FACTORS INFLUENCING PHENOTYPIC EXPRESSIONS OF CYTOPLASMIC MALE-STERILITY IN THE SUGAR BEET (*Beta vulgaris* L.)

By

George J. Hogaboam

A THESIS

Submitted to the School of Graduate Studies of Michigan State University of Agriculture and Applied Science in partial fulfillment of the requirements for the degree of

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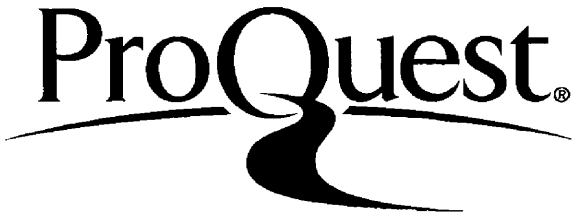
Department of Farm Crops

1956



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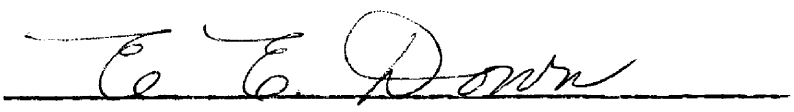
Approved 

ABSTRACT

Owen (1) reported cytoplasmically inherited male-sterility in sugar beets in which there was a partial restoration of pollen production due to the action of dominant nuclear genes (X and/or Z). Therefore, the male parent of the male-sterile line must be recessive for x and/or z. Such a male parent plant was designated by Owen[2,3] as a type O plant.

In our search for type O plants, many plants were found to be segregating for the pollen restorer gene or genes but no true breeding type O plants were found. The present investigation was undertaken to evaluate some of the factors influencing male-sterile phenotypes.

The effects of 46-50 degrees Fahrenheit temperatures applied during microsporogenesis were studied without successfully altering the phenotypes of the progeny SL9460M.

An attempt to alter the degree of pollen restoration through changes in the auxin concentration within the plant was without success for the concentrations of indole acetic acid and maleic hydrazide used in the experiment.

A study of the S_1 progeny of a plant, containing male-sterile cytoplasm yet producing pollen, indicated that a gene for pollen restoration and the gene for monogermness were carried on the same chromosome. Furthermore, this progeny revealed the indefinite phenotypic expression of the heterozygous pollen restorer genotype.

Breeding experiments with another progeny lead to the discovery of a mutation which would complement the restoring action of the

pollen restoring gene to the extent that the misclassification of the heterozygous pollen restoring genotypes was improbable. The dominant complementary gene (Sh) was found to be lacking in pollen restoring ability when the dominant pollen restorer gene (X) was absent in male sterile cytoplasm.

References

1. Owen, F. V. Cytoplasmically inherited male sterility in sugar beets. Jour. Agric. Res. 71: 423-440. 1945.

2. Owen, F. V. Utilization of male sterility in breeding superior-yielding sugar beets. Proc. Amer. Soc. Sugar Beet Tech. 157-161. 1948.

3. Owen, F. V. The sugar beet breeder's problem of establishing male-sterile populations for hybridization purposes. Proc. Amer. Soc. Sugar Beet Tech. 191-194. 1950.

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INTRODUCTION

The small, perfect flowers and indeterminate flowering habit of the sugar beet make it impossible to undertake large scale controlled cross-pollinations without the cumbersome use of marker genes for identification of true hybrids. Although self-sterility has been used to enforce cross- and sib-pollinations, this character complicates inbreeding procedures and does not result in an all hybrid progeny.

Large scale production of truly hybrid seed appeared possible with Owen's (49) discovery of cytoplasmically inherited male-sterility. Ideally, this made it possible to develop emasculated equivalents of the desired female parent of a cross by repeatedly backcrossing the desired hermaphroditic plant to one with male-sterile cytoplasm. However, Owen reported partial pollen restoration through the action of nuclear genes. He assumed two dominant pollen restoring genes, X and/or Z, in this type of cytoplasmic male-sterility. Therefore, it is also necessary to have the pollen parent of the emasculated equivalent (female) line recessive for the pollen restorer gene or genes. Type O plants (69, 70) then, have the cytoplasmic-genetic formula of N xx or Nxxzz and owe their pollen production ability to the N (normal) cytoplasm as contrasted to the S (male-sterile) cytoplasm.

In our search for type O plants, many plants were found to be segregating for the pollen restorer gene or genes but no true breeding type O plants were found. Conversations with other sugar beet breeders have revealed similar difficulties. The present investigation was undertaken to evaluate the factors influencing male-sterile phenotypes.

LITERATURE REVIEW

The complete or partial failure of microsporogenesis in plants may be caused by several factors, of which some operate externally and other internally.

Failure due to external factors have been reported by several investigators. Takenaka (66) reported a temperature effect on failure of microsporogenesis in Colchicum autumnale. Miyazawa (43) found pollen sterility in scarlet runner beans (Phaseolus multiflorus) growing at abnormally high temperature. Jones (32) reported sex reversals to neutral tassels of Zea mays by decreasing photoperiod. Pollen abortion as a result of photoperiod abnormalities was observed in soy beans (Soja max) by Nielsen (47). An entire series of failures in microsporogenesis was observed by Madsen (39) with an experimental group of Cosmos sulphureus CAV in which the photoinductive cycles and post inductive photoperiods were altered. Naylor and Davis (45) found that dilute concentrations of maleic hydrazide (0.025%) applied at the time of microsporogenesis caused sterility of the staminate inflorescences in maize while the pistillate flowers remained fertile. Rehm (52) states ". . . treatments with growth regulating substances has resulted in male sterility in cucurbitaceous plants and the tomato for periods of one to several weeks." Wittwer and Hillyer (68), working with cucurbits, were able to produce plants with the usual number of pistillate flowers and no staminate flowers by spraying with maleic hydrazide (250-350 PPM) at the time the first true leaf was expanding.

The internal causes of failure in microsporogenesis may be considered under three categories, namely: nuclear gene action, cytoplasm-nuclear gene interaction, and cytoplasmic action.

Nuclear Gene Action

Male-sterility due to recessive mutants has been reported in many plants, a partial listing is as follows: Alfalfa-Childers (10); cayenne pepper - Martin and Crawford (41); field corn - Eyster (18,19, 20), Beadle (6,7,8,9), Singleton and Jones (63), Emerson, Beadle, and Fraser (17); rape - Morinaga and Kuriyama (44); sorghum - Stephens (65), Hadjinov (26); sugar beets - Owen (50); Tomato - Lesley and Lesley (36), Rick (55,56), Mancini (40). Male sterility due to dominant genes has been reported in alfalfa by Childers (10), and in Origanum vulgare by Lewis and Crowe (38).

