# VARIABILITY OF CHARACTER EXPRESSION IN THE CYTOPLASMIC MALE STERILITY OF SUGAR BEETS[1)]

By

Toshiro KINOSHITA and Man-emon TAKAHASHI

(Plant Breeding Institute, Faculty of Agriculture,
Hokkaido University. Sapporo, Japan)

Received October 30, 1971

## Introduction

Cytoplasmic male sterility is useful in producing single- or double-cross hybrids and triploid varieties in sugar beets. It is important to know the mechanism by which heritable and environmental factors influence the character expression of the cytoplasmic male sterility for the utilization of this character in the breeding works.

In the present paper, variations of pollen sterility during the flowering period and within an individual plant were examined using diploid and tetraploid strains. The change of phenotypic expression of the male sterility by the influence of low temperature and starvation culture was also examined. In addition, cytological observations were made on the anther development in the male sterility affected by the treatment of low temperature.

## Materials and Methods

The strains which were used in the present study, are listed in Table 1. The plants were cultured either in the experimental field under natural conditions or in the greenhouse. A low temperature cabinet (3° to 5°C) with illumination was used for the experiments of the low temperature treatment.

**Table 1.** The strains used in the study

| Strain | Ploidy | Type of cytoplasm | Description |
|---|---|---|---|
| H-19 | 2X | *N* | A selection from monogerm strain, 'M-10' |
| H-19 MS | 2X | *S* | Isogenic line of H-19 with cytoplasmic male sterility and monogermity |
| H-19 (4X) | 4X | *N* | Tetraploids induced by colchicine treatment from 'H-19' |
| H-19 MS (4X) | 4X | *S* | Tetraploids induced by colchicine treatment from 'H-19 MS' |
| H-4002 | 4X | *N* | Tetraploids induced by colchicine treatment from 'H-2002' |
| 4M-50 | 4X | *S* | Tetraploids induced by colchicine treatment from cytoplasmic male sterile strain, M-50 |

