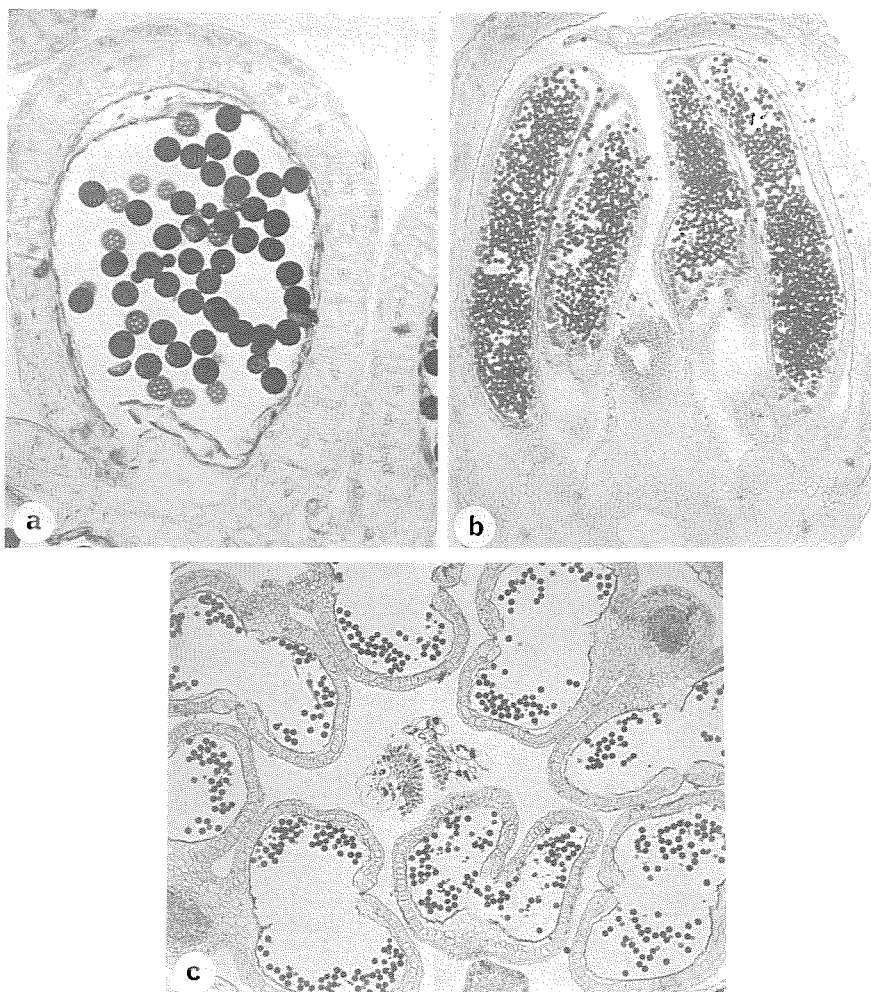


PLATE XIV

Transverse and longitudinal sections of anthers in the N type of tetraploid cytoplasmic-genetic male sterility.

- a. Dark staining young pollens, remains of degenerating tapetum and characteristic banded appearance of endothecium.  $\times 200$ .
- b. Longitudinal section of the anther enclosing healthy young pollens.  $\times 50$ .
- c. Anthers just prior to anthesis showing mature pollens, lip cells connected by thin threads of cells and conspicuous striation of the endothecium.  $\times 50$ .