Cytoplasm-Nuclear Gene Interaction

According to Jones (30) cytoplasmic pollen abortion was first discovered by Correns in 1908. Bateson and Gairdner (5) in 1921 reported two types of segregation for male-sterility in flax. In 1929, Gairdner (24) defined cytoplasmic male-sterility in flax which was accompanied by pollen restorer genes. von Wettstein's (67) report agreed with that of Gairdner. Rhoades (53) first described cytoplasmic male-sterility in corn in 1933. Jones (29) in 1951 demonstrated that nuclear genes would restore pollen production in cytoplasmic male-sterile corn. Gabelman (23) partially characterizes the cytoplasmic factor (plasmagene) causing male-sterility in corn. He states

"The reproduction and distribution of this particle are quite similar to the reproduction and distribution of chromosomes. The similarity in reproduction holds both at meiosis and mitosis. The similarity of distribution holds quite well at mitosis but is completely random at meiosis. It must, therefore, have many characters in common with the chromosome." Newlin (46), working with maize, found that the nuclear genes for pollen restoration in male-sterile cytoplasm may vary in their effect under different environmental conditions. Rhoades (54) found that the iojap (ij) gene in corn caused cytoplasmic mutations, one of which was cytoplasmic male-sterility. He stated "Evidence of the particulate nature of this cytoplasmic factor is the occurrence of partially male-sterile plants with diverse percentages of pollen abortion. It is believed that these partially sterile plants arise from egg cells with a cytoplasm containing both normal and mutated particles. Completely male-sterile plants presumably possess only mutated particles while male-fertile individuals have non-mutated particles."

Cytoplasmic male-sterility in onions, described by Jones and Clarke (31) in 1943 as modified by the action of nuclear genes, may also be modified by temperature during the period from the tetrad stage to the first post meiotic division according to Barham and Munger (4).

In 1945, Owen (49) reported cytoplasmically interited male-sterility in sugar beets in which there was a partial restoration of pollen production due to the action of nuclear genes.

Lamm (35) indicated a male-sterility in potatoes which was probably due to interaction between the cytoplasm of S. tuberosum and one or more genes of S. acaule. Shams-Ul-Islam Khan (34) working with potatoes concluded: "Pollen sterility is a complex phenomenon, involving chromosomal, genic, physiological and environmental factors."

Michaelis (42) in experiments with Epilobium concluded that nuclear genes and plasmic units react together to form a complicated genetic system.

Cytoplasmic Action

Clayton (11) in 1950, working with hybrids whose genome complement consisted of strikingly non-homologous genomes and replacing (by successive back crosses) the female genomes of the hybrid with the male genomes, obtained cytoplasmic male-sterile tobacco from the cross Nicotiana debenyi by N. tabacum. Clayton stated, "Male-sterility appeared in BC_1, increased in BC_2 and was complete in BC_3."--"The gradual development suggested progressive incompatibility between cytoplasmic and nuclear constituents."

Fukasawa (22) was able to do essentially the same thing to make a cytoplasmic male-sterile durum wheat in his studies on restoration and substitution of nucleus in Aegilotricum (a Aegilops ovata by Triticum durum hybrid). He found that where extra chromosomes of Ae. ovata occurred, in addition to the 14 durum bivalents, only partial sterility resulted.

After reviewing the literature, it appears that a delicate balance must be maintained between the plasmagenes in the cytoplasm and the

chromogenes in the nucleus in order to have normal microsporogenesis. Although either plasmagenes or chromogenes may mutate to cause male-sterility, sooner or later a pollen restorer mutant will be found. External environment may alter the balance sufficiently to cause male-sterility or pollen-restoration, as the case may be. Apparently, only when the plasmagenes come from a widely divergent source from that of the chromogenes may one expect a lasting type of cytoplasmic male-sterility (one without pollen restoration).

EXPERIMENTAL METHODS

The following experiments were conducted to determine the role of various factors in pollen production in sugar beets containing male-sterile cytoplasm:

Experiment 1 -- Aceto-carmine slides were made of various anther types to determine possible microscopic characteristics which could differentiate anther types.

Experiment 2 -- Cytoplasmic male-sterile annual plants were subjected to varying lengths of cold treatments at different stages of seed stalk development to determine effects of cold temperature on pollen production.

Experiment 3 -- Branches of cytoplasmic male-sterile plants were treated in different ways with auxins and anti-auxins to determine possible auxin relationships to pollen production.

Experiment 4 -- A plant containing male-sterile cytoplasm, yet producing pollen, was selfed to observe the genetic segregation within the same cytoplasm and to observe possible linkages with the genes controlling monogermness.

Experiment 5 -- Observations were made on a number of single plant crosses to white anthered cytoplasmic male-sterile plants in an attempt to discover additional gene actions modifying the phenotypic expression of the major pollen restorer genes. Such an action is reported in the progeny I5-1-7E2.

Experiment 1

Materials and Methods

Several anthers were collected from each of several plants known to contain male-sterile cytoplasm. A description as to color, fullness of anther, and physiological age of each anther was recorded by plant number. An aceto-carmine smear was made of each freshly collected anther and immediate microscopic examination was made. Notes were made on abundance, size, shape, and sculpturing of pollen grains.

Results

White anthered plants.

Some anthers contained no pollen grains, although normal tetrads were observed. Other anthers contained sculptured pollen grains which were empty, with collapsed walls, and smaller than normal pollen grains which were unstained.

Yellow anthered plants.

The anther contents ranged from small, empty, collapsed, unstained, sculptured pollen grains to apparently normal viable pollen grains.

Conclusions

Since some empty, small, sculptured pollen grains with collapsed walls were found in anthers from nearly every white anthered plant examined, it was felt that the presence or absence of pollen grains was not a safe criterion for differentiating genotypes in male-sterile cytoplasm. For this reason, microscopic examination of anthers was discontinued and a search for ways to develop greater visual

differences between white and yellow anthers was continued.

Experiment 2

Male-sterile plants had been observed which were thought to be white anthered only to be classified as yellow anthered plants with a subsequent observation. Based on Owen's suggestion of the possible influence of cold on pollen restoration an experiment was initiated to study the effect of cold temperature on the production of pollen in male-sterile cytoplasm.

