CAUSAL GENES AND CHARACTER EXPRESSION OF MALE STERILITY IN BEETS [1)]

By

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Introduction

It has generally been recognized that the employment of the male sterility character facilitates controlled pollination in producing hybrid seeds of plants on a large scale; this is the case with commercial hybrid seeds of sugar beets. Recently, triploid varieties of sugar beets have been produced by crossing diploid male sterile strains with tetraploid pollinators (ELLERTON & HENDRIKSEN 1959).

The nature of the inheritance of the male sterility character and its utilization in hybridization work have been studied mainly by OWEN (1945, 1948, 1952), KNAPP (1855) and HOGABOAM (1957), and it was assumed that the expression of this character is due to either an interaction of chromogenes and cytoplasmic genes or to a single chromogene. Further tetraploids were obtained in male sterile sugar beets, and some new and more potent sources of cytoplasmic male sterility were discovered from some wild species (SAVITSKY 1954, OLDEMEYER 1957).

In this report, there are presented some data derived from genetical and cytological studies on the male sterility character of sugar beets, together with some information on the utilization of this character in actual breeding work.

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Material and Methods

In the present studies two strains with male sterility, K- and M-strains were used. K-strains are the progenies of plants which were male sterile; they were obtained from a well known Japanese variety, Hon-iku-192. M-atrains originally came out of the cytoplasmic male sterile plants in a foreign variety introduced in 1954. As noted in the previous report (NAGAO, TAKAHASHI & KINOSHITA 1960), one of the M-strains, M-2-8, carried a cytoplasmic male sterility character together with a monogerm characters. When a complete-sterile type of the M-2-8 was crossed with plants from an introduced monogerm strain "M-10"[1], their F_1s were complete-sterile without exception, indicating that the pollen parents were so-called "type-O", the most appropriate pollen parent for producing male sterile progeny. The plants of type-O from "M-10" were propagted by sib-mating and were bred true as a tester strain (H-19) for detecting both the male sterility and the monogerm characters. On the other hand, the M-2-8 was crossed back to H-19 and the isogenic strain H-19MS was produced, which is also useful in that it carries the complete-sterile and the monogerm characters. Autotetraploid plants were induced from M-strain by applying colchicine solution; they were named 4M-strain.

Materials used for the cytological investigtions on microsporogenesis were fixed in acetic alcohol. Preparations were made by usual paraffin method. Serial sections were cut at 10 μ thick and were stained with Heidenhain's iron-alum haematoxylin.

Experimental Results

A. Classification of the male sterility

Types of abnormality in anthers and pollen grains from the progenies of K- and M-strains were classified into four classes as shown in Table 1 and

TABLE 1. Classification of male sterility

Types of male sterility	Complete-sterile C. S.	Semi-sterile b S. S. b	Semi-sterile a S. S. a	Normal (S)* N	Normal (N)* Control
Color of anther	White	Orange	Yellow	Yellow	Yellow
Dehiscing	—	—	—	—	—
Fertility (%)	0	0	5-90	50-90	80-95
Germination rate	0	0	30	90	100

* type of cytoplasm.

1) "M-10" are distinguished from the M-strains with male sterility, by using the quotation-marks.

Fig. 1, 2. In this table and figures the complete-sterile type (C. S.) is characterized by white and shrunken anthers in which the majority of pollen grains were disintegrated and the residue was sticking to the inner wall of the anther. Semi-steril type-b (S. S. b) possesses non-dehisced reddish-yellow anthers in which a large part of the pollen grains were abortive and small, though the extine of pollen was well developed. Semi-sterile type-a (S. S. a) seems to be a most variable phenotype. Both well dehisced and non-dehisced anthers are contained

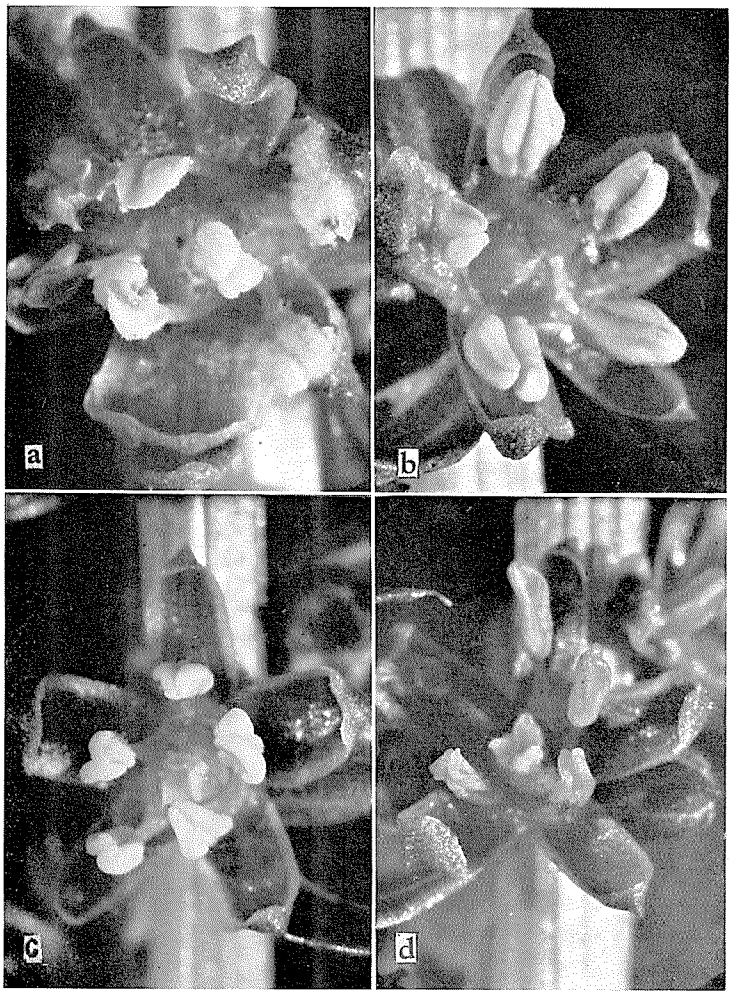

Fig. 1. Flowers representing each of the four types of male sterility in beets. × 8.