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

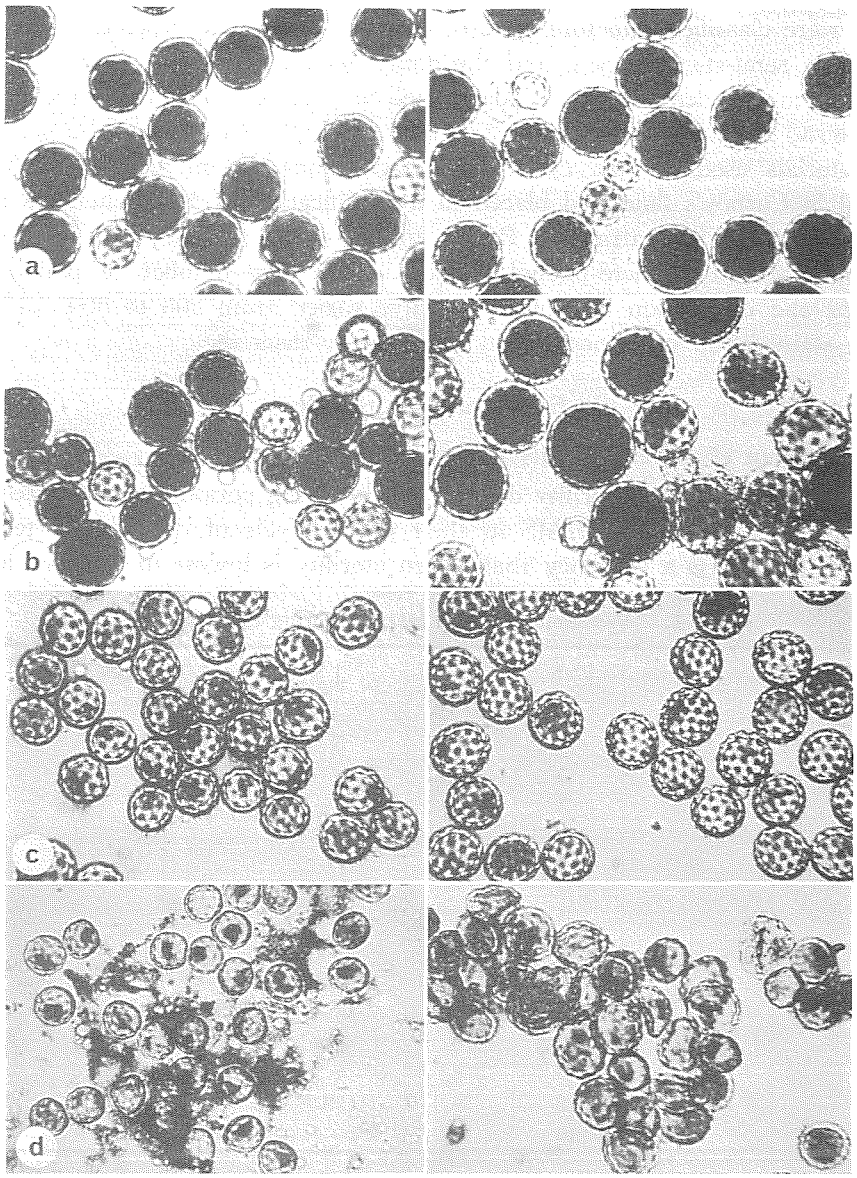


**Fig. 1.** Pollen grains of norml anad male sterile plants at diploid and tetraploid levels. ×370.

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).

Types of anthers and pollen grains from the progenies of male sterile 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 as shown in Fig. 1, according to the demarcation-standard used in the previous report (NAGAO and KINOSHITA, 1962).

Materials used for the cytological observations on microsporogenesis were prefixed in Carnoy's fluid and placed in a modification of Karpechenko's haematoxylin followed by safranin. Pollen grains were stained with cotton blue pigment dissolved in lacto-phenol solution. The total number of pollen grains used for the calculation of pollen sterility, varied from 200 to 500. The two classes, stained and unstained pollen grains were discrete.

## Experimental Results

### 1. Variation of pollen sterility during flowering periods

Variation of pollen sterility during the flowering period was examined using the strains, H–19 and H–19 MS in their ploidy levels of diploid and tetraploid (Table 2). There is a tendency that pollen sterility is lowest in full bloom while

**Table 2.** 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 (4X) | 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* (4X) | 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.

increases as 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 flower appeared from October to November. As shown in Table 3, the difference of pollen sterility is 14.3% at its maximum in normal cytoplasm strain H-19, while a higher variability was observed in the plants of N and S.S.a type at diploid and teraploid levels.

**Table 3.** 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 |

**2. Intra-individual variation of pollen sterility**

Another experiment was conducted to examine the variability of pollen sterility whthin a single plant. 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 from the lowest branch of the plant. In every flower bud, three anthers 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 4, 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 plants of S.S.a type 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 was examined between every different branch, between every different flower, between every different anther and between the two anther lobes. This is shown in Table 5. Considerable differences of pollen sterility were observed in plants of S.S.a type and a plant of S.S.b type