Materials and Methods

Twenty-four containers were planted with seed lot SL9460M, an annual cytoplasmic male sterile seed lot which Owen considered to be homozygous recessive for pollen restorer genes. To control nutritional variability nutrient solution and sand culture was used. Supplemental incandescent lights from 6pm to 6am were used from seedling emergence, December 18, 1953, to the completion of the experiment, March 26, 1954. The beets were thinned to ten per container. The cold treatment was started on February 9, 1954, when seed stalks ranged in height up to six inches. In the cold room, continuous incandescent lighting was maintained over the plants. The temperature at the crown of the beets was maintained between 46 and 50 degrees Fahrenheit.

The variables in this experiment were: the stage of development of seed stalk at time of exposure and duration of cold treatment. The schedule of treatments is shown in Table 1.

Results

The opening flowers were observed daily to determine any sectors of yellow anthers on the plants which could be related to the period of cold exposure. A few scattered yellow anthers were observed on some plants in almost every treatment including the control which had no cold exposure. Neither the yellow anthers nor the white anthers were confined to any specific sectors on the plant.

Table 1. Assignment of Treatments and Schedules to Groups (Containers).

Group No.	Type of Treatment	Treatment Started	Group No.	Type of Treatment	Treatment Started
1	48 hrs. cold	2-9-54	13	96 hrs. cold	2-9-54
2	48 hrs. cold	2-11-54	14	96 hrs. cold	2-13-54
3	48 hrs. cold	2-13-54	15	96 hrs. cold	2-17-54
4	48 hrs. cold	2-15-54	16	96 hrs. cold	2-21-54
5	48 hrs. cold	2-17-54	17	96 hrs. cold	2-25-54
6	48 hrs. cold	2-19-54	18	96 hrs. cold	3-1-54
7	48 hrs. cold	2-21-54	19	control, no cold treatment	
8	48 hrs. cold	2-23-54	20	control, no cold treatment	
9	48 hrs. cold	2-25-54	21	288 hrs. cold	2-9-54
10	48 hrs. cold	2-27-54	22	288 hrs. cold	2-9-54
11	48 hrs. cold	3-1-54	23	144 hrs. cold	2-9-54
12	48 hrs. cold	3-3-54	24	144 hrs. cold	2-21-54

Conclusions

Contrary to expectations, this population was not true breeding for all white anthered plants. The lack of any consistent pattern of anther color on the treated plants as well as the presence of yellow anthers on the check plants indicates that the cold treatments given in this experiment were not effective in altering the anther color of

the plants in the population SL9460M. Although this experiment does not rule out the possibility that cold temperature may influence pollen restoration in plants having male-sterile cytoplasm, it can be said that a temperature of 46° to 50° F does not have a pronounced effect on pollen restoration.

Experiment 3

A branch of a plant, containing S cytoplasm but having both white and yellow anthers, was observed to have a preponderance of yellow, full, and dehiscing anthers near the tip which had reassumed an upright position following accidental inversion. A temporary change in the auxin concentration during development of the anthers was postulated to account for the apparent shift to yellow, full, dehiscing anthers. This experiment was designed to test the possible role of auxin in pollen restoration.

Materials and Methods

Indole acetic acid (IAA) was used as the auxin source at a concentration of 1,000 parts per million. Maleic hydrazide (MH) at a concentration of 200 parts per million was used as a growth inhibitor. Two methods of application were used. In the first method the ends of branches were submerged in the treatment solutions for varying lengths of time, one set of branches had the tips removed while another set of branches was left intact. Eight plants were used to test the effects of submersion of branch tips in IAA and MH. The design of experiment is shown in Table 2.

The second method of application involved spraying the terminal portions of the branches. Branches of white anthered and yellow anthered plants were sprayed with IAA while other branches of the same plants were sprayed with MH. These marked branches were observed during their subsequent blooming period.

The tips of branches on many plants of both white and mixed white and yellow anther types were tied in an inverted position in an effort to substantiate the original observation.

Table 2. Schedule of treatments by plant number for branch tips to be submersed in Auxin, Anti-Auxin, and Water.

Hours Submersion	MH	IAA	Water
1.5	5, 3	5, 3	5, 3
24	1, 10	1, 10	1, 10
48	2, 4	2, 4	2, 4
72	8, 9	8, 9	8, 9

Results

Necrosis of the tips of the branches occurred after 24 hours submersion in the IAA solution, hence all IAA submersion treatments were discontinued after 24 hours. The internodes of the IAA-treated plants elongated considerably, probably because of an increase in auxin content of the cells. IAA and MH had no visible effect on anther color. No alteration in anther color was noted from the spray treatments as compared to unsprayed branches.

The tips of seed branches tied in the inverted position responsed geotropically but without an alteration in anther color.

Tips of seed stalk branches were removed from several white and several yellow anthered plants to alter the auxin concentration. No effects were noted from this treatment. Perhaps leaflets also should have been removed to further alter the auxin relationship.

Conclusion

Although these experiments do not exclude the possibility of auxins having an effect on anther color the evidence fails to indicate that they play a major role.

Experiment 4

Several cytoplasmic semi-male-sterile plants considered to be heterozygous for monogermness, Mm, were selfed to study the anther types of the segregates and possible linkages between the pollen restorer gene or genes and the gene controlling monogermness. Only one of the selfed plants, 13H15-169, set enough seed to permit a thorough study of its progeny. The progeny from this plant were classified for monogerm and multi-germ type plants and also for anther types according to the following eight classes:

Y_1 -all anthers yellow and dehiscing.

Y_2 -all anthers yellow, no dehiscing observed.

Y_3 -most anthers yellow and dehiscing but some anthers, at least, were not definitely yellow.

Y_4 -most anthers yellow, no dehiscing observed, some anthers "white".

Y_5 -most anthers "white" but some anthers yellow and partially full.

Y_6 -most anthers "white" but some anthers yellow and shrunken.

W_1 -anthers dark and shrunken with no yellow visible or anthers a light buff color not typical of white anthers.

W_2 -anthers white with no yellow coloration observed.

The number of plants in each phenotypic class in the selfed progeny of 13H15-169 are presented in Table 3. The data indicate that the plant 13H15-169 was heterozygous at the locus for anther color and at another locus for monogermness.

Table 3. Number of plants in each phenotype.