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

in the same flower of a single plant, at different rates of mixing. The pollen grains were functional while the fertility of the pollen varied remarkably in each anther. Nomal type (N)[1] has well dehisced anthers and as high pollen fertility as that of control strains.

In regard to the classification of male sterility, HOGABOAM (1957) and

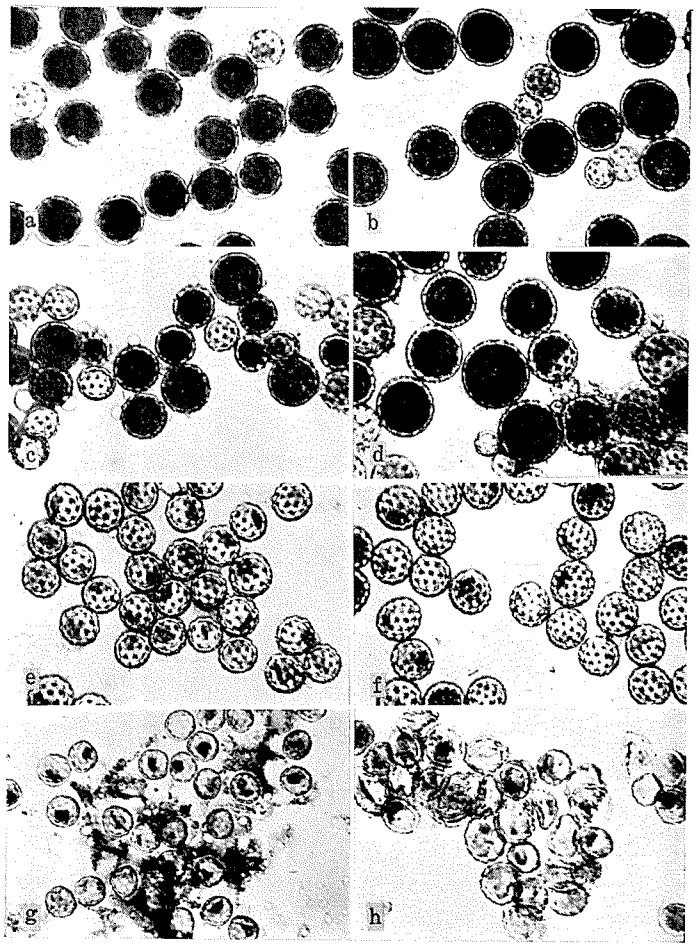

Fig. 2. Pollen grains of diploid and tetraploid male sterile beets. × 100.
a, b. Normal type of diploid and tetraploid.
c, d. Semi-sterile-a type of diploid and tetraploid.
e, f. Semi-sterile-b type of diploid and tetraploid.
g, h. Complete-sterile type of diploid and tetraploid.

1) N type differs in meaning from the normal cytoplasm "*N*", which is contrasted with the sterile cytoplasm "*S*", as explained below.

OLDEMYER (1957) set out 8 or 10 classes from their observations. However, according to succesive observations during the flowering period, S. S. a type and N type were changeable in respect to dehiscing anthers and the pollen fertility, and even in a normal plant of control strains, pollen fertility, decreased about 10–15% in the beginning and ending of the flowering. Therefore, on the basis of repeated observations throughout the blooming period, the present authors made the above classification into four types which seems to correspond with that by OWEN (1945) and KNAPP (1955).

B. Mode of inheritance

As mentioned above, cytoplasmic inheritance of male sterility character in M-strain has already been recognized, while in the K-strains, genetical nature of their male sterility character is completely unexplored. To get some information on this point, reciprocal crossings were made between N type from K–3 strain and plants from the variety, Hon-iku–192. As shown in Table 2, no

TABLE 2. Phenotypic ratios of male sterility in the progenis of reciprocal crosses between N type (*S* cytoplasm) and pollinators from Hon-iku–192 (*N* cytoplasm)

Cross combination	Percentage of male sterile type				Number of Plants
	C. S.	S. S. b	S. S. a	N	
K-3-4 (N)×Hon-iku-192-2	27	25	29	19	48
Reciprocal	0	0	0	100	29
K-3-4 (N)×Hon-iku-192-4	22	26	19	33	27
Reciprocal	0	0	0	100	29
K-3-4 (N)×Hon-iku-192-5	0	38	7	55	42
Reciprocal	0	0	0	100	19
K-3-2 (N)×Hon-iku-192-2	—	—	—	—	—
Reciprocal	0	0	0	100	35

male sterile plants appeared in the progenies from ♀ Hon-iku–192 × N type, while various types of male sterility were found in the progenies from ♀ N type × Hon-iku–192. It follows from this fact that the male sterility of K-strains depends upon a male sterile cytoplasm.