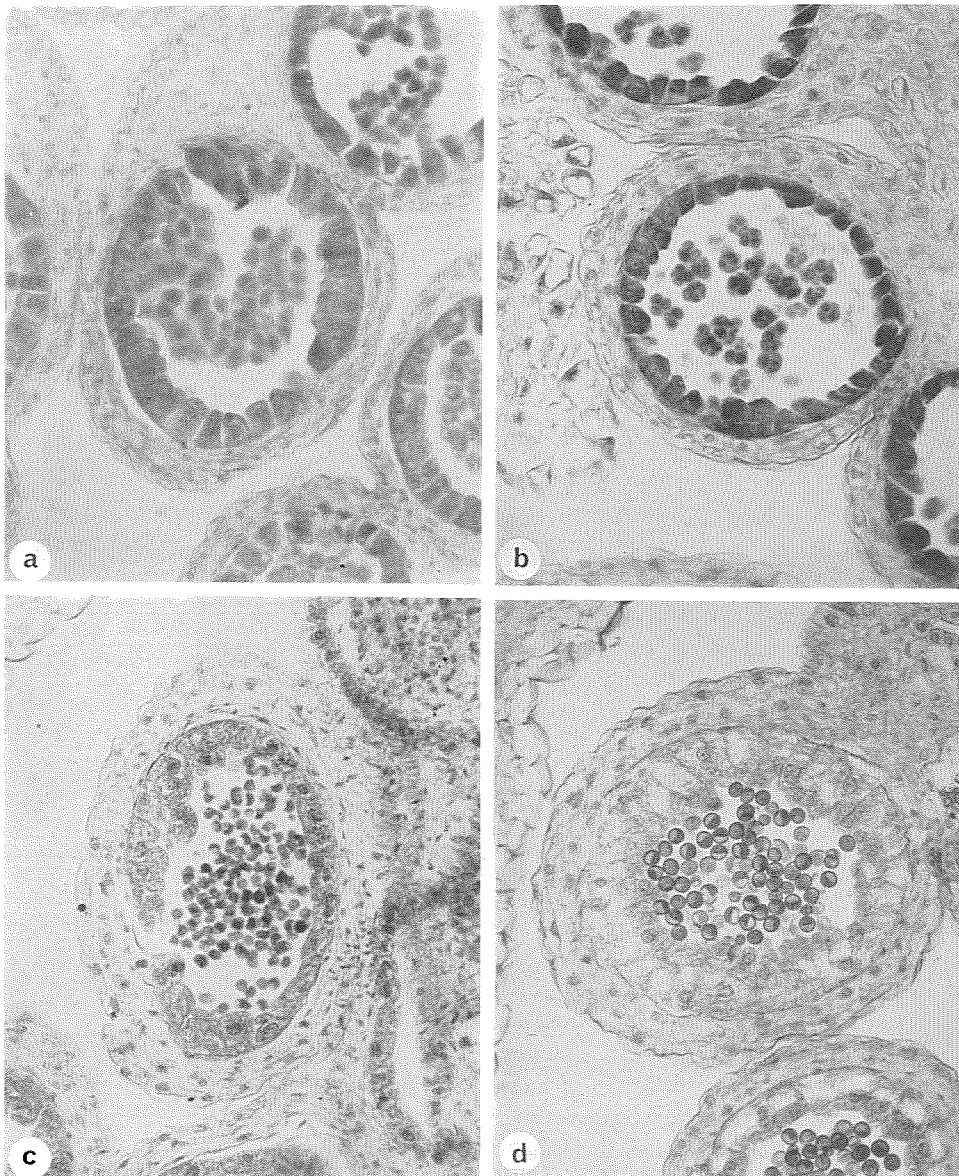


PLATE XV

Transverse sections of anthers in the C. S. type of tetraploid cytoplasmic-genetic male sterility.  $\times 200$ .

- a. Tapetal cells transforming into irregular shapes in meiosis.
- b. Tetrad stage; tapetum indicates a sign of plasmodium formation.
- c. Formation of tapetal plasmodium at the stage of liberation of microspores from the quartets.
- d. Tapetal plasmodium enclosing young microspores.