	Anther Types								
	Y_1	Y_2	Y_3	Y_4	Y_5	Y_6	W_1	W_2	Total
monogerm	6	0	2	1	0	3	4	33	49
double germ	37	2	14	4	10	3	15	53	138
total	43	2	16	5	10	6	19	86	187

If the cytoplasmic-genetic constitution of plant 13H15-169 for anther color and monogermness is assumed to be S XxMm, then there were far too many white anthered plants in the progeny to permit an explanation that one dominant X gene will always result in a yellow anthered plant. However, if one postulates: first, that the homozygous dominant (XX) genes in male sterile cytoplasm (S) produce plants which have all yellow full anthers; second, that the homozygous recessive (xx) genes in S cytoplasm give all white anthers; and third, that the

heterozygous (Xx) plants have anther type classifications which range from the Y_3 category (most anthers yellow full) through the white category, then the homozygous dominant Y_1 and Y_2 categories (all yellow, full anther phenotype) can be tested versus the combination of the other phenotypes using a 1:3 ratio. Such a chi-square test gave a good fit, with a probability figure of 70% to 80% (Table 4). Another possible explanation for anther color would be a duplicate recessive epistasis ratio, in which xx is epistatic to Z, and zz is epistatic to X. Such a gene action would give a ratio of 9 yellow to 7 white. The fit of this ratio to the data is dependent upon the genotype of the W_1 anther color phenotype. If the W_1 anther color type is classed as yellow, the chi-square test gives a fit of P = 50-70%, however if the W_1 anther color is considered as actually white then the chi-square test gives a fit of P = less than 1%. Other data to be given in experiment 5 concur with the postulates of the first explanation of the inheritance of anther color.

The chi-square test in Table 4 indicates P value of 70% for one set of alleles controlling the monogerm character, which is in agreement with the findings of Savitsky (58). The dominant multigerm allele involved in this progeny was probably the M^1 allele described by Savitsky (59) since no more than two flowers per cluster were observed on the parent plant or its progeny.

When the two factors, anther color and monogermness, were tested together in a chi-square test the P value was reduced from 70% for each independent ratio to 10-20% for the combined independent

Table 4. Chi-Square tests of 13H15-169 S_1 progeny.

Class	Observed	Calculated	(o-c)	$\frac{(o-c)^2}{c}$	
1:3 segregation all yellow full anthers v.s. "white anthers"					
Yellow (y_1-y_2)	15	46.75	-1.75	0.0771	
"White" (y_3-w)	142	140.25	1.75	0.0218	
	187	187.00	0.00	x^2=0.0989	P=70-80%
Multigermness v.s. Monogermness 3:1 segregation					
Multigerm	138	140.25	-2.25	0.036	
Monogerm	49	46.75	2.25	0.108	
	187	187.00	0.00	x^2=0.144	P=70%
Combined germ type and anther color (independent inheritance)					
Yellow multi-	39	35.07	3.93	0.4404	
"White" multi	99	105.21	-6.21	0.3665	
Yellow mono-	6	11.69	-5.69	2.7696	
"White" mono-	43	35.07	7.93	1.7931	
	187	187.04	-0.04	x^2=5.3696	P=10-20%
Combined germ type and anther color (Linkage in coupling phase with 37.5% crossover)					
Yellow multi-	39	40.18	-1.18	0.0347	
"White" multi	99	100.08	-1.08	0.0116	
Yellow mono-	6	6.57	-0.57	0.0495	
"White" mono	43	40.17	2.83	0.1994	
	187	187.00	0.00	x^2=0.2952	P=95-98%

inhertiance ratio. If a linkage in the coupling phase, with 37.5% crossover, is assumed, then the P value is increased to 95-98%. Pleiotropic action of the monogerm gene in the recessive mm genotypes to give more "white" phenotypes would be another possible explanation for the reduction of the P values upon combining the independent ratios. The linkage theory is preferred.

Experiment 5

The anther color segregation was checked for a number of two plant (one normal cytoplasm hermaphroditic male plant and one male-sterile cytoplasm, white anthered female plant) crosses in an attempt to discover more about the action of the pollen restorer genes. An unusual gene action was noted in the progeny I5-1-7E2. The 128 plants in this progeny were given permanent numbers to identify them in subsequent studies. The plants were individually classified at four different dates as to anther color, fullness of anthers, and dehiscence. They were first classified in the field June 29, 1954 when they were past full bloom. These same stecklings sent out a second growth of seed stalks as the first crop of seed was maturing. The anthers were classified again August 27 which was approaching the end of the second blooming period. The plants were potted in the fall and moved to the greenhouse where the third reading was made February 24, 1955, just previous to full bloom and the fourth, March 3 as the terminal blooming period approached. The following anther classification was used:

1_y -yellow, full, dehiscing.

2_y -yellow, full, delayed dehiscence.

3_y -anthers of earliest blooms lacking definite yellow colorations and shrunken in appearance, however, in later blooms yellow partially full and dehiscing anthers appear.

4_y -same as 3_y except with delayed dehiscence.

5_y -yellow, shrunken, no dehiscence. Anthers of earliest blooms may lack definite yellow coloration.

6_w -anthers white, shrunken, sometimes darkened before blooming.

Four branches of each plant moved to the greenhouse were bagged just prior to blooming. Of the 128 plants, 4 died after the first reading of anther type; 23 of the 128 were always judged to be 1_y and of these, 4 set no selfed seed, 2 set seed with imperfect embryos, and the other 17 set normal selfed seed but only 10 produced enough for progeny testing; 28 of the 128 had yellow full anthers in at least one of the classification dates and also fell within the categories 3_y to 6_w at least one time; 17 of the 128 had at least one classification of 5_y and either a 5_y or 6_w the other times, 10 of these were 5_y at all classifications; while the remaining 56 of the 128 were always observed to be in the 6_w category. The complete classification of all plants on the different dates are given in Table 5 along with the identified genotypes from Table 9. From Table 5 it is apparent that anther classifications varied considerably between different reading dates for several of the individual plants.

A complementary factor controlling the expression of fullness of anthers was indicated during the June 29, 1954 reading and re-evaluated at subsequent readings. This new gene locus has been designated the Sh locus for the generally shrunken anthers of the homozygous recessive phenotype. Depending on the genotypes of the parents, the

Table 5. Anther classification by dates, notes on set of selfed seed, and genotypes (where known) of the 128 plants in progeny I5-1-7E2.