OWEN (1945, 1950) described two different chromogenes, *X* and *Z*, which are responsible for restoration of pollen fertility in the sterile cytoplasm resultant from the presence of plasmagene *S*; HOGABOAM (1957) reported a new gene, *Sh*, which enhances the pollen producing ability in case of coexistence with

X and/or *Z* gene. In order to ascertain the presence of these sorts of modifying genes in the authors' materials, complete-sterile plants from the K- and M-strains were crossed with pollinators from the varieties, Hon-iku-192 and Hon-iku-399. Among the F_1 progenies of the crosses, the highest degree of pollen restoration was observed in the F_1 from a cross of M-2-8 × Hon-iku-192-5, in which the majority of plants were N or S. S. a type (Table 5). Three plants which were 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_1s were produced from crosses between H-19MS and the four F_1s. With regard to the phenotypic variation of the male sterility character in F_2s and B_1s, they were classified into four types as shown in Table 3. a, b.

TABLE 3. Inheritance of male sterility

a. F_2: '56-106 M-2-8 C.S.×Hon-iku-192-5

Cross combination		F_2 Segregation					Goodness of fit		
		N	S. S. a	S. S. b	C. S.	Total	χ^2	d. f.	p
1	Obs.	79	25	84	19	207			
	Cal.	87.33	29.11	77.63	12.94	207.01	4.739	3	0.10–0.20
2	Obs.	25	9	13	3	50			
	Cal.	21.09	7.03	18.75	3.13	50.00	2.853	2	0.20–0.30
3	Obs.	38	9	17	2	66			
	Cal.	27.84	9.28	24.75	4.13	66.00	7.100	2	0.02–0.05
Cal. ratio (9 : 6 : 1) (3 : 1)		27	9	24	4				

b. B_1: H-19 MS×'56-106 F_1 (M-2-8 C.S.×Hon-iku-192-5)

Cross combination	N	S. S. a	S. S. b	C. S.	Total
H-19 MS×'56-106 F_1-4	13	5	17	6	41
〃 × 〃 F_1-7	12	4	19	7	42
〃 × 〃 F_1-8	4	0	5	7	16
〃 × 〃 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)……χ^2 = 38.447 d. f. = 3 p = 0.01.
(3 : 1) ……χ^2 = 0.978 d. f. = 1 p = 0.30–0.50.

Applying OWEN's genetic scheme of the two genes, *X* and *Z*, to the F_2 segregations, one may expect to find that the genotypes of the semi-sterile-a (S. S. a), semi-sterile-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 respectively, in an F_2 segregation ratio of 9 : 6 : 1. In addition to this, and if an another modifier, such as *Sh*, is involved in these cross combinations and exerts its enhancing effect on restoration of pollen fertility—that is turns S. S. a into N—, a monogenic ratio of 3 : 1 would be expected between normal (N) and semi-sterile-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 a 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. In actual observation of the B_1 was not a close fit to the above ratio; however, if B_1 plants were classified into two groups as (N, S. S. a, S. S. b) : (C. S.), an expected digenic ratio of 3 : 1 is fairly well satisfied in this examination.

On the whole, therefore by the experimental results from the use of F_2 and B_1 generations, it is confirmed that the complete-sterile type is dependent upon the double recessive state of the pollen restoring genes, *X* and *Z*. As to the verification of the presence of an enhancer, though no conclusive results were obtained in B_1 examination, the greater part of the segregation mode in F_2s lead the authors to the assumption that in their cross combinations there remains a high possibility of the presence of such an enhancer as *Sh*. Thus for the present it is concluded that at least the two different genes are concerned with the pollen restoration under condition of coexistence with a plasmagene *S*; they are designated as *X* and *Z* adopting OWEN's gene symbols.

C. Selection of pollen parent

Under the genic assumption of the existence of *X* and *Z*, nine genotypes are expected to exist in phenotypic plants of normal cytoplasm, viz. plants with *N* cytoplasm (Table 4). From amongst them, a double recessive with *X* and *Z*, that is $N\,xx\,zz$ is most desirable to be the pollen parent or pollinator in producing plants of the complete sterile type ($S\,xx\,zz$), because the progeny of the cross $S\,xx\,zz \times N\,xx\,zz$ results in 100% of the complete-sterile type. OWEN applied to this type of $N\,xx\,zz$ the name "type-0" and this type of plants was selected from some commercial varieties by some workers (OWEN 1948, PETERSON 1952, KNAPP 1955).

In order to select the plants of type-O Japanese varieties Hon-iku-192 and

TABLE 4. Segregating ratios expected from the cross C. S. type (*S xxzz*)×Pollinator (*N* cytoplasm)

Genotype of pollinator		Phenotypic ratio of offspring (%)		
		C. S.	S. S. b	S. S. a
0	*N xx zz*	100	—	—
I	*N Xx zz* or *xx Zz*	50	50	—
II	*N Xx ZZ*	25	50	25
III	*N Xx ZZ* or *XX Zz*	—	50	50
IV	*N XX zz* or *xx ZZ*	—	100	—
V	*N XX ZZ*	—	—	100

Hon-iku-399, the authors crossed complete sterile types of K- or M-strains with some pollinators selected at random from the said varieties. The frequencies of the male sterile types which appeared in the progenies are shown in Table 5a. The genotypes of the pollinators were estimated from the theoretical ratios given in Table 4, and the fitness of the genotypes to the authors' results was confirmed by the chi square tests.