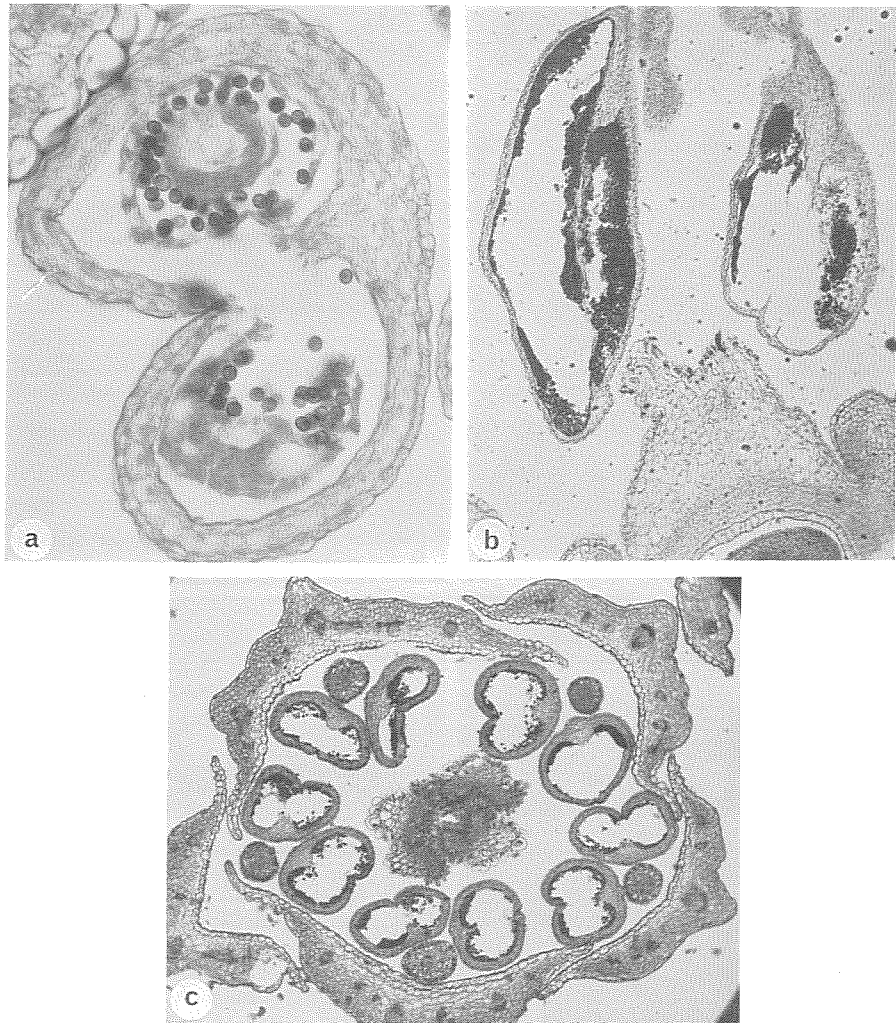
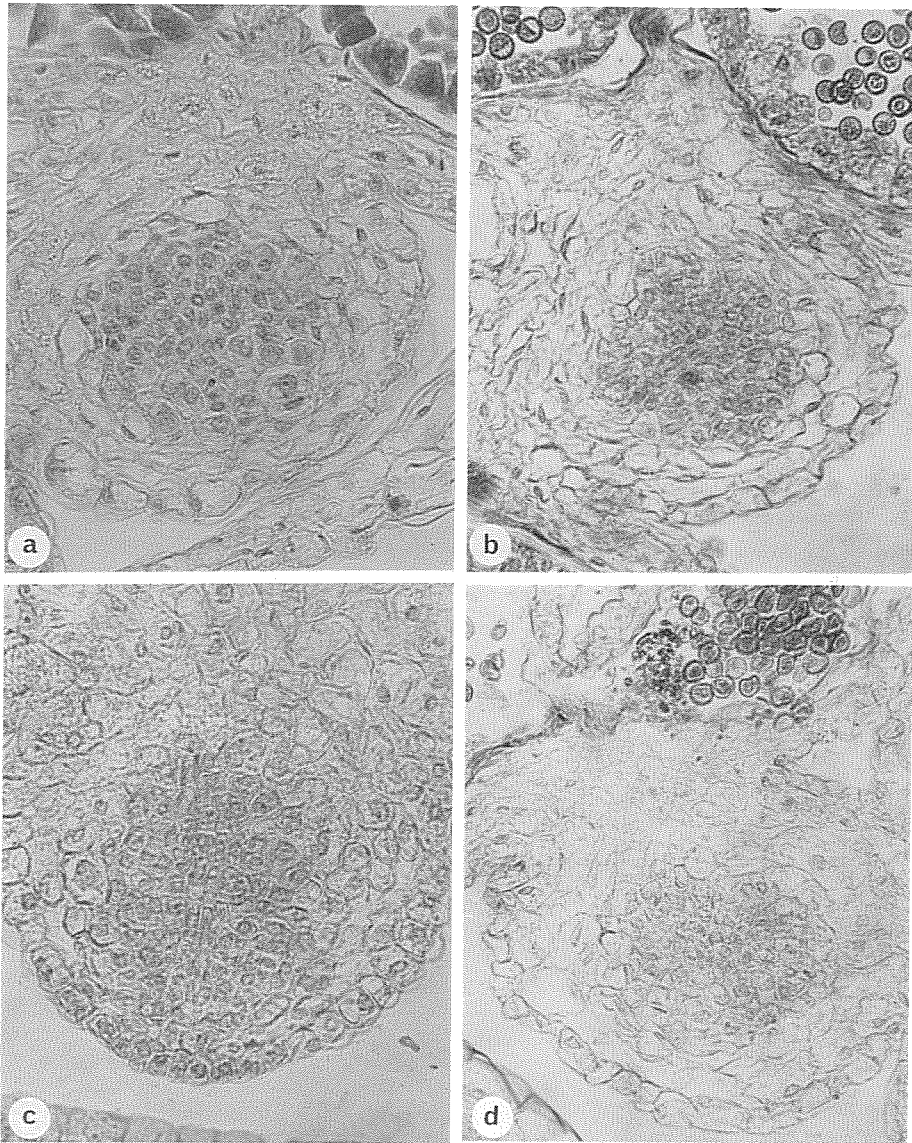


PLATE XVI

Transverse and longitudinal sections of anthers in the C. S. type of tetraploid cytoplasmic-genetic male sterility.

- a. Degeneration of tapetal plasmodium and breakdown of the partition between two locules.  $\times 200$ .
- b. Longitudinal section of anthers containing a strand of blackened matter and degenerated microspores.  $\times 50$ .
- c. Transverse section of a flower prior to anthesis showing locules containing black strands of degenerated microspores.  $\times 50$ .



## PLATE XVII

Transverse sections of the connectives in N and C. S. types of tetraploid cytoplasmic-genetic male sterility.  $\times 350$ .

- a. The connective at the young microspore stage subsequent to the tetrad in the N type.
- b. The connective at the young microspore stage subsequent to the tetrad in the C. S. type.
- c. The differentiation of vascular bundles in the connective at a later stage of microsporogenesis in the N type.
- d. The degeneration of the parenchyma tissue and poor development of vascular bundles following the abnormality of tapetum and microspores in the C. S. type.