Pl. No.	Self seed set	Class by month 6 8 2 3	Pl. No.	Self seed set	Class by month 6 8 2 3	Constant all Dates by plant number (1_y) (5_y) (6_w)				Pl. No.	Self seed set	Class by month 6 8 2 3	Identified genotype (Table 9)
2	m	3 3 1 1	75	u	3 4 1 1	15m	5n	1	50	114	21	g	1 1 1 1	S XxShSh
3	n	3 6 v 5	77	m	3 3 1 1	42m	28n	4	54	115	51	g	1 1 v 1	S XxShSh
10	m	3 v 1 1	79	d	6 d d d	56m	33u	6	64	116	55	g	1 1 1 1	S XxShSh
14	m	3 4 1 1	80	d	4 d d d	69m	44u	7	65	118				
16	n	6 6 5 5	88	u	3 5 4 3	87u	52u	8	66	121	9	g	1 1 1 1	S XxShsh
											17	g	1 1 1 1	S XxShsh
24	m	4 3 1 1	91	n	3 5 4 3	95m	60n	11	68	122	47	g	5 1 1 1	S XxShsh
27	m	3 3 4 5	94	u	6 5 6 5	97n	73u	13	70	123	53	g	1 1 1 1	S XxShsh
31	m	4 3 1 1	96	u	6 5 5 5	100n	81n	18	71	124	57	g	3 1 1 1	S XxShsh
34	m	1 3 1 1	103	d	3 d d d	102n	84n	19	74	125	82	g	1 1 1 1	S XxShsh
35	m	2 4 6 5	110	u	1 4 1 1	109m	93u	20	76	127	85	g	1 1 4 3	S XxShsh
											98	g	1 1 1 1	S XxShsh
38	m	3 1 1 1	112	n	5 6 6 5	111u		22	78		106	g	1 1 1 1	S XxShsh
40	m	5 5 5 3	117	u	3 5 5 5	119m		23	83		113	g	1 1 1 1	S XxShsh
45	n	6 5 5 5	120	m	5 6 5 5	126n		25	86					
48	m	5 1 1 1	128	m	1 4 4 3			26	89		12	g	5 5 3 3	S Xxshsh
58	n	5 6 5 5						29	90		61	g	5 5 5 4	S Xxshsh
59	m	1 3 1 1						30	92		43		6 6 6 6	S xxShsh
62	d	6 d d d						32	99		49		6 6 v 6	S xxShsh
63	u	3 5 5 3						36	101		104		6 6 6 6	S xxShsh
67	m	5 3 3 3						39	107		105		6 6 6 6	S xxShsh
72	u	3 4 1 1						41	108					
											37		6 6 6 6	S xxshsh
											46		6 6 6 6	S xxshsh

n = no selfed seed set
u = selfed "seeds" but embryos undeveloped
m = good selfed seed but not enough for progeny test
g = enough good selfed seed for progeny test
d = plant dead
v = plant not blooming when classification was made

presence of the Sh gene may change the expected backcross ratio of 1:1 for the X-x locus to a ratio of 3 yellow full: 1 yellow shrunken: 4 white or a ratio of 1 yellow full: 1 yellow shrunken: 2 white. For example, S xxShsh by N XxShsh would give a 3:1:4 ratio because Sh fails to act in the presence of xx in S cytoplasm and for the same reason S xxShsh by N Xxshsh gives a 1:1:2 ratio. The data in Table 6 show a good fit to a 3:1:4 ratio indicating the genotype of the female parent of I5-1-7E2 to be S xxShsh and that of the male parent to be N XxShsh.

To further test the action of the Sh gene, the progenies from open pollinated white anthered plants (classified 6_w at all classifications) in the isolation plot containing the I5-1-7E2 progeny were indexed in the spring of 1955. Since the only source of pollen in this isolation plot was from the yellow dehiscing anthers, the genotypes of the white anthered females could be determined, assuming even distribution of pollen, from the segregation ratios observed in plants grown from the open pollinated seed. The derivations of the ratios for each genotype are given in Table 7.

Since the size of progeny required to differentiate the expected ratios was large, classification was completed for only six of the open pollinated white anthered progenies. Of these six progenies, four fit the 5:1:6 ratio for S xxShsh mother plants and two fit the 2:1:3 ratio for S xxshsh mother plants. The chi-square tests for these ratios are given in Table 8.

Table 6. Chi-Square tests of I5-1-7E2 progeny by dates of classification fit to a 3:1:4 ratio

Date	Class	Observed	Calculated	o-c	$\frac{(o-c)^2}{c}$	
6-29-54	1-4_y	47	48	-1	0.0208	
	5_y	19	16	3	0.5625	
	6_w	62	64	-2	0.0625	
		128	128	0	x^2=0.6458	P=70-80%
8-27-54	1-4_y	42	45.38	-3.38	0.2517	
	5_y	19	15.13	3.87	0.9899	
	6_w	60	60.50	-0.50	0.0041	
		121	121.01	-0.01	x^2=1.2457	P=50-70%
2-23-55	1-4_y	41	41.25	-0.25	0.0152	
	5_y	17	13.75	3.25	0.7682	
	6_w	52	55.00	-3.00	0.1636	
		110	110.00	0.00	x^2=0.9470	P=50-70%
3-3-55	1-4_y	46	44.25	1.75	0.0692	
	5_y	19	14.75	4.25	1.2246	
	6_w	53	59.00	-6.00	0.6102	
		118	118.00	0.00	x^2=1.9040	P=30-50%
Probable	1-4_y	51	46.5	4.5	0.4355	
True	5_y	17	15.5	1.5	0.1452	
Phenotype	6_w	56	62.0	-6.0	0.5806	
		124	124.0	0.0	x^2=1.1613	P=50-70%

Table 7. Derivation of Ratios Expected from Sib-Pollination of White Anthered Plants in I5-1-7E2 Progeny with the Yellow Dehiscing Anthered Plants in the same Progeny.

Parents				Offspring
Phenotypes	Genotypes			
Male			Male Gametes	
1_y-4_y	1 S XxShSh		1(2 XSh : 2 xSh)	
1_y-4_y	2 S XxShsh		2(1 XSh : 1 xSh : 1 Xsh : 1 xsh)	
5_y	1 S Xxshsh		Pollen Production Negligible	
		Male Gametes	4 XSh 4 xSh 2 Xsh 2 xsh	
Female		Female Gametes	Genotypes of Progeny	Phenotypic Ratios expected
6_w	1 S xxShSh	xSh	4XxShSh 4xxShSh 2XxShsh 2xxShsh	1(1_y-4_y) : 1(6_w)
6_w	2 S xxShsh	xSh	4XxShSh 4xxShsh 2Xxshsh 2xxshsh	5(1_y-4_y) : 1(5_y): 6(6_w)
		xsh	4XxShsh 4xxShsh 2Xxshsh 2xxshsh	
6_w	1 S xxshsh	xsh	4XxShsh 4xxShsh 2Xxshsh 2xxshsh	2(1_y-4_y) : 1(5_y) : 3(6_w)
5_y*	? S Xxshsh	Xsh	4XXShsh 4XxShsh 2XXshsh 2Xxshsh	7(1_y-4_y) : 2(5_y) : 3(6_w)
		xsh	4XxShsh 4xxShsh 2Xxshsh 2xxshsh	

* 5_y appears as 6_w under certain conditions.