As shown in Table 5a, the heterozygous conditions of pollinators (I, II, and III) were found more frequently than the homozygous conditions of pollinators (0, IV and V). Besides this, in cases where 192–11 and 192–12 were used as pollinators, the genotype of the complete-sterile plants seemed to affect the segregation mode of the progenies more strongly than the environment. So it is necessary to male further investigation in order to reach reasonable explanation on the genic constitution of the complete-sterile phenotypic plants. The frequency of the male sterile types in the progenies from the same complete-sterile plants pollinated with Hon-iku–192 and Hon-iku–399 were summed up and the respective average frequency of each sterile type is shown in Table 5b. It is noted that there was similar frequency of occurrence of the male sterile types in the F_1s from the above three kinds of cross combinations, regardless of which strain, K- or M-, was used as the female parent, and of which strain, 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-b type, too; in these crosses the total average frequency of C.S. and S.S.b was about 75%, showing 10% of decrease below the findings in the corresponding experiments on the complete-sterile type (Table 6).

These results show that even if plants from random sampling in the above varieties are used as the pollinators, a relatively high percentage (more than 75%) of C.S. and S.S.b which are safe in pollen shedding is able to transmit

TABLE 5. Phenotypic ratios of male sterility observed in the progenies of the crosses C. S. type (S *xx zz*) × Pollinators (N cytoplasm)

a. C. S. type × Pollinator (Hon-iku-192 or Hon-iku-399)

Cross combination	Percentage of male sterile type				Number of plants	Pollinator	
	C. S.	S. S. b	S. S. a	N		Expected genotype	χ^2
K-3- 6×192- 4	74	16	0	11	19	I	4.26
M- 19×192-10	51	43	3	3	35	"	0.03
K-3- 6×192- 1	51	35	8	5	37	"	0.03
K-3- 6×192- 2	40	56	4	0	45	"	1.80
M- 19× "	61	39	0	0	18	"	0.89
K-3- 6×192-12	73	27	0	0	22	"	4.55
M- 19× "	70	16	11	3	37	"	6.08
M- 2× "	33	38	10	19	21	"	2.33
M- 17× "	23	59	9	9	22	"	0.55
M- 22×399- 3	63	23	13	0	30	I	2.13
M- 2×399- 7	50	50	0	0	16	"	0
K-3- 2×192-13	34	60	6	0	35	II	1.61
K-3- 6×192- 3	26	52	15	7	27	"	0.11
K-3- 2×192-15	20	71	6	3	35	"	0.47
K-3- 6× "	25	57	11	7	44	"	1.23
M- 2× "	0	89	7	4	27	"	—
M- 2×399- 2	13	73	3	10	30	II	—
M- 2×399- 4	13	78	7	2	46	"	1.04
M- 2×399- 8	14	31	23	31	35	III	0.26
K-5- 2×192-11	15	37	32	17	41	III	0.02
M- 19× "	3	73	10	15	40	"	10.00 *
M- 2× "	0	41	12	46	41	"	1.20
M- 16× "	0	50	29	21	24	"	0
M- 3× "	0	35	25	40	20	"	1.80
K-3- 6×192- 6	0	70	20	10	10	IV	—
M- 19× "	0	88	13	0	24	"	—
M-2- 8×192-55	0	0	25	75	12	V	—

* Significant at the 1% level.

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 (»)× »	5	37	52	7	4	154
M- 2 (»)×399	5	31	51	9	9	157
Mean		36.3	49.3	8.3	6.3	

TABLE 6. Phenotypic ratios of male sterility in the progenies of the crosses S.S.b type×pollinators (Hon-iku-192)

Cross combination	Percentage of male sterile type				Number of Plants
	C. S.	S. S. b	S. S. a	N	
K-3-11 (S. S. b)×192	9	68	18	5	22
» -12 (»)× »	2	81	14	4	57
» -16 (»)× »	26	26	40	10	20
Mean	12.3	58.3	24.3	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
» -32a (»)× »	27	73	0	0	11
» -32b (»)× »	15	65	8	12	26
» -32c (»)× »	0	35	25	40	20
Mean	9.2	68.0	10.0	11.7	—

and continue existence in progenies. However in a case where it is desired to obtain 100% incidence of male sterele plants, the type-O plants should be found and employed as the pollinators.

D. Maintenance of the male sterility

As to the maintenance of K-strains, in view of the present authors' experience, it is rather difficult to breed true the type-O plants from population of the original variety, Hon-iku-192. Therefore and as a short cut method, the authors 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. The frequency of the said four types in K-3 and K-5 strains was as follows:

Strain	C. S.	S. S. b	S. S. a	N	Number of plants
K-3	2%	30%	17%	51%	84
K-5	3%	38%	20%	39%	127

TABLE 7. Phenotypic ratios of male-sterility observed in the progenies from the crosses among male-sterile types of "*S*" cytoplasm

a) Normal (*S*)×Normal (*S*)