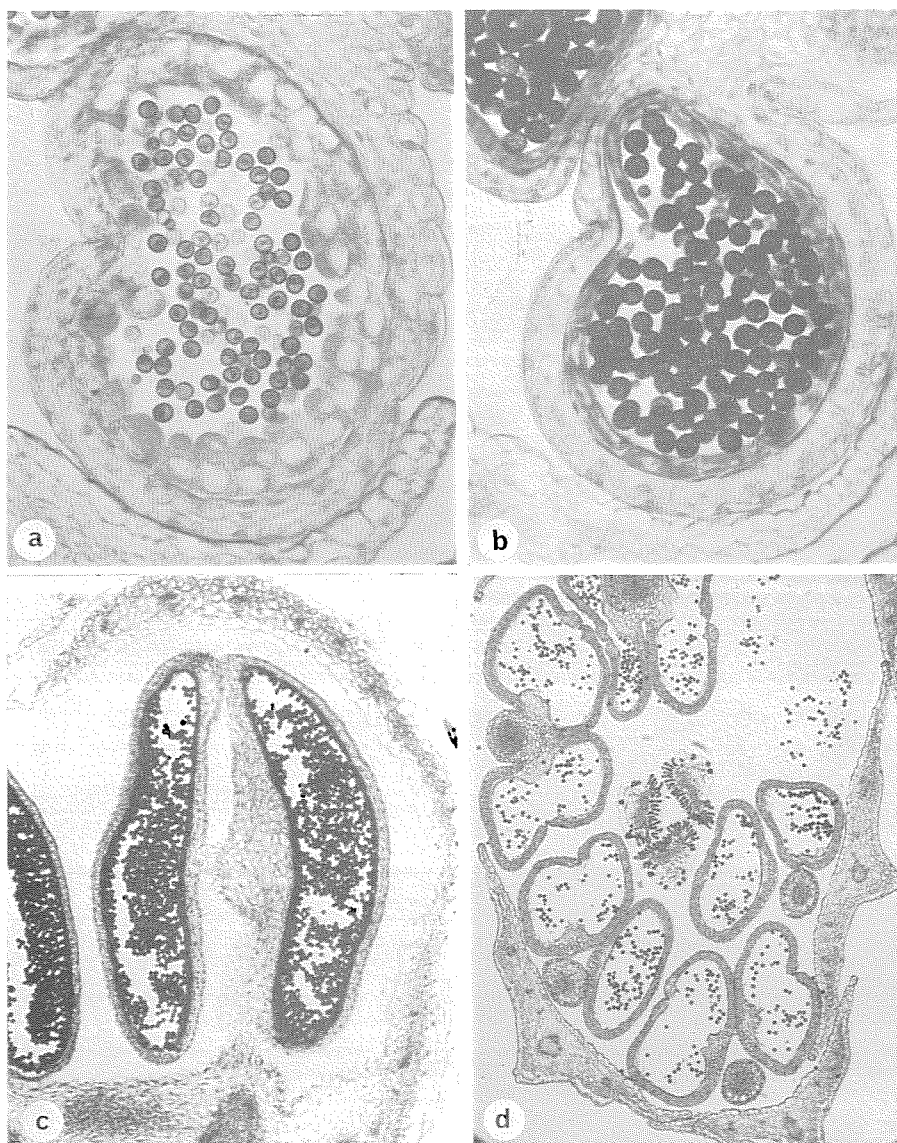
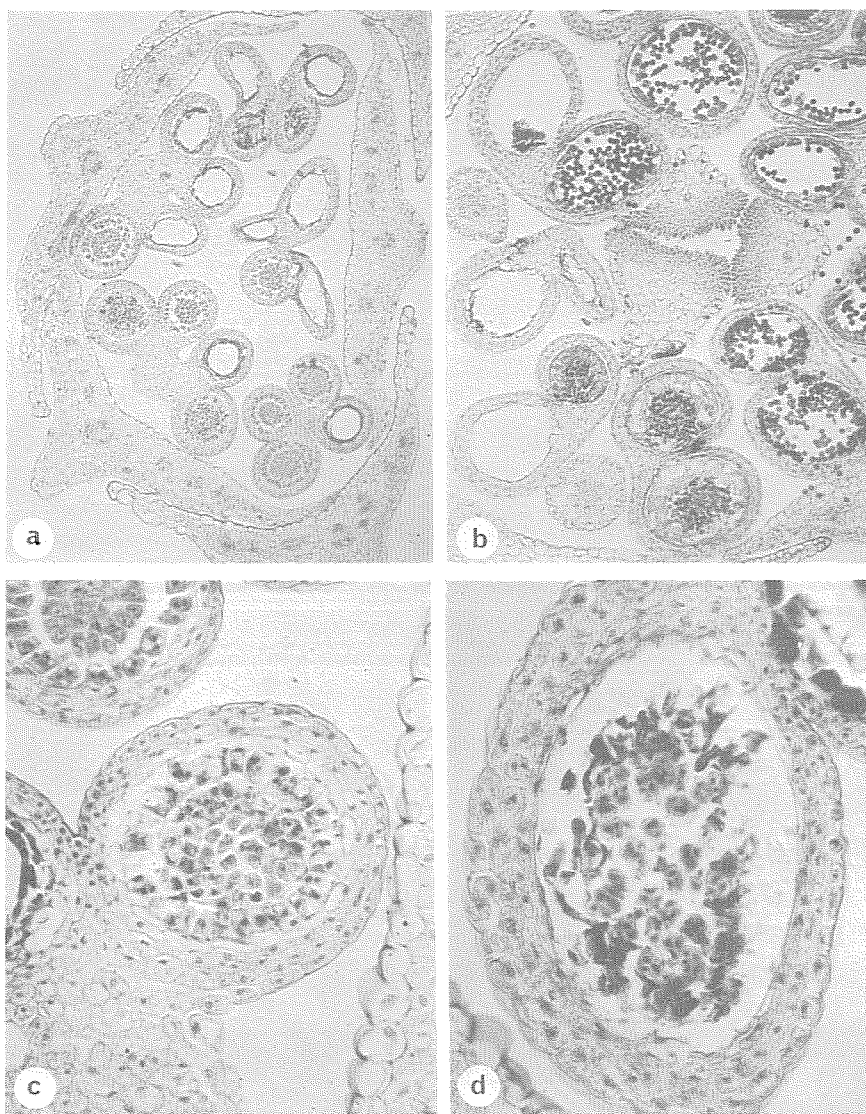


