Sugar Beet Male Sterility

R. K. OLDEMEYER[1]

Cytoplasmic male-sterility is the only practical, if not the only tool for enforcing hybridization on a large scale in sugar beets. Little if any genetic research has been done regarding this complex character in sugar beets since Owen's reports (6)[2] (7) in 1945 and 1950. Limited plantings of commercial sugar beet inbred x variety hybrids utilizing cytoplasmic male sterility have been made recently by some commercial companies. Corn breeders are now successfully using cytoplasmic male sterility for the commercial production of hybrids. Several sources of maize cytoplasm are available, (2) (5) and the one in use is capable of causing castration over a broad range of inbreds (8) (5). Very effective emasculation can be accomplished by the cytoplasm avialable in sorghums (9).

Sugar beet plants having genotypes, which with sterile cytoplasm, can be castrated, are rather rare in open-pollinated populations. This limits the proportion of inbred lines which can be made male sterile, and prevents the full exploitation of the male-sterile character unless a long and an intensive program is undertaken to transfer suitable genotypes to the necessary inbreds.

The purpose of this paper is to (a) report observations and results of experiments of the Great Western Sugar Company for the last two years regarding cytoplasmic male sterility and (b) point up the necessity for basic research regarding male sterility.

Indexing Variation Attributed to the Male-Sterile Parent

Intrasource variation overshadowed intersource variation as to castration of progeny from the same pollen parent when a Great Western source and a USDA (Owen) source of male sterility were compared. These results prompted a design of an experiment to determine the prevalence and degree of intrasource variation. The index of the progeny of one male-sterile plant was compared to the index of progeny from another male-sterile plant, when the pollen parent was the same.

The male-sterile population from which the test plants were chosen was heterogeneous, consisting of male-sterile plants found in the variety GW304 pollinated with the variety GW359, both varieties being very heterozygous and heterogeneous.

The use of male-sterile plants for indexing was confined to plants showing no visible signs of pollen in the anther, all other plants being rogued in the isolation plot. Pollination was accomplished by bag interchange, the pollen bags being brought from another isolation. One pollinator was used on as many as 10 different male-sterile plants. Pollinations were arranged so that one male-sterile parent had as many as 8 pollinations in common with other male-sterile plants.

---

[1] Plant Breeder, Agricultural Experiment Station, The Great Western Sugar Company, Longmont, Colorado.

[2] Numbers in parentheses refer to literature cited.

Table 1.—Numbers and Percentage of Sterile, Intermediate and Fertile Progeny Produced by Various Male-Sterile Plants with Common Pollinators.

	Sterile Plant Number												
Male Parent	**B623-1**						**vs**	**B623-2**					
	Sterile		**Inter.**		**Fertile**			**Sterile**		**Inter.**		**Fertile**	
	No.	**%**	**No.**	**%**	**No.**	**%**		**No.**	**%**	**No.**	**%**	**No.**	**%**
51-412-1	18	78	5	22	0	0		17	53	15	47	0	0
-457-1	3	8	11	31	22	61		3	9	12	36	18	55
-457-3	8	29	15	54	5	17		8	20	22	55	10	25
-500-1	20	57	9	26	6	17		19	48	18	45	3	8
-517-3	3	23	9	69	1	8		8	23	22	69	10	8
-622-1	7	25	14	50	7	25		11	31	19	54	5	14
GWI-5-2	9	47	10	53	0	0		22	79	6	21	0	0
-7-1	29	81	7	19	0	0		17	65	7	27	2	8
Total		348		324		128			328		354		118

Total $\chi^2 = 12.953$, P $= .7\text{-}.5$ for 14 d.f.