Crosses	Percentage of male sterile types				Number of plants	Expected type of crosses
	C. S.	S. S. b	S. S.. a	N		
K-3-11×K-3-12	3	53	23	23	40	I
» -15× » -17	3	9	9	79	34	I
» - 1× » -22	3	6	6	84	32	I
» -10× » -12	0	49	22	29	51	II
» - 4× » - 5	0	34	28	38	32	II
» -10× » -11	0	14	35	52	29	II
» - 3× » - 2	0	10	30	60	10	II
» -26× » -13	0	7	28	65	43	III
Mean	1.1	22.8	22.6	53.8	—	—
K-5- 9×K-5-11	7	17	7	69	29	I
» - 5× » - 3	0	36	50	14	14	II
» -22× » - 4	0	26	13	61	31	II
» - 4× » - 6	0	25	5	70	20	II
» - 3× » - 4	0	18	18	65	17	II
» - 4× » - 5	0	8	8	85	13	III
» - 1× » - 2	0	6	19	75	16	III
» - 8× » - 9	0	5	30	65	20	III
Mean	0.9	17.6	18.8	63.0	—	—

b) S. S. a×Normal (*S*)

Crosses	C. S.	S. S. b	S. S. a	N	Number of plants	Expected type of crosses
K-5- 1×K-5- 2	22	22	28	28	36	I
» -12× » -10	5	33	19	43	21	I
K-3- 9×K-3-10	0	17	39	44	41	II
» - 7× » - 8	0	14	20	66	35	II
K-5- 7×K-5- 9	0	7	50	43	30	III
Mean	5.4	18.6	31.2	44.8	—	—

c) S.S.b×Normal (*S*)

Crosses	C. S.	S. S. b	S. S. a	N	Number of plants	Expected type of crosses
K-3-17×K-3-18	16	32	16	36	25	IV
» -21× » - 2	0	54	39	8	13	V
» -21× » -14	0	50	29	21	14	V
» -16× » -17	0	42	42	16	19	V
» -23× » - 5	0	28	28	43	21	VI
» -12× » -15	0	14	38	48	21	VI
Mean	2.7	36.7	32.0	28.7	—	—

In order to confirm the genic constitutions of plants in a population with *S* cytoplasm and to gain information on the mode of transmission of male sterility, crosses were mode among three types of plants with S.S.b, S.S-a and N, within K-3 and K-5 strains. The frequency of each of the four types in the progenies of the cross N × N, S.S.a × N and S.S.b × N were as shown in Table 7.

Theoretically seven kinds of segregation modes would be expected through out the above three cross combinations, with respect to two chromogenes, *X* and *Z* (Table 8). From the result of this actual examination as shown in Table 7, six segregation modes out of the above seven could safely be estimated to exist. In this table it is also pointed out that the average frequencies of C.S. and S.S.b in the progenies from N × N and S.S.a × N decreased when they were compared with those of C. S. and S.S.b in the original populations, whereas

TABLE 8. Segregating ratios expected from the crosses among male sterile types of "*S*" cytoplasm

a) Normal (*S*)×Normal (*S*), S.S.a×Normal (*S*)

Type of crosses	Genotype		Phenotypic ratio of offspring (%)		
	female	male	C. S.	S. S. b	S. S. a
I	*XxZz*	*XxZz*	6.25	37.5	56.25
II	*XxZZ* *XXZz*	*XxZZ*, *XxZz* *XXZz*, *XxZz*		25	75
III	*XXZZ* *XXZZ* *XxZZ* *XXZz*	*XXZZ*, *XxZZ* *XXZz*, *XxZz* *XXZz* *XxZZ*			100

b) S. S. b×Normal (*S*)

Type of crosses	Genotype		Phenotypic ratio of offspring (%)		
	female	male	C. S.	S. S. b	S. S .a
IV	*Xxzz* *xxZz*	*XxZz* *XxZz*	12.5	50	37.5
V	*XXzz* *zzZZ* *Xxzz* *xxZz*	*XXZz*, *XxZz* *XxZZ*, *XxZz* *XXZz* *XxZZ*		50	50
VI	*Xxzz* *xxZz*	*XzZZ* *XXZx*		25	75
VII	*XXzz* *xxZZ* *Xxzz* *xxZz*	*XXZZ*, *XxZZ* *XXZZ*, *XXZz* *XXZZ* *XXZZ*			100

slight increases of C.S. and S.S.b frequencies were observed in the progeny of the cross S.S.b × N. In the progeny of S.S.b × N, about 60% of the plants were pollen producers, while in the cross of S.S.b × Hon-iku-192 (*N* cytoplasm) only 25% of the progeny were pollen producing plants. However, in the cross of S.S.b × N, the recovery of the male fertile plants is a beneficial phenomenon for the making of repeated inter-crossing 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 male sterility characteristic within the respective strains or populations.

E. Male sterility in tetraploid level

The data in the preceeding sections of this chapter were obtained by the use of beets of the diploid chromosomal level. In this section, the authors present some data on the tetraploid male sterile strain (4M-strain) which was induced from M-strain by colchicine treatment.

The utilization of tetraploid male sterility may be important for producing tetraploid and triploid hybrids (SAVITSKY 1954). Therefore, it is needful to clarify the influence of the chromosome doubling in respect to the character expression of male sterility. It is known that in tetraploid plants of sugar beets the size of the flowers and pollen grains is increased, while the pollen fertility and germination rate of the pollen are significantly lower than those

of diploid plants (NAGAO & TAKAHASHI 1952).