PLATE XVIII

Transverse sections of anthers in the S.S. b type of tetraploid cytoplasmic-genetic male sterility.

- a. Enlargement of tapetum at the stage of the exine-formation of microspores.  $\times 200$ .
- b. Persisting tapetum and abortive pollen grains at a later stage of microsporogenesis.  $\times 200$ .
- c. Longitudinal section of anthers showing persistent tapetum.  $\times 50$ .
- d. Transverse section of a flower prior to anthesis showing abortive pollens, a lack of stomium and a feeble development of endothecium.  $\times 50$ .



## PLATE XIX

Transverse sections of anthers and flowers in a plant of S.S. a type of tetraploid cytoplasmic-genetic male sterility.

- a. The lack of uniformity in the anther development showing various abnormalities of microsporangia.  $\times 50$ .
- b. The mixture of nearly normal, tapetal abnormality and empty microsporangia within a flower.  $\times 50$ .
- c. Degeneration of tapetal cells and microspore mother cells prior to meiosis.  $\times 200$ .
- d. Degeneration of tapetal plasmodium enclosing the quartets.  $\times 200$ .

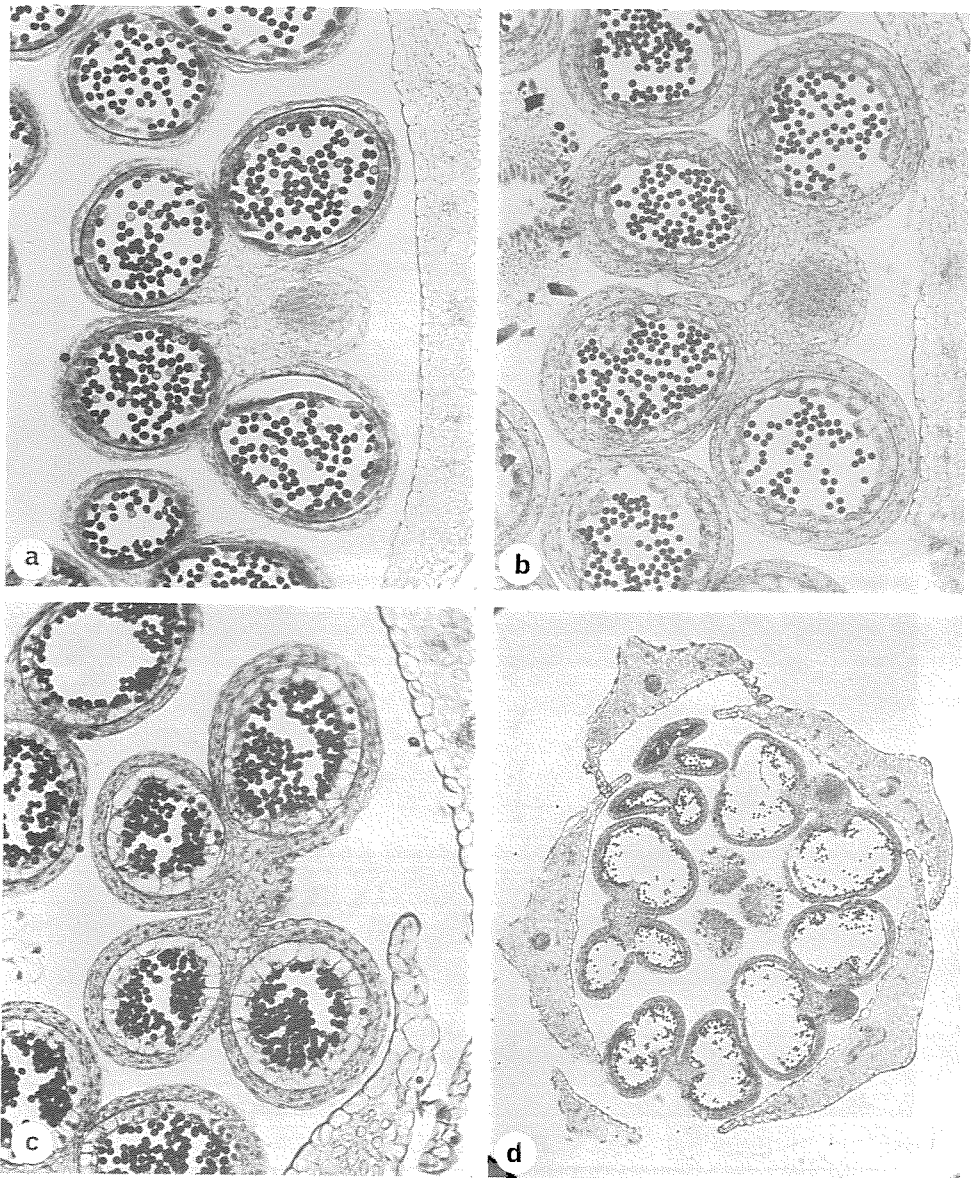


PLATE XX

Transverse sections of anthers in N and MS-I (equivalent with C. S. type) types of diploid genetic male sterility.

- a. Densely stained microspores and degenerating tapetum in the N type.  $\times 100$ .
- b. Hypertrophied tapetum and microspores losing their contents in the MS-I type.  $\times 100$ .
- c. Highly vacuolated tapetal cells and degeneration of microspores in the MS-I type.  $\times 100$ .
- d. Collapsed microsporangium containing shrunk microspores in the MS-I type prior to anthesis.  $\times 50$ .