	B623-1						**vs**	**B623-3**					
51-412-1	18	78	5	22	0	0		14	44	18	56	0	0
-457-1	3	8	11	31	22	61		2	5	15	38	23	58
-457-3	8	29	15	54	5	17		15	38	16	40	9	23
-622-1	7	25	14	50	7	25		18	64	7	25	3	11
GWI-5-2	9	47	10	53	0	0		10	24	23	56	8	20
-6-2	1	3	23	57	16	40		1	2	14	35	25	63
-7-1	29	81	7	19	0	0		7	33	10	48	4	19
Total		271		286		143			210		298		194

Total $\chi^2 = 32.543$, P $= .01\text{-}.001$ for 13 d.f.

	B623-2						**vs**	**B623-3**					
51-412-1	17	53	15	47	0	0		14	44	18	56	0	0
-457-1	3	9	12	36	18	55		2	5	15	38	23	58
-457-3	8	20	22	55	10	25		15	38	16	40	9	23
-622-1	11	31	19	54	5	14		18	64	7	25	3	11
GWI-5-2	22	79	6	21	0	0		10	24	23	56	8	20
-7-1	17	65	7	27	2	8		7	33	10	48	4	19
Total		257		240		102			208		263		131

Total $\chi^2 = 34.085$, P $<.001$ for 11 d.f.

	B623-1						**vs**	**B623-7**					
51-412-1	18	78	5	22	0	0		15	44	15	44	4	12
-457-3	8	29	15	43	5	17		6	40	9	60	0	0
-622-1	7	25	14	50	7	25		2	6	27	77	6	17
GWI-6-2	1	3	23	57	16	40		1	2	5	12	34	85
-7-1	29	81	7	19	0	0		19	46	21	51	1	2
Total		216		202		82			138		244		116

Total of $\chi^2 = 37.562$. P $<.001$ for 10 d.f.

Seed from crosses was planted at Salem, Oregon, in August, 1953. Visual readings were made on progenies in June, 1954. Progenies were divided into three classes: sterile, no pollen production; intermediate, trace to considerable pollen but no shedding; fertile, considerable to normal pollen which shed. Families (progenies from individual crosses) were indexed without the reader knowing the pedigree. From 10 to 40 offspring were included per family, with the average about 30.

The χ^2 method was used to determine the probability that any two male-sterile plants indexed a series of male plants alike. A number of male-sterile plants was compared in all possible combinations as to the proportion of sterile, intermediate and fertile progeny they produced. To make a comparison of any two male-sterile plants, a series of contingency tables was constructed, one for each male plant they had in common. The total χ^2 from the contingency tables measures the probability that the two male-sterile plants indexed the male plants the same. All but seven of the 30 comparisons had to be rejected as being different, P less than .05.

Contributing to the total χ^2 (Table 1) is the general trend for one male-sterile plant to produce more or less offspring of one classification than another male-sterile plant. This general trend is demonstrated by comparing the total percentage of sterile offspring (the most distinct class) produced by each male-sterile plant, comparisons limited to crosses with common pollinators. The ratios of steriles produced by one male-sterile plant as compared to the steriles produced by another are summarized in Table 2.

Table 2.—Ratios of the Total Percentages of Sterile Progeny for All Combinations of Male-sterile Parents. Only Steriles from Families Whose Inbred Pollinator Was Used on Both Male-sterile Plants Included in the Totals.

Male-Sterile Comparison	Sterile Ratio	Male-Sterile Comparison	Sterile Ratio
1 vs 2	1.06	3 vs 4	0.83
3	1.29	5	0.85
4	1.47	6	1.09
5	1.68	7	1.30
6	1.79	14	0.60
7	1.57	4 vs 5	1.71
14	2.38	6	0.92
2 vs 3	1.24	7	2.53
4	1.41	14	1.13
5	1.39	5 vs 6	1.42
6	1.19	7	1.22
7	1.24	14	0.66
14	2.16	6 vs 7	1.21

The ratio, 1.06, for 1 vs. 2 (Table 2) is obtained by dividing the total percentage in the sterile columns (Table 1) of B623-1, 348, by 328, the total percentage of steriles of B623-2. This indicates B623-1 produced a greater proportion of steriles than B623-2. Arranged in descending order in the production of steriles, the plants are 1, 2, 4, 14, 5, 3, 6, and 7. The only exception to this order is that 6 produced more than 4, but in all cases 4 produced more than 14, 5, 3, and 7; and 5 and 3 produced more than 6.