The mode of abnormality of anthers and pollen grains in 4M-strain was classified into four types, similarly to the diploid case (Fig. 2).[1)]

In the original population of the 4M-strain the majority of the plants were S. S. b type. Some plants of this type were back-crossed two times—successively—with pollinators from 4398 strain, tetraploid induced from diploid variety Hon-iku-398. Table 9 presents the data on progenies of the second back cross.

TABLE 9. Phenotypic ratios of male-sterility in the progenies from the second back-crosses of S.S.b type in 4M-strain pollinated with 4398-strain

Cross combination	Percentage of male sterile type				Number of Plants
	C. S.	S. S. b	S. S. a	N	
4M-48 (S. S. b)×4398	0	94	0	6	16
〃 -50 (〃)× 〃	7	89	2	2	135
〃 -59 (〃)× 〃	4	91	4	0	45
〃 -60 (〃)× 〃	0	90	5	5	21
Mean	2.8	91.0	2.8	2.3	

As the maternal plants were S. S. b type, so these data were compared with those of in Table 6 which presents the results of the similar experiments with diploid strains.

In the tetraploid progenies, a large percentage of the plants fell into S. S. b type while the plants with C. S., S. S. a and N types were quite few. In comparison with those of the diploid progenies shown in Table 6, the relative frequencies of each type between diploid and tetraploid levels were remarkably different. The total average frequencies of C. S. and S. S. b were 93% in the tetraploid whereas in the diploid they were only 70-78%.

These findings suggest that with regard to the triploid breeding and to the production of pure triploid seeds, the utilization of tetraploid male sterility is more efficient than that of diploid male sterility. Therefore, it is needful to ascertain the genetical mode of the pollen restoring genes in tetraploid level, together with the securing of the tetraploid male sterile strains of high combining ability.

1) Pollen fertility of the normal tetraploid plants was 80-90%, in comparison with 90-95% of the normal diploid. Therefore, the difference of fertility between diploid and tetraploid is fairly smaller than that between N and S. S. a in diploid strains, and there is no need of making special allowance for the grouping of sterility types in tetraploid strains as was done in diploid.

F. Cytological observations

It is generally accepted that a close relationship exists between the development of tapetal cells and the breakdown of microsporogenesis in the male sterile plants, regardless of causation of the sterility, whether genetic or environmental (WHYTE 1929, CLAUSEN 1930, SAKAI 1943, RICK 1948, CHILDERS 1952, TAKEBE 1952, etc.). In sugar beets, ARTSCHWAGER (1947) has reported that the pollen abortion of cytoplasmically inherited male sterility is associated with either the abnormal development of tapetal plasmodium or a cellular tapetum; HOSOKAWA et al. (1954) also observed similar phenomena.

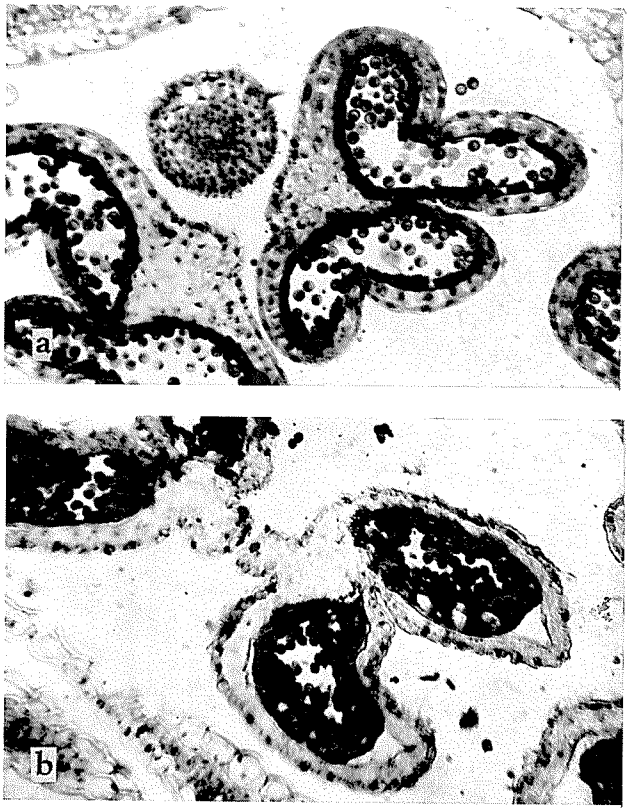

Fig. 3. Cross sections of anthers in normal and complete-sterile tetraploid beets at about a week before anthesis, showing microspores and tapetal tissues. × 100.

a. Normal type: a layer of tapetum and pollen grains with developed extines.

b. Complete-sterile type: abnormally developed tapetum and degenerated microspores.

The authors made preliminary examination on the microsporogenesis of the male sterility, paying special attention to comparative observation between diploid and tetraploid levels. The authors' results with respect to the diploid male sterility character coincide with the observations of ARTSCHWAGER and other workers, and therefore detailed accounts are ommited here.