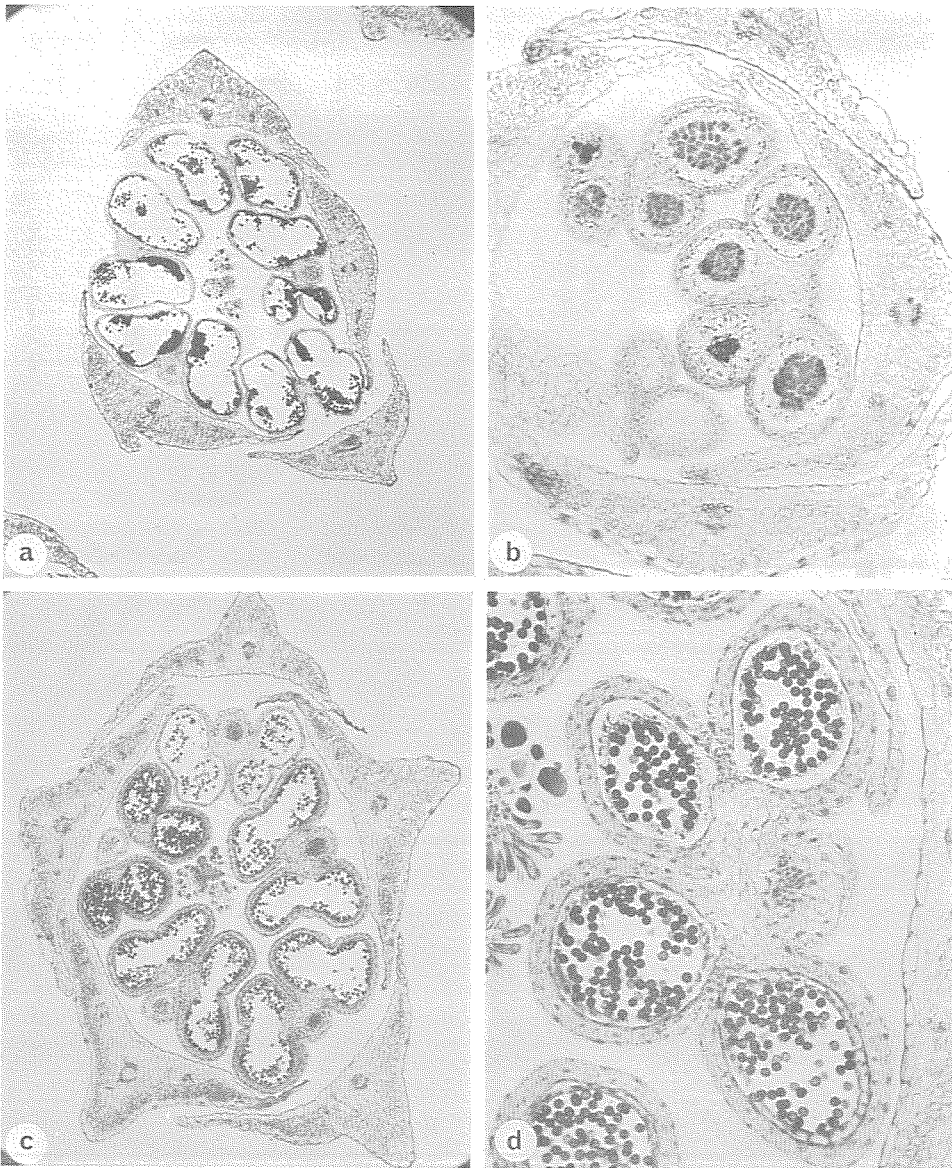


PLATE XXI

Transverse sections of anthers in MS-I, II and III types of diploid and tetraploid genetic male sterility.

- a. Degenerated microspores, a thin line of endothecium and under-developed calyces in a small flower of the MS-I type in diploids.  $\times 50$ .
- b. Degeneration of tapetal cells enclosing the quartets in the MS-I type of diploids.  $\times 50$ .