Table 8. Chi-Square tests of open pollinated progenies from white anthered plants.

Plant No.	Best fitting ratio	Observed	Calculated	o-c	$\frac{(o-c)^2}{c}$	
43	5 (1_y-4_y)	45	43.75	1.25	0.036	
	1 (5_y)	8	8.75	-.75	0.064	
	6 (6_w)	52	52.5	-.50	0.048	
		105	105.00	0.00	x^2=0.148	P=90-95%
105	5 (1_y-4_y)	46	40.415	5.585	0.772	
	1 (5_y)	9	8.083	0.917	0.104	
	6 (6_w)	42	48.498	-6.498	0.871	
		97	96.996	0.004	x^2=1.747	P=30-50%
104	5 (1_y-4_y)	45	37.5	7.5	1.500	
	1 (5_y)	7	7.5	-0.5	0.033	
	6 (6_w)	38	45.0	-7.0	1.089	
		90	90.0	0.0	x^2=2.622	P=20-30%
49	5 (1_y-4_y)	53	42.5	10.5	2.594	
	1 (5_y)	9	8.5	.5	0.029	
	6 (6_w)	40	51.0	-11.0	2.373	
		102	102.0	0.0	x^2=4.996	P=5-10%
37	2 (1_y-4_y)	31	32	-1	0.031	
	1 (5_y)	15	16	-1	0.063	
	3 (6_w)	50	48	2	0.083	
		96	96	0	x^2=0.177	P=90-95%
46	2 (1_y-4_y)	20	31.668	-11.668	4.299	
	1 (5_y)	22	15.834	6.166	2.401	
	3 (6_w)	53	47.502	5.498	0.636	
		95	95.004	-0.004	x^2=7.336	P=2-5%*

* Plant 46 occurred in the isolation plot next to plant 47 later identified as a SXx Sh sh genotype, which, when crossed to a Sxx sh sh plant, would give a 1:1:2 ratio very close to the observed ratio.

An additional test of the interactions of the X-x and Sh-sh alleles was obtained when the selfed progenies of the yellow anthered I5-1-7E2 plants were classified in the greenhouse during the winter of 1955-56. Sufficient plants were obtained from 15 of the 38 lots of selfed seed to index them for anther type. The results of this classification are given in Table 9. Three of the progenies had parents of the S XxShSh genotype; ten of them had parents of the S XxShsh genotype; and two progenies had parents (plants 12 and 61) of the S Xxshsh genotype. The anther classification of plant 12 was 5_y (yellow shrunken) in the first two readings, and 3_y (yellow "full") in the last two. Plant 61 had an anther classification of 5_y in the first three readings and 4_y in the fourth. This evidence corroborates experiment 4 in that the S Xx genotype, without the dominant Sh gene, has a variable phenotypic expression ranging from empty white to yellow, partly full and dehiscing anthers with viable pollen.

The variable phenotypic expression of the S Xx genotype has made the task of indexing for true breeding type O (N xx) hermaphrodites very difficult, since the observed ratios in the progenies may be unreliable and the genotype of the female parent of the progeny may have been S Xx instead of S xx. Although there is still some phenotypic variability in the yellow anthered plants containing the dominant Sh factor, the presence of this factor seems to insure sufficient pollen in the anthers to avoid misclassification as a white anthered plant.

Anther classifications should not be made too early in the blooming period, since anthers of the early opening flowers may be nearly

Table 9. Results of Chi-Square Tests to Determine Genotypes From the Selfed Progeny of the Yellow Anthered Plants in the I5-1-7E2 Progeny

Plant No.	Observed Ratio 1_y-4_y	5_y	6_w	Best fitting Ratio 1_y-4_y	5_y	6_w	P Value	Probable Genotype
21	36	0	11	3	0	1	80%	S XxShSh
51	21	0	5	3	0	1	50%	S XxShSh
55	97	0	29	3	0	1	80-90%	S XxShSh
9	28	4	14	5	1	2	50-70%	S XxShsh
17	32	1	8	5	1	2	5-10%	S XxShsh
47	63	8	22	5	1	2	30-50%	S XxShsh
53	54	2	21	5	1	2	2-5%	S XxShsh
57	63	7	26	5	1	2	30%	S XxShsh
82	87	6	26	5	1	2	1-2%	S XxShsh
85	39	12	30	5	1	2	2-5%	S XxShsh
98	68	8	15	5	1	2	5-10%	S XxShsh
106	36	4	25	5	1	2	2-5%	S XxShsh
113	14	3	12	5	1	2	10-20%	S XxShsh
12	12	8	21	1	"3"		50-70%	S Xxshsh
61	9	3	13	1	"3"		30-50%	S Xxshsh

white even though subsequent flowers will have yellow, full anthers. Some flowers have been observed to contain both white and nearly full yellow anthers. White anthered plants used as females to index for type O plants should be checked several times to see that they remain white anthered plants.

The Sh allele may be valuable in cytoplasmic male-sterile indexing work, since the progeny from the cross S xxShSh by N ------ should not have the yellow shrunken category which is difficult to detect. The Sh gene may be thought of as a complimentary gene, since it will enhance pollen production if one of the genetic factors for pollen restoration is present in male-sterile cytoplasm.

Summary of Experiments 4 and 5

A study of the phenotypic expression of various semi-male-sterile genotypes in cytoplasmic male-sterile sugar beets has revealed a new genetic factor Sh which, when present in the dominant condition, enhances the pollen-producing ability of the S Xx to such an extent that its classification as a white anthered phenotype is improbable.

The S Xxshsh genotype has a variable expression of anther color which leads to faulty phenotypic ratios.

Linkage between the X-x locus for pollen restoration in male-sterile cytoplasm and the M-m locus for monogermness is indicated. However, pleiotropic action of the monogerm gene in the recessive mm genotypes to produce more white anthered phenotypes is not precluded.