It has been known in ordinary, that is in normal cytoplasmic tetraploid beets, that several chromosomal aberrations occur in the course of their meiosis (RASMUSSON & LEVAN 1939, MATSUMURA et al 1942, NAGAO & TAKAHASHI 1952, SAVITSKY 1952, etc.). In the present examination of the authors, however, it is pointed out that three were no marked differences in regard to the meiosis between complete sterile type and normal fertile type of tetraploid plants. In complete-sterile type of tetraploid, the peri-plasmodium was formed around the pollen tetrads and after that a part of the plasmodium began to swell up, containing some nuclei and large vacuoles. The abnormality of tapetum was in sharp contrast to that for normal type in tetraploid (Fig. 3). With the

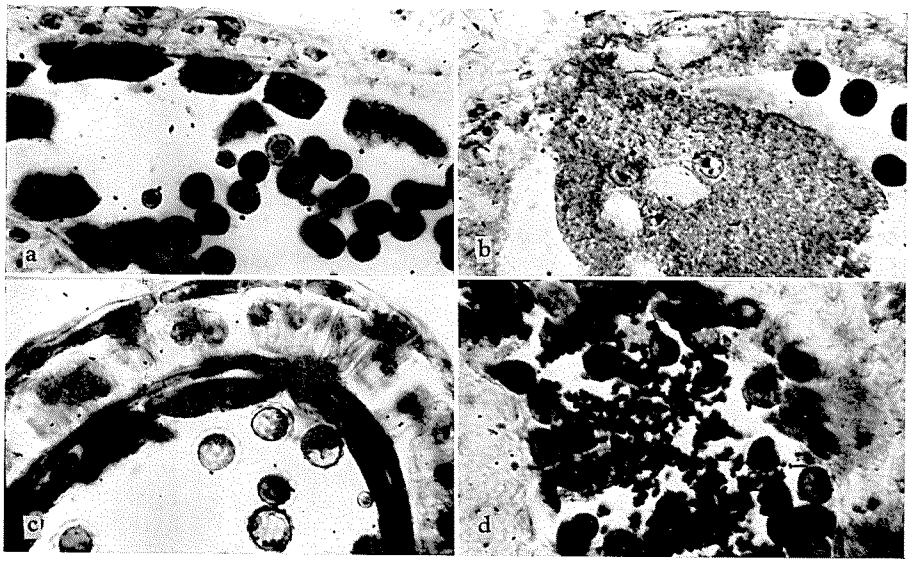

Fig. 4. Cross sections of locules in normal and complete-sterile tetraploid beets. × 280.

a. Normal type: early stage of degenerating tapetum, and dark stained microspores after the tetrad stage.

b. Complete-sterile type: corresponding stage with a. in development of microspores, whereas tapetum is making pseudopodium-like incursions.

c. Normal type: completely degenerated tapetum, endothecium with wall thickening and pollen grain with thickened extine.

d. Complete-sterile type: degeneration of microspores and tapetal plasmodium.

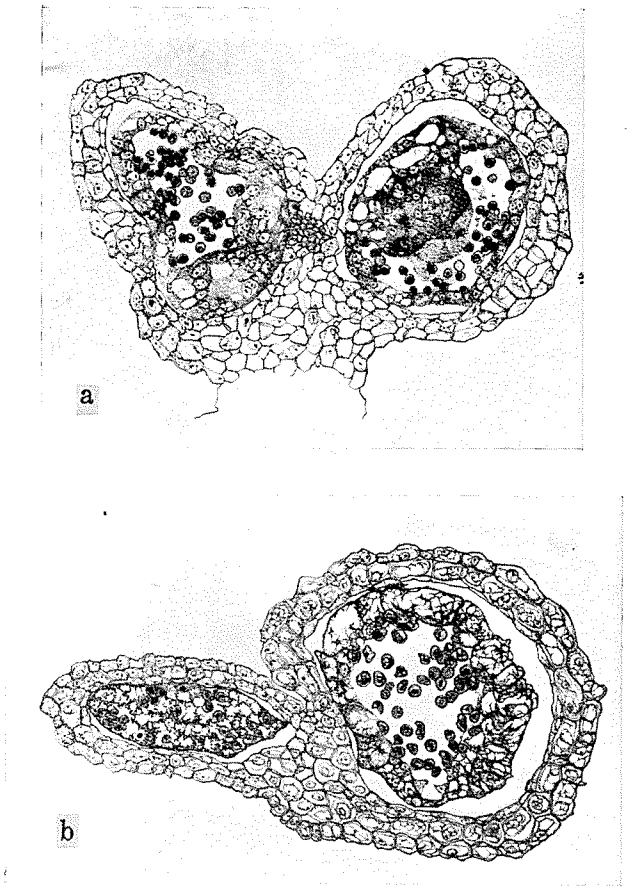

Fig. 5. Cross sections of locules in complete-sterile tetraploid beets. (sketch by camera lucida). × 130.
a. Maximum development of periplasmodium.
b. Degeneration of microspores and tapetal plasmodium.

growth of the plasmodium, the microspores were pushed into a narrow space and finally destroyed. After the plasmodium had reached its maximum development, it degenerated with microspores (Fig. 4, 5). In semi-sterile-a and -b types, though there was variation even within a single flower, the course of tapetal development did not differ from that of the normal type, as a whole, while the microspores began to degenerate after the formation of the extine and the germ core. On the whole, the abnormality of tapetal development in a tetraploid strain corresponds with that of a diploid, and therefore, at present, no close relation between the tapetal abnormality and the chromosome doubling is recognized.

Summary

1. Cytoplasmic inheritance was confirmed in the male sterility derived from Japanese sugar beet variety Hon-iku-192, together with a genetic strain H-19MS, which carries complete male sterility and monogerm characters.

2. It was ascertained that two duplicate genes, X and Z, were responsible for restoration of pollen fertility when they co-exist with a plasma gene S for male sterility.