Another group of male-sterile plants was tested with plants from the variety A1183 (SL89) which was near "O" type. Only sterile and intermediate offspring resulted. Comparing the total of the sterile and intermediate offspring produced by common pollinators, as was done in the previous group of male-sterile plants, 14 of 21 comparisons had to be rejected, P less than .05. This again indicates little if any likeness exists between the plants as regards production of sterile progeny. The ratios of total percentages of sterile progeny produced by a set of common pollinators are tabulated in Table 3. Male-sterile plants in descending order of the percentage of male-sterile progeny produced, 59, 49, 103, 111, 108, 115, and 18, show no deviation from the order.

There are two possible causes for male-sterile plants producing different percentages of male-sterile progeny and falling into a continuous series as to percentage of sterile progeny when crossed to the same pollinators. The male-sterile plants might differ in (a) plasma genes, either in number or nature or (b) in content of minor modifying genes, genes not necessarily involved in differential interactions.

Testing for variations in plasma genes would involve backcrossing a homozygous inbred to several homozygous male-sterile plants suspected of having different plasma genes and observing the progeny during and at the end of a number of backcrosses. Differences in the male-sterile character of the progeny from the same inbred, but crossed to different male-sterile parents, could then be attributed to variation in plasma genes.

Also contributing to the total χ^2 (Table 1) is that variation which does not conform to the general trend for any one male-sterile plant to produce more or less sterile offspring. The general trend for B623-1 was to produce a greater percentage of steriles than B623-2, but the index of GWI-5-2 by these two plants was the opposite. Another example of the same variation is the indexing of plant 51-457-3 by plants B623-1 and B623-3. Such variation is evident, more or less, in all comparisons and is probably the result of genetic interaction in which the genes of a male plant complement the genes of one male-sterile plant differently than the genes of another male-sterile plant as regards their influence on pollen production.

The results of this study do not necessarily refute Owen's (6) postulation of 2 major factor pairs for the control of cytoplasmic male sterility in sugar beets. The major variations found between the indexes of different pollen plants on the same male-sterile plant may be due to differences in the two factor pairs. However, it is hardly conceivable that all the variation found in this group of male-sterile plants, as regards the fertility of their progeny, can be attributed to some of the male-sterile tester plants being of a heterozygous Z genotype, xxZz, as reported by Owen (7). There is no doubt that the mechanisms involved in the conrol of cytoplasmic male sterility are more complicated than the two factor pair control postulated by Owen; the variability found in this study, whether caused by genes of minor action or by differences in cytoplasm, may have been present to cause the descrepancies noted by Owen (3).

Miscroscopic Pollen Examination

Pollen from a number of plants having male-sterile cytoplasm was stained with acetocarmine and examined with a microscope in order to have a basis upon which to coordinate visual readings with the actual fertilities. All stages of pollen abortion were present in a continuous series, from abortion in the tetrad stage, through normal pollen. The following classes were arbitrarily assigned:

Class	Microscopic description	Visual description
1.	Aborted, tetrad stage	No color in anther, sterile
2.	Aborted, without exine	No color in anther, sterile
3.	Aborted, little exine	Light yellow anther, sterile
4.	Aborted, well developed exine	Intermediate in fertility
5.	Less than 1% sound	Intermediate in fertility
6.	1-5% sound	Intermediate in fertility
7.	5-25% sound	Intermediate in fertility
8.	25-75% sound	Intermediate to fertile
9.	75-95% sound	Fertile
10.	95-100% sound	Fertile

Visual identification of the sterile group of classes, 1 through 3, is quite accurate. An experienced reader could differentiate the group of classes 4, 5, and 6, from class 7 with little overlapping. Fertile plants, classes 9 and 10, are quite difficult in some cases, to differentiate from classes 7 and 8. However, plants in the fertile classes generally have anthers which are packed with pollen. Only plants in classes 7 through 10 would reduce cross pollination significantly in a field of commercial hybrid seed, particularly if relatively self-sterile lines are used.