**Table 4.** Variation of pollen sterility among different branches of a single plant

| Ploidy | Strain | Plant No. | Type of cyto-plasm | Pheno-type of M.S. | Pollen sterility at five branches (%)* | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | 1 | 2 | 3 | 4 | 5 | Mean |
| 2X | H-19 | 1 | *N* | N | 7.7 | 5.1 | 5.0 | 1.2 | 2.3 | 4.3 |
| | | 2 | " | " | 7.7 | 13.1 | 17.4 | 1.1 | 1.4 | 8.1 |
| | | 3 | " | " | 14.2 | 4.1 | 4.5 | 3.6 | 17.3 | 8.7 |
| 2X | H-19 MS | 1 | *S* | N | 4.8 | 4.5 | 2.2 | 3.0 | 2.9 | 3.5 |
| | | 2 | " | " | 9.1 | 3.8 | 4.0 | 1.8 | 1.9 | 4.1 |
| | | 3 | " | " | 14.0 | 11.1 | 2.1 | 7.3 | 16.5 | 10.2 |
| | | 4 | " | S.S.a | 22.2 | 22.0 | 29.2 | 59.4 | 84.8 | 43.5 |
| | | 5 | " | " | 61.4 | 41.4 | 44.7 | 61.9 | 47.5 | 51.4 |
| | | 6 | " | S.S.b | 73.4 | 67.6 | 91.0 | 51.6 | 89.3 | 74.6 |
| | | 7 | " | " | 100 | 100 | 100 | 100 | 100 | 100 |
| | | 8 | " | " | 100 | 100 | 100 | 100 | 100 | 100 |
| | | 9 | " | C.S. | 100 | 100 | 100 | 100 | 100 | 100 |
| | | 10 | " | " | 100 | 100 | 100 | 100 | 100 | 100 |
| 4X | H-19 (4X) | 1 | *N* | N | 29.1 | 12.8 | 8.0 | 9.7 | 8.9 | 13.7 |
| | | 2 | " | " | 17.0 | 7.9 | 15.8 | 7.1 | 57.1 | 21.0 |
| | | 3 | " | " | 31.3 | 17.7 | 31.6 | — | — | 26.9 |
| 4X | H-19 MS (4X) | 1 | *S* | S.S.a | 52.8 | 17.2 | 8.0 | 53.2 | 96.5 | 45.5 |
| | | 2 | " | S.S.b | 90.1 | 93.6 | 89.4 | 96.6 | 92.8 | 92.5 |
| | | 3 | " | " | 100 | 98.1 | 99.7 | 99.9 | — | 99.4 |

* Five branches were chosen at regular intervals from the lowest (1) to the highest (5).

**Table 5.** Average difference of pollen sterility between flowers, anthers and lobes of a single plant

| Ploidy | Strain | Plant No. | Type of cyto-plasm | Pheno-type of M.S. | Difference of pollen sterility (%) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Between branches | | Between flowers | | Between anthers | | Between lobes | |
| | | | | | mean[1] | range | mean[2] | range | mean[3] | range | mean[3] | 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 (4X) | 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 (4X) | 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

in diploids. In some anthers, pollen sterility differred up to 55% 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 indicate 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 the S.S.b and C.S. types are stable throughout different parts of a single plant. An intra-individual variation of pollen sterility at the blooming is representatively shown in Table 6. Small or large groups of lobes appear in partial fertility, while most of the lobes remain in the S.S.b type. In other cases, "islands of sterility" (DUVICK 1965) appear

**Table 6.** 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 (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 (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".

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 plants at the late blooming stage (Table 7). 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. 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 8). 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 7.** 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 (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 (Top) | a | (99) | (99) | (100) | (100) | (100) | (100) | 100.0 |
| | 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".

**Table 8.** Pollen sterility of yellow anther (dehiscent) and brown anther (non-dehiscent) in H-19

| Ploidy | Strain | Plant No. | Type of cytoplasm | Phenotype | Pollen sterility (%) | |
|---|---|---|---|---|---|---|
| | | | | | Yellow anthers | Brown anthers |
| 2X | H-19 | 1 | *N* | S.S.a | 69.6 (2-100)[2] | 69.1 (14-100) |
| | | 2 | *N* | S.S.a | 55.3 (3-100) | 69.2 (15- 99) |
| 4X | H-19 (4X) | 1 | *N* | S.S.a | 6.8 (1- 14) | 39.4 ( 3-100) |
| | | 2 | *N* | 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)