- c. A lack of uniformity in abnormality of anther development within a single flower of the MS-II type in diploids.  $\times 50$ .
- d. Adherence of vacuolated tapetum and its degeneration in the MS-III type of tetraploids.  $\times 100$ .

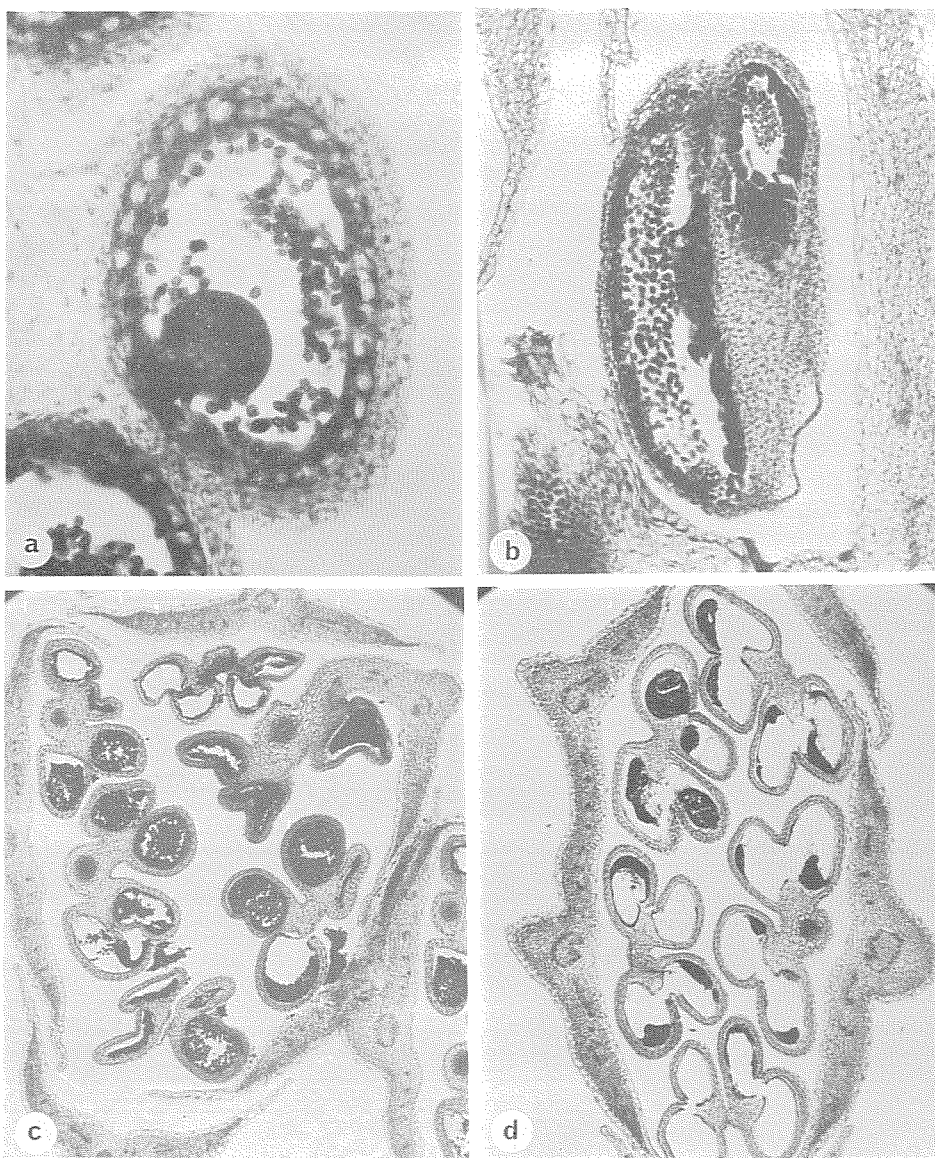


PLATE XXII

Transverse and longitudinal sections of anthers in the male sterile plants induced by gamma irradiation.