GENERAL SUMMARY AND CONCLUSIONS

Since cytoplasmic male-sterility in the sugar beet is the result of a cytoplasm-gene intereaction, one should expect to find pollen restoration in varying degrees brought about by mutations in either the nuclear or the cytoplasmic components. Environmental changes also could be responsible for pollen restoration.

The effects of cold temperatures applied during microsporogenesis were studied without successfully altering the phenotypes of the progeny SL9460M.

An attempt to alter the degree of pollen restoration through changes in the auxin concentrations within the plant was without success.

A study of the S_1 progeny of a plant, containing male-sterile cytoplasm yet producing pollen, indicated that a gene for pollen restoration and the gene for monogermness were carried on the same chromosome. Furthermore, this progeny revealed the indefinite phenotypic expression of the heterozygous pollen restorer genotype.

Breeding experiments with another progeny lead to the discovery of a mutation which would complement the restoring action of the pollen restoring gene to the extent that the misclassification of the heterozygous pollen restoring genotypes was improbable. The dominant complementary gene (Sh) was found to be lacking in pollen restoring ability when the dominant pollen restorer gene (X) was absent in male-sterile cytoplasm.

Although no cytoplasmic mutation for pollen restoration were identified in this study, the observation that some flowers contain

both empty white and yellow full anthers might be explained on a cytoplasmic basis. Also, it is not clear why plants considered white anthered at one classification have yellow anthers on another date while neighboring plants concurrently change from yellow to white categories.

BIBLIOGRAPHY

1. *Anonymous, from the report of the Wisconsin Agricultural Experiment Station as quoted in Plant Breeding Abstracts: 22: abst. 1717. 1952.

2. *Artschwager, Ernst. Pollen degeneration in male-sterile sugar beets with special reference to the tapetal plasmodium. Jour. Agric. Res.: 75: 191-197: 1947.

3. *Baker, H. G. The inheritance of certain characters in crosses between Melandrium dicicum and M. album. Genetica 25: 126-156. 1950.

4. Barham, W. S. and Munger, E. M. The stability of male-sterility in onions. Amer. Soc. Hort. Sci. Proc. 56: 401-409. 1950.

5. Bateson, M. A. and Gairdner, A. E. Male sterility in flax, subject to two types of segregation. Jour. Genetics 11: 269-275. 1921.

6. Beadle, G. W. Genetical and Cytological studies of mendelian asynapsis in Zea mays. Cornell Agric. Expt. Sta. Mem. 129: 1-23. 1930.

7. Beadle, G. W. A gene in maize for supernumerary cell divisions following meiosis. Cornell Agric. Expt. Sta. Mem. 135: 1-12. 1931.

8. Beadle, G. W. Genes in maize for pollen sterility. Genetics. 17: 413-431. 1932.

9. Beadle, G. W. A gene in Zea mays for failure or cytokinesis during meiosis. Cytologia, 4: 269-287. 1933.

10. Childers, W. R. Male sterility in Medicago sativa L. Sci. Agric. 32: 351-64. 1952.

11. Clayton, E. E. Male sterile tobacco. J. Hered. 41: 171-175. 1950.

12. *Correns, C. Title not available Ber. Deut. Botan. Ges. 36: 686-701. 1908.

13. *Correns, C. Bestemmung, Veerburg und Verteilung des Geschlechtes bei der hoheren pflanger. Handb. Vererbangsw. 2: 126-138. 1928.

*Supplementary references

14. *Currence, T. M. A combination of semisterility with two simple inherited characters that can be used to reduce the cost of hybrid tomato seed. Proc. Amer. Soc. Hort. Sci. 44: 403-406. 1944.

15. *Domingo, W. E. The development of domestic castor bean production Econ. Bot. 7: 65-75. 1953.

16. *Edwardson, J. R. The restoration of fertility to cytoplasmic male-sterile corn. Agron. J. 47: 457-461. 1955.

17. Emerson, R. A., G. W. Beadle, and A. C. Fraser. A summary of linkage studies in maize. Cornell Univ. Agric. Expt. Sta. Memoir 180: 1-83. 1935.

18. Eyster, L. A. Hertiable characters of maize. VII. Male sterile. J. Hered. 12: 138-141. 1921.

19. Eyster, W. H. Heritable characters of maize. XXXIX. Male sterile. J. Hered. 22: 117-119. 1931.

20. Eyster, W. H. Heretible characters of maize. Male Sterile. J. Hered. 22: 99-102. 1931.

21. *Frankel, O. H. Studies in Hebe. II. The significance of male sterility in the genetic system. J. Genetics 40: 171-184. 1940.

22. Fukasawa, Hirosuke. Studies on restoration and substitution of nucleus in Aegilotricum 1. Appearance of male-sterile durum in substitution crosses. Cytologia 18: 167-175. 1953.

23. Gabelman, W. H. Reproduction and distribution of the cytoplasmic factor for male sterility in maize. Proc. Nat. Acad. Sci. 35: 634-639. 1949.

24. Gairdner, Alice E. Male sterility in flax. II. A case of reciprocal crosses differing in f_2. J. Genetics 21: 117-124. 1929.

25. *Galinat, W. C. Glumeless hybrid sweet corn and the use of Vg gene in producing crossed corn. J. Hered. 42: 115-116. 1951.

26. Hadjinov, M. I. Sterility in varietal hybrids of sorghum. Bull. Appl. Bot. Gen. Pl. Breed. series II, no. 7, pp. 442-446, 1937.

27. *Horovitz, S. and Obregon, P. El caracter de "Anteras indehiscentes" en maiz y su posible utilizacion en la produccion de hibridos. (The character "indehiscent anthers" in maize and its possible use in the production of hybrids). Agron. Trop. Rev. Inst. Nac. Agric., Venezuela 1:77-82. 1951, from Plant Breed. Abst. 22: abst. 353.

28. *Jenkins, T. J. Self-fertility in perennial rye grass. Bull. Welsh Pl. Breed. Sta. 12: 100-119. 1931.

29. Jones, D. F. The interrelation of plasmagenes and chromogenes in pollen production in maize. Genetics 35: 507-512. 1950.

30. Jones, D. F. The cytoplasmic separation of species. Natl. Acad. Sci. Proc. 37: 408-410. 1951.

31. Jones, H. A. and Clarke, A. E. Inheritance of male sterility in the onion and the production of hybrid seed. Proc. Am. Soc. Hort. Sci. 43: 189-194. 1943.