**Table 9.** Modification of pollen sterility after treatment by low temperature

a. Experiment in 1963

| Ploidy | Strain | Plant No. | Type of cytoplasm | Phenotype of M.S. | Pollen sterility (%) | | Change of phenotype |
|---|---|---|---|---|---|---|---|
| | | | | | Before treat. | After* treat. | |
| 2X | H-19 | 1 | *N* | N | 1.4 | 25.1 | N→N |
| | | 2 | | " | 1.9 | 23.2 | N→N |
| | | 3 | | " | 2.2 | 26.2 | N→N |
| | | 4 | | " | 2.3 | 24.3 | N→N |
| | | 5 | | " | 2.7 | 58.3 | N→S.S.a |
| | | 6 | | " | 3.3 | 59.5 | N→S.S.a |
| | | 7 | | " | 4.2 | 46.2 | N→S.S.a |
| | | 8 | | " | 7.8 | 26.2 | N→N |
| | | 9 | | " | 10.3 | 25.7 | N→N |
| 2X | H-19 MS | 1 | *S* | N | 3.8 | 85.3 | N→S.S.b |
| | | 2 | | " | 18.4 | 100 | do |
| | | 3 | | " | 26.7 | C.S. | N→C.S. |
| | | 4 | | S.S.b | 100 | C.S. | S.S.b→C.S. |
| | | 5 | | C.S. | C.S. | C.S. | C.S.→C.S. |
| | | 6 | | " | C.S. | C.S. | do |
| 4X | 4M-50 | 1 | *S* | N | 14.0 | 100 | N→S.S.b |
| | | 2 | | S.S.a | 33.0 | 91.6 | S.S.a→S.S.b |
| | | 3 | | " | 45.2 | 85.1 | do |
| | | 4 | | " | 51.3 | 100 | do |
| | | 5 | | " | 54.3 | 100 | do |
| | | 6 | | S.S.b | 73.9 | 100 | S.S.b→S.S.b |
| | | 7 | | " | 87.6 | 100 | do |
| | | 8 | | " | 100 | 100 | do |
| | | 9 | | " | 100 | 100 | do |
| | | 10 | | " | 100 | 100 | do |
| | | 11 | | " | 100 | 100 | do |
| | | 12 | | " | 100 | C.S. | S.S.b→C.S. |

* Highest pollen sterility after treatment by low temperature.

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 9 a and b, 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. As

b. Experiment in 1965

| Ploidy | Strain | Plant No. | Type of cytoplasm | Phenotype of M.S. | Pollen sterility (%) | | Change of phenotype |
|---|---|---|---|---|---|---|---|
| | | | | | Before treat. | After treat. | |
| 2X | H-19 | 1 | *N* | N | 1 | 10 | N→N |
| | | 2 | | " | 3 | 96 | N→S.S.b |
| 2X | H-19 MS | 1 | *S* | N | 1 | 23 | N→N |
| | | 2 | | " | 3 | 88 | N→S.S.b |
| | | 3 | | " | 5 | 96 | N→S.S.a |
| | | 4 | | " | 9 | 39 | N→S.S.a |
| | | 5 | | " | 10 | 94 | N→S.S.b |
| | | 6 | | " | 13 | 83 | do |
| | | 7 | | " | 16 | 100 | do |
| | | 8 | | " | 11 | C.S. | N→C.S. |
| | | 9 | *S* | S.S.a | 23 | 100 | S.S.a→S.S.b |
| | | 10 | | " | 33 | 61 | S.S.a→S.S.a |
| | | 11 | *S* | S.S.b | 76 | C.S. | S.S.b→C.S. |
| | | 12 | | " | 80 | C.S. | do |
| | | 13 | | " | 80 | 100 | S.S.b→S.S.b |
| | | 14 | | " | 81 | C.S. | S.S.b→C.S. |
| | | 15 | | " | 93 | 100 | S.S.b→S.S.b |
| | | 16 | | " | 99 | C.S. | S.S.b→C.S. |
| | | 17-27[1] | | " | 100 | 100 | S.S.b→S.S.b |
| | | 28-32[2] | | " | 100 | C.S. | S.S.b→C.S. |
| | | 33-36[3] | *S* | C.S. | C.S. | C.S. | C.S.→C.S. |