- a. Tapetum showing a pseudopodium-like incursion.  $\times 200$ .
- b. Longitudinal section of anthers showing abnormality of tapetum.  $\times 50$ .
- c. Degeneration of tapetum and microspores. A sepal is united with an anther lobe.  $\times 50$ .
- d. Flower prior to anthesis showing locules containing black strands of degenerated microspores. Two sepals join each other forming four sepals.  $\times 50$ .

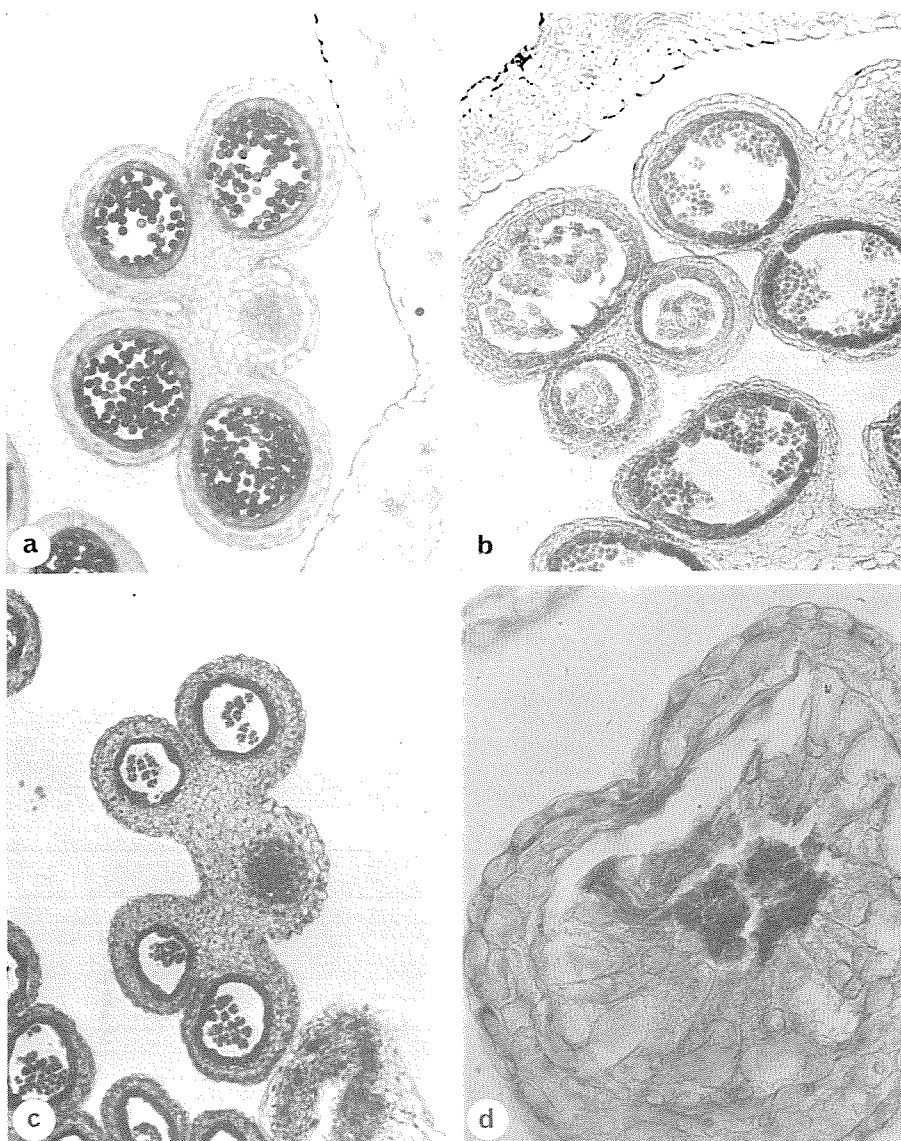


PLATE XXIII

Transverse sections of anthers in the male sterility affected by the treatment of low temperature and the gametocide.

- a. Persisting tapetum at a later stage of microsporogenesis in the S. S. a type produced from a normal plant of H-19 (*N* cytoplasm strain) after the treatment of low temperature.  $\times 100$ .
- b. Mixture of different division stages of meiosis within a single flower which was observed in the C. S. type produced from the S. S. b type in H-19 MS after the treatment of low temperature.  $\times 130$ .
- c. Thickened anther walls and small microsporangia containing a smaller number of the quartets, which was observed in a plant of 4M-50 (tetraploid *S* cytoplasm strain) after the treatment of low temperature  $\times 100$ .
- d. Degeneration of hypertrophied tapetum and crushed microspores in the male sterility induced by the gametocide.  $\times 230$ .