New and More Potent Sources of Cytoplasmic Male Sterility

A more potent source of cytoplasm, which would castrate plants with a wider range of genotypes than is possible with the present male-sterile cytoplasm, would make an inbred-hybrid breeding program much more flexible. Inasmuch as two new sources in wild *Beta* have been discovered by Great Western, new and as yet undiscovered sources probably exist.

A partially male-sterile *B. macrocarpa* Guss. (A1171) x sugar beet hybrid when backcrossed to sugar beet yielded a high percentage of male-sterile plants. A wild leaf beet introduced from Turkey, PEI 206411, was highly male sterile. Preliminary studies of these sources indicate they are very similar to, if not the same as the sources now available. In addition to these two new sources, there exists in Great Western stocks several additional sources which have not yet been studied.

Several instances of sterile cytoplasm existing in other species and races of genera have been reported for *Nicotiana* (4) and *Linum* (1). Caspari (3) reviewed the work of Michaelis who also found the phenomenon in *Epilobium*. In the above instances when the nuclear germ plasm of one species was placed, by backcrossing, into the cytoplasm of another, cytoplasmic male sterility resulted. A limited survey will be made by Great Western for male-sterile cytoplasm in other races of *B. vulgaris* and other species of *Beta*.

Summary

1. A group of sugar beet pollinator plants was used on a series of male-sterile plants so that the male-sterile plants had pollinators in common. An analysis of the progeny produced by different male-sterile parents with the same pollinator indicated genic interaction, influencing degree of fertility in male-sterile cytoplasm, exists between two parents and is not the same from one parental combination to another. Consistent differences in the proportion of sterile progeny existed between male-sterile parents over a range of common pollinators indicating either minor differences in genes not involved in interactions or variation in plasma genes.

2. Plants in a heterozygous population having male-sterile cytoplasm varied as to pollen abortion in a continuous series from abortion in the tetrad stage of development to normal pollen production. A classification based on microscopic examination of pollen is proposed.

3. Two new sources of male-sterile cytoplasm, one in *Beta macrocarpa* and one in a wild beet from Turkey, were discovered by Great Western. Preliminary investigation would indicate little difference between the action of the new sources and the sources already available.

References

(1) BATESON, W. and GAIRDNER, A. E. 1921. Male sterility in flax, subject to two types of segregation. Nature 21:269-275.

(2) BRIGGLE, LELAND W. 1954. A comparison of cytoplasmic-genotypic interactions in a group of cytoplasmic male sterile corn types. PhD dissertation, Iowa State College. 141 pp.

(3) CASPARI, ERNST. 1948. Cytoplasmic inheritance. Advances in Genetics pp. 1-66. Academic Press, Inc., New York.

(4) EAST, E. M. 1931. Studies on self fertility IX. The behavior of crosses between self-sterile and self-fertile plants. Genetics 17:175-202.

(5) JONES, D. F. and MANGELSDORF, P. C. 1951. The production of hybrid corn seed without detasseling. Conn. Agr. Exp. Sta. Bul. 550.

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

(7) OWEN, F. V. 1950. The sugar beet breeder's problem of establishing male-sterile populations for hybridization purposes. Proc. Am. Soc. Sugar Beet Tech. 191-194.

(8) ROGERS, JOHN S. and EDWARDSON, JOHN R. 1952. The utilization of cytoplasmic male-sterile inbreds in production of corn hybrids. Agron. Jour. 44:8-13.

(9) STEPHENS, J. C. and HOLLAND, R. F. 1954. Cytoplasmic male sterility for hybrid sorghum seed production. Agron. Jour. 46:20-23.