1) 11 plants 2) 5 plants 3) 4 plants

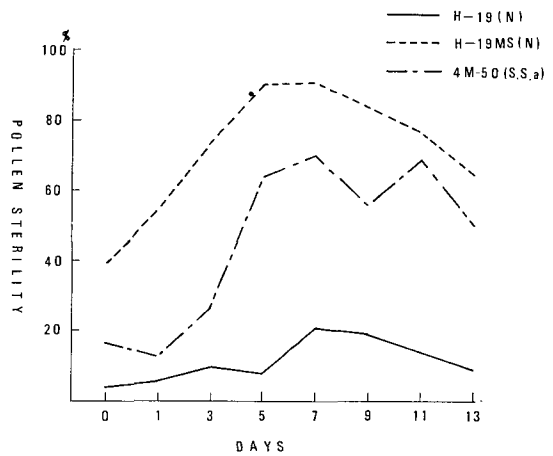


**Fig. 2.** Change of pollen sterility after the low temperature treatment.

**Table 10.** Modification of pollen sterility by deficiency of fertilizer

a. Experiment in 1967

| Ploidy | Strain | Plant No. | Fertilized soil | | Vermiculite* | |
|---|---|---|---|---|---|---|
| | | | Phenotype of M.S. | Pollen sterility (%) | Phenotype of M.S. | Pollen sterility (%) |
| 2X | H-19 | 1 | N | 4 | N | 9 |
| | | 2 | N | 3 | S.S.b | 95 |
| 2X | H-19 MS | 1 | N | 5 | N | 0 |
| | | 2 | S.S.b | 100 | C.S. | 100 |
| | | 3 | C.S. | C.S. | C.S. | C.S. |

* Fertilizer was not applied.

shown in Fig. 2, 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.

The effect of nutritional conditions was examined for the plants of H-19 and H-19 MS. 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 10 a and b).

### 4. Anther development in the male sterility affected by the treatment of low temperature

Pollen sterility and dehiscence of anthers were affected to some extent following low temperture treatment in most of the plants (Table 9 a and b). 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.

As shown in Table 9 a, 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 befor 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 (Fig. 3 a).

The plant No. 3 of H-19 MS (a 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

b. Experiment in 1968

| Ploidy | Strain | Plant No. | Type of cytoplasm | Fertilized soil | | Vermiculite | |
|---|---|---|---|---|---|---|---|
| | | | | Phenotype of M.S. | Pollen sterility (%) | Phenotype of M.S. | Pollen sterility (%) |
| 2X | H-19 | 1 | *N* | N | 2 | N | 1 |
| | | 2 | | " | 2 | " | 2 |
| | | 3 | | " | 2 | " | 8 |
| | | 4 | | " | 4 | " | 25 |
| | | 5 | | " | 18 | S.S.a | 56 |
| | | 6 | | " | 20 | N | 3 |
| | | 7 | | S.S.a | 44 | " | 27 |
| 4X | H-4002 | 1 | *N* | N | 7 | S.S.a | 66 |
| | | 2 | | " | 11 | N | 28 |
| | | 3 | | " | 13 | " | 21 |
| | | 4 | | " | 21 | " | 12 |
| | | 5 | | " | 29 | " | 28 |
| | | 6 | | " | 29 | S.S.a | 43 |
| | | 7 | | S.S.a | 31 | N | 26 |
| | | 8 | | " | 40 | S.S.a | 35 |
| | | 9 | | " | 40 | " | 44 |
| | | 10 | | " | 41 | N | 28 |
| | | 11 | | " | 45 | S.S.a | 60 |
| | | 12 | | " | 48 | " | 41 |
| 4X | 4M-50 | 1 | *S* | N | 21 | S.S.a | 56 |
| | | 2 | | S.S.a | 37 | " | 58 |
| | | 3 | | " | 54 | " | 56 |
| | | 4 | | " | 62 | S.S.b | 93 |
| | | 5 | | S.S.b | 72 | S.S.a | 45 |
| | | 6 | | " | 79 | S.S.b | 73 |
| | | 7 | | " | 94 | " | 77 |
| | | 8 | | " | 96 | " | 100 |
| | | 9 | | " | 97 | " | 95 |
| | | 10 | | " | 98 | " | 98 |
| | | 11 | | " | 99 | " | 77 |
| | | 12 | | " | 100 | " | 99 |
| | | 13 | | " | 100 | " | 100 |
| | | 14 | | " | 100 | " | 100 |