3. Besides these genes, there was a possibility of the existence of another chromogene which exerts an enhancing effect on pollen fertility.

4. There remains a difficult problem to find out the so-called type-O ($Nxx\,zz$) from two Japanese varieties, Hon-iku-192 and Hon-iku-399, because of the fact that in these populations, plants with heterozygous condition of X and Z genes are discovered more frequently than plants with homozygous condition of these genes.

5. Thus, in maintaining the male sterile strain, the inter-crossing between male sterile group (C. S. and S. S. b) and male fertile group (S. S. a and N) within the strain of S cytoplasm, are effective from the practical point of view.

6. Tetraploids were induced by colchicine treatment from a diploid male sterile strain. The high frequency occurrence of the male sterile types (C. S. and S. S. b) in the progeny of tetraploid male sterile strains suggests a possibility of making efficient utilization of this strain or population in producing triploid beets.

7. The abnormality of tapetal cells is associated with the pollen abortion of the male sterile plants, both in diploid and tetraploid levels.

Literature Cited

ARTSCHWAGER, E. (1947): Pollen degeneration in male-sterile sugar beets with special reference to the tapetal plasmodium. Jour. Agr. Res., 75 : 191–197.

CHILDERS, W. R. (1952): Male sterility in *Medicago sativa* L. Sci. Agr., 32 : 361–364.

CLAUSEN, J. (1930): Male sterility in *Viola orphanidis*. Hereditas, 13 : 53–72.

ELLERTON, S. and HENDRIKSEN, A. J. TH. (1959): Note on the probable cause of the occurrence of tetraploid plants in triploid varieties of sugar beets. Euphytica, 8 : 99–103.

HOGABOAM, G. J. (1957): Factors influencing phenotypic expression of cytoplasmic male sterility in the *Beta vulgaris* L. Proc. Amer. Soc. Sugar Beet Tech., 9 : 457–465.

HOSOKAWA, S., TAKEDA, T., OTANI, Y. and IKEHATA, M. (1954): Cyto-histological studies on male sterility of sugar beet with special reference to pollen degeneration and tapetal plasmodium. Jap. Jour. Breed., 4 : 196–202. (in Japanese).

KNAPP, E. (1955): Zur plasmonisch kontrollierten Pollensterilität der Zuckerrüben. Der

Züchter, 25 : 231–236.

MATSUMURA, S., MOCHIZUKI, A. and AKEMINE, T. (1942): Genetische und zytologische Untersuchungen bei Beta-Arten. II. Colchicininduzierte polyploide Pflanzen und ihre Nachkommen bei *Beta vulgaris*. Rep. Kihara Inst. Biol. Res. No. 1 : 16–23. (in Japanese).

NAGAO, S. and TAKAHASHI, M. (1952): Studies in polyploid varieties of sugar beets. II Development and germination of pollens. Bull. Res. Farm Fac. Agr. Hokkaido Univ. 10 : 11–27. (in Japanese).

————, ———————— and KINOSHITA, T. (1961): Studies on some heritable characters of sugar beets, from the standpoint of breeding. I. Monogerm. Rep. Sugar Beet Res. Serv. Fac. Agr. Hokkaido Univ., 2 : 171–179. (in Japanese).

OLDEMEYER, R. K. (1957): Sugar beet male sterility. Jour. Amer. Soc. Sugar Beet Tech. 9 : 381–386.

OWEN, F. V. (1945): Cytoplasmically inherited male-sterility in sugar beets. Jour. Agr. Res., 71 : 423–440.

———— (1948): Utilization of male sterility in breeding superior yielding sugar beets. 1948 Proc. Amer. Soc. Sugar Beet Tech., : 156–161.

———— (1950): The sugar beet breeder's problem of establishing male-sterile populations for hybridization purposes. 1950 Proc. Amer. Soc. Sugar Beet Tech.: 191–194.

———— (1952): Mendelian male sterility in sugar beets. 1952 Proc. Amer. Soc. Sugar Beet Tech.: 371–376.

PETERSON, D. F. (1952): Inbred seed production in progeny classification for type O plants in open-pollinated varieties. 1952 Proc. Amer. Soc. Sugar Beet Tech.: 351–353.

RASMUSSON, J. and LEVAN, A. (1939): Tetraploid sugar beets from colchicine treatments. Hereditas 25 : 97–102.

RICK, C. M. (1948): Genetics and development of nine sterile tomato mutants. Hilgardia, 18 : 599–633.

SAKAI, K. (1949): Cyto-histological and thremmatological studies on sterility of rice in nothern parts of Japan, with special reference to abnormal hypertrophy of tapetal cells due to low-temperature. Rep. Hokkaido Agr. Exp. Station 43 : 1–48. (in Japanese).

SAVITSKY, H. (1952): Polyploid sugar beets-cytological study and methods of production. 1952 Proc. Amer. Soc. Sugar Beet Tech.: 470–476.

———— (1954) Obtaining tetraploid monogerm self fertile, self sterile, and male sterile beets. Proc. Amer. Soc. Sugar Beet Tech. 8 (2) : 50–58.

TAKEBE, K. (1952): Cytological studies on pollen degeneration in male-sterile onions. Jour. Hort. Soc. Japan 21 : 73–75. (in Japanese).

WHYTE, R. O. (1929): Studies in *Ranunculus*. II. The cytological basis of sex. in *R. acris*. Jour. Genet. 21 : 183–191.