32. Jones, Kenneth L. Studies on Ambrosia. IV. Effects of short photoperiod and temperature on sex expression. Amer. J. Bot. 34: 371. 1947.

33. Josephson, L. M. Effect of Maleic hydrazide in delaying flowering in corn. Agron. J. 43: 404-405. 1951.

34. Khan, Shams-UI-Islam. Pollen sterility in Solanum tuberosum L. Cytologia, Tokyo, 16: 124-130. 1951 from Pl. Breed. Abst. 22: abst. 2029.

35. Lamm, R. Investigations on some tuber-bearing Solanum hybrids. Hereditas 39: 97-112. 1953.

36. Lesley, J. W. and Margaret M. Lesley. Unfruitfulness in the tomato caused by male sterility. J. Agric. Res. 58: 621-630. 1939.

37. *Lewis, D. Male sterility in natural populations of hermaphrodite plants. The New Phytologist 41: 56-63. 1941.

38. Lewis, D. and Crowe, L. K. Male sterility as an outbreeding mechanism in Origanum vulgare. Heredity 6: 136 (abst.) 1952.

39. Madsen, G. C. Influence of photoperiod on microsporgenesis in Cosmos sulphurius cav. var. Klondike. Bot. Gaz. 109:120. 1947.

40. Mancini, E. La produzione delle sementie ibride. (The production of hybrid seed). Riv. Ortoflorofrut ticolt. Ital., Fironze 1952: 77: 12-21. from Pl. Breed. Abst. 22: abst. 1579.

41. Martin, J. A. and Crawford, J. H. Several types of sterility in Capsicum frutescens. Proc. Amer. Soc. Hort. Sci. 57: 335-338. 1951.

42. Michaelis, P. Interactions between genes and cytoplasm in Epilobium. Cold Springs Harbor Symposia on Quantitative Biology 16: 121-129. 1951.

43. Miyazawa, A. Sterility of Phaseolus Multiflorus under high temperature. Annual Report of the National Institute of Genetics, Japan, 1949-50: 1:33. 1951.

44. Morinaga, T. and Kuriyama, H. Idengaku Zashi Jap. J. Genet. 1949: 24: 35-36. Heritable sterility in Brassica (B. napus) Pl. Breed. Abst. 23: abst. 440. 1953.

45. Naylor, A. W. and Davis, E. A. Maleic hydrazide as a plant growth inhibitor. Bot. Gaz. 112 (1): 112-126. 1950.

46. Newlin, O. J. Inheritance of fertility restoration in cytoplasmic male-sterile Zea mays. Diss. Abs. 15: 1478. Oct. 1955.

47. Nielson, C. G. Effects of photoperiod on microsporogenesis in biloxi soybeans. Bot. Gaz. 104:99. 1942.

48. *Offerijns, F. J. M. Meiosis in the pollen mother cells of Canna glauca, "pure yellow." Genetica 20: 59-65. 1938.

49. Owen, F. V. Cytoplasmically inherited male-sterility in sugar beets. Jour. Agric. Res. 71: 423-440. 1945.

50. Owen, F. V. Mendelian male sterility in sugar beets. Proc. Amer. Soc. of Sugar Beet Technologists. 371-376. 1952.

51. Owen, F. V. Interpretation of cytoplasmically inherited male sterility in sugar beets. Proc. Eighth Internat. Cong. Genet. (abstract) 638-639. 1949.

52. Rehm, S. Male sterile plants by chemical treatment. Nature, London. 170: 38-39. 1952.

53. Rhoades, M. M. The cytoplasmic inheritance of male sterility in Zea mays. J. Gen. 27: 71-93. 1933.

54. Rhoades, M. M. Genic induction of an inherited cytoplasmic difference. Proc. Nat. Acad. Sci. 36: 634-635. 1950.

55. Rick, C. M. A new male-sterile mutant in the tomato. Science 99: 543. 1944.

56. Rick, Charles M. A survey of cytogenetic causes of unfruitfulness in the tomato. Genetics 30: 347-362. 1945.

57. *Sakai, K. (Hypertrophy of the rice tapetum with special reference to its significance for breeding for resistance to cold). Jap. J. Genet. 1946: 21: 68-69. (Japanese) from Pl. Breed. Abst. 22: abst. 422.

58. Savitsky, V. F. Monogerm sugar beets in the United States. Proc. Am. Soc. Sugar Beet Tech. 156-159. 1950.

59. Savitsky, V. F. Inheritance of the number of flowers in flower clusters of Beta vulgaris L. Proc. Amer. Soc. Sugar Beet Tech. 3-15 in part 2. 1954.

60. Schaffner, J. H. Sex reversal and production of neutral tassels in Zea mays. Bot. Gaz. 90: 279. 1930.

61. *Schwartz, D. A case of male sterility in maize involving gene-cytoplasm interaction. Genetics 36: 575 (abst.). 1951.

62. *Schwartz, D. The interaction of nuclear and cytoplasmic factors in the inheritance of male sterility in maize. Genetics 36: 676-696. 1951.

63. Singleton, W. R. and Jones, D. F. Heritable characters of maize. XXXV. Male sterile. J. Hered. 21: 266-268. 1930.

64. *Smith, H. H. Relation between sterility and morphological characters in an interspecific Nicotiana cross. Genetics 36: 576 (abst.). 1951, and Genetics 37: 26-38. 1952.

65. Stephens, J. C. Male sterility in sorghum: its possible utilization in production of hybrid seed. Jour. Amer. Soc. Agron. 29: 690-695. 1937.

66. Takenska, Y. Sterility and autotoxicosis of colchicum autumnals. Annual Report of the National Institute of Genetics, Japan, 1949-50: 130-31. 1951.

67. von Wettstein, F. Untersuchungen zur plasmatischen Vererbung. I. Linum. (Investigations on plasmatic inheritance. I. Linum). Biol. Zbl. 65: 149-166. 1946, from Pl. Breed. Abst. 23: pp. 293.

68. Wittwer, S. H. and I. G. Hillyer. Chemical induction of male-sterility in cucurbits. Science 120: 893-894. 1954.

69. Owen, F. V. Utilization of male sterility in breeding superior-yielding sugar beets. Proc. Amer. Soc. Sugar Beet Tech. 157-161. 1948.

70. Owen, F. V. The sugar beet breeder's problem of establishing male-sterile populations for hybridization purposes. Proc. Amer. Soc. Sugar Beet Tech. 191-194. 1950.