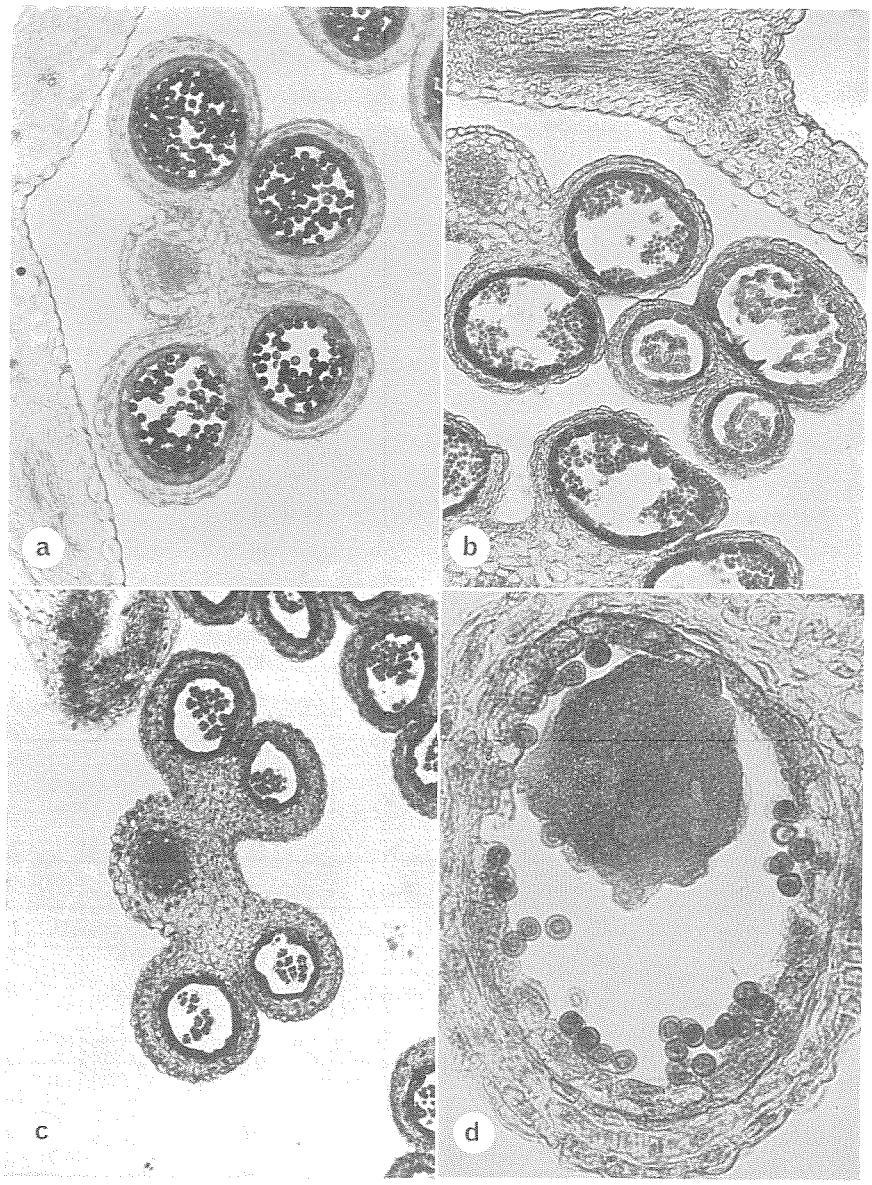


**Fig. 3.** Transverse sections of anthers in the male sterility affected by the treatment of low temperature.

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. ×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. ×130.

c. Thickened anther walls and smaller microsporangia containing a small number of the quartets, which was observed in a plant of 4M-50 (tetraploid *S* cytoplasm strain) after the treatment. ×100.

d. Tapetal plasmodium showing a pseudopodium-like incursion in the C.S. type produced from the S.S.b type in 4M-50 after the treatment. ×350.

develoment 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 (Fig. 3 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 (Fig. 3 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 microspors were observed in the material after the treatment, while the tapetal enlargement was not so pronounced in the material before the treatment (Fig. 3 d).

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.

## Discussion

Investigations on the stability of a male sterile character for the internal and environmental conditions is a fundamental problem in the use of male sterility. GABELMAN (1956) suggested that variability in the male sterility may be due to environmental factors, genetic factors, and their interactions.

In the authors' experiments, variations of pollen sterility during the flowering period 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 this experiment, the phenotype of male sterility was decided on the basis of repeated observations throughout the blooming period and questionable plants were re-examined in the next flowering season. 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 entirity of the plant. GABELMAN's

hypothesis on the particulate male sterile factor was not supported from the authors' results on the intr-aindividual variations of pollen sterility in *S* and *N* cytoplasm plants. Similar results were obtained in partially fertile plants both in Texas and normal cytoplasm in corn (DUVICK 1965). 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 the halves split 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 (1965) 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 lattitudes. A genetical influence is assumed to be shown in common for modifiability and variability in the same way. In onions, male sterility is not influenced even under long and normal photoperiods and temperatures below 21°C (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).

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 were subjected to low and high temperatures. SAKAI (1943, 1949 a, b) 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 phenotype 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 about 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.

## Summary

As a basis for the practical use of the male sterility, the phenotypic variability was studied on the cytoplasmic male sterility at diploid and tetraploid levels.

A considerable variation of pollen sterility was observed during the flowering period or between different seasons, in most of the S.S.a type plants and some plants of the N and S.S.b types. It was demonstrated that the pollen sterility in semisterile type plants is unstable under different environmental and internal conditions. A remarkable difference of pollen sterility was observed among different parts within a single plant, especially in the S.S.a type plants. It seems that the phenotypic expression of the male sterile character are affected prominently depending on some internal factors which interact with environmental conditions during the developmental process of the plant. The degree of the male sterility was also significantly affected by low temperature and starvation culture. In an extreme case, the N type changed into C.S. type after a 3 day treatment under low temperature (5°C).

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 pesedopodium-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.

## Literature cited


BARHAM, W. S. and MUNGER, H. M. (1950): The stability of male sterility in onions. Proc. Am. Hort. Sci. **56**: 401–409.

CORTESSI, H. A. (1967): Investigations made into male sterile. Euphytica **16**: 425–432.

DUVICK, D. N. (1960): Effect of fertilizer applications on degree of pollen sterility of partially fertile cytoplasmic male-sterile corn. Abst. Annual Meeting Amer. Soc. Agronomy, Cincinnati, Ohio. 1959: 57. Ref. Plant Breed. Abst. **30**, 1220 (1960).

DUVICK, D. N. (1965): Cytoplasmic pollen sterility in corn. Adv. Genet. **13**: 1–56.

GABELMAN, W. H. (1956): Male sterility in vegetable breeding. Brookhaven Symp. Biol. **9**: 113–122.

HOGABOAM, G. J. (1957): Factors influencing phenoytypic expression of cytoplasmic male sterility in the sugar beet (*Beta vulgaris* L.). J. Am. Soc. Suger Beet Tech. **9**: 457–465.

NAGAO, S. and KINOSHITA, T. (1962): Causal genes and character expression of male sterility in beets. J. Fac. Agr., Hokkaido Univ. **52**: 51–69.

OHTA, T. and MATSUMURA, S. (1960): Cytological and histological observations on mlae sterility in sugar beet. (Japanese with English summary). Seiken Ziho **11**: 78–82.

ROHRBACH, U. (1965): *Beiträge zum Problem der Pollensterilität bei Beta vulgaris* L. III. Untersuchungen über die Wirkung der Umwelt auf den Phänotyp des Merkmals "Pollensterilität". Z. Pflanzenzücht. **54**: 111–129.

SAKAI, K. (1943): Cyto-histological observations on pollen abortion caused by cool temperature during the summer of 1943 in rice cultivation of Hokkaido (Japanese). Rep. Hokkaido Agr. Exp. Station **40**: 2–17.

SAKAI, K. (1949 a): Cytological study on pollen abortion caused by cool temperature in rice (Japanese). Nogaku **3** (8): 418–423.

SAKAI, K. (1949 b): Cyto-histological study on pollen abortion caused by cool temperature and the related problems in rice breeding (Japanese). Rep. Hokkaido Agr. Exp. Station **43**: 2–46.

SAX, K. (1937): Effect of variations in temperature on nuclear and cell division in *Tradescantia*. Am. J. Bot. **24**: 218–225.

STEIN, H. and GABELMAN, W. H. (1959): Pollen sterility in *Beta vulgaris* associated with red pigmentation of the anthers. J. Am. Soc. Sugar Beet Tech. **10**: 612–618.

VASIL, I. K. (1965): Some aspects of the physiology of anthers. In: Plant Tissue Culture, Ed. P. R. WHITE and A. R. GROVE. Barkley, California. p. 341–356.