Variability in Partial Male-Fertile Sugarbeet[1]

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The inheritance of cytoplasmic male sterility (CMS) in sugarbeet, *Beta vulgaris* L. has been attributed to the complementary action of two homozygous recessive gene pairs *(xxzz)* interacting with sterile, *S,* cytoplasm (14). Even at the time of publication, however, Owen (14) recognized that his proposal did not give a complete answer for the inheritance of this character. Certain exceptions mainly associated with the breeding behavior of partial male-fertile (PF) plants were pointed out. Later Owen (15) suggested that the *X* factor pair exerted a major influence while the *Z* factor pair had a minor influence on fertility. He noted that genes *X* or *Z* with *S* plasm give intermediate partial male-fertile types and that preliminary tests often do not distinguish between type O (maintainer) pollinators of *Nxxzz* genotype and pollinators carrying the dominant *Z* allele *(NxxZz* genotype).

Hogaboam (8) studied the phenotypic expression of various partial male-fertile beets. He proposed that a dominant fertile factor *(Sh)* enhanced the pollen-producing ability of plants with heterozygous *Xx* genotypes and altered the phenotypic ratios one would expect on the basis of Owen's complementary gene hypothesis.

Exposure of germinated seed of CMS annual beet material to a temperature of 55 C resulted in male-fertile plants. Cleij (2) hypothesized that this was caused by the conversion of *S* to *N* plasm without change in the *x* and *z* genes.

Other investigators (12,18) have concurred that essentially there are two major genes governing CMS in the sugarbeet. They noted, however, that certain modifier genes play an important role in the development of type O and pollen-restorer lines. In some cases, lines designated type O on the basis of crosses with the annual tester, SLC O3 CMS, have produced partial male-fertile progenies when they were crossed with certain biennial CMS lines.

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Variability in the degree of fertility attributed to genic interactions has been observed when various CMS lines are crossed to the same pollinator (12,13). Such variability in partail male-fertile progenies has also been attributed to the effects of temperature, nutrition, photoperiod, and other environmental factors (3,12,13,17,18).

Scientists have noted that the early opening sugarbeet flowers and subsequent later flowers on the same plant are often different in fertility (3,8,15).

Studies of CMS in carrot (7), sorghum (9,11), and corn (1,4) reveal similar findings. In segregating populations, male-sterile versus fertile classes gave good fit to monogenic or digenic ratios; however, wide variation was observed in the fertile class. Gabelman (6) proposed that this fertility variation in corn was caused by the reproduction and segregation of a cytoplasmic particle, which was distributed the same as chromosomes during mitosis, but distributed completely at random during meiosis. Conversely, Maunder and Pickett (9) concluded that the variation in fertility in sorghum was under genic control and that segregation of cytoplasmic factors was not the reason for partial male fertility. Several other research workers (1,2,3,5,8,16) have also attributed the fertility variation in various crops to genetic modifiers or environmental influences. Miller and Pickett (11) reported that the inheritance of partial male fertility in sorghum was complex and involved both inter- and intra-allelic interaction. Beckett (1) and Duvick (4) also found the inheritance of partial male fertility in corn to be complex. Duvick reported linkage of CMS modifier genes in corn with genetic markers on chromosomes 2, 3, 4, 7, 9, and 10, for the populations which he studied.

In 1964 an attempt was begun in our Logan laboratory to learn more about the variation that occurs within partial male-fertile sugarbeets. We endeavored to isolate stable partial-fertile genotypes and to study the segregation of progenies derived from a single partial male-fertile plant.

Materials and Methods

The S_1 progenies of three annual CMS hybrids, which previously were observed to segregate male-sterile and partial male-fertile progenies were selected for study to determine the feasibility of obtaining genetically stable lines with different degrees of male fertility. These populations were planted in January, 1964, in soil beds in a greenhouse maintained at 22 to 27 C. Each plant of each line was carefully observed for pollen dehiscence during the last week of March. A composite sample of pollen, or anthers, was taken from several flowers of each plant, stained with aceto-carmine, and observed microscopically to determine the degree of fertility. This pollen analysis was

repeated each week for 2 weeks following the initial reading and each plant was classified according to its highest fertility reading. Selfed progenies from individual plants having varied degrees of fertility in each of the three populations were subsequently evaluated for correlation with the fertility of their respective parents.

In another greenhouse study, plants from partial male-fertile F_1 hybrids were repeatedly sampled at weekly intervals to determine the variation among branches of an individual plant. The terminal branch of the seedstalk was sampled first, then subsequently lower branches of the inflorescence were sampled as the flowers in the central portion of each branch reached anthesis. Fertility was again determined by microscopic observation of a sample of pollen from several flowers on each branch.

Noting the great amount of variation in the above populations, we decided to study the inheritance of partial male sterility beginning with a single F_1 hybrid plant of SLC 133 CMS $\times$ SLC 130.

The inbred SLC 133 CMS was released to the sugarbeet industry in 1960 as a fairly good male-sterile inbred line that had shown excellent combining ability in previous years. When white-anther plants of SLC 133 CMS were crossed with type O lines, SLC 129 or SLC 128, 100% male-sterile offspring were obtained. Conversely, crosses of similar SLC 133 CMS with CT 9 or SLC 130 inbreds resulted in male-sterile and partially male-fertile progenies.

Each flower on one selected partial male-fertile F_1 plant (9136) was tagged with a small numbered jewelry tag and the characteristics of each individual flower were noted. The plant was bagged to insure self-pollination and the resultant F_2 seed was planted in 4-inch pots in the greenhouse. When plants were 3 months old they were transferred to a cold chamber (7°C) for a 2-month photo-thermal induction period, then returned to the greenhouse. All F_2 plants were observed for pollen dehiscence, and fertility was carefully determined by repeated microscopic observations of pollen samples. Pollen-producing plants were bagged and allowed to self and male-sterile segregates were crossed to the biennial SLC 129 or to the annual SLC 03 type O pollinators. In the latter crosses, part of the inflorescence of each male-sterile plant was bagged separately for a check, to certify that the plant remained male sterile during the entire flowering and seed development period. The selfed and crossed progenies were subsequently evaluated for fertility over an extended period of time.

Annual plants from male sterile X SLC 03 crosses were cut back after fertility classification and allowed to develop new

seedstalks. These were then observed for fertility and cut back again. This procedure was continued for 10 months with an average of nine readings on each plant. All plant material was grown in the greenhouse at approximately 25°C with 8 hours of supplemental incandescent light. All readings were made when 50 to 75% of the flowers on a plant were open.

Results and Discussion

Variation and Stability of Partial Male-Fertile Plants

There was poor correlation between the fertility of the selected individual parent plants of B4921, B4923, and B4924 and their progenies (Table 1.) The offspring of plants selected

Table 1.—Fertility segregation of progenies from three annual semi-male-fertile populations grown in the greenhouse at Logan, Utah in 1964.

Progeny number	Parent fertility percent	Percent fertility (upper class units)												Total number plants
		MS	10	20	30	40	50	60	70	80	90	100	Av.	
B 4921-3	1	7	3	1	0	0	0	0	0	1	0	1	17.7	13
B 4921-11	40	13	1	3	1	0	1	1	1	1	1	0	19.6	23
B 4923-2	10	2	4	5	1	2	1	1	3	2	3	0	41.7	24
B 4923-4	50	0	5	1	1	1	1	2	4	3	4	0	54.1	22
B 4923-5	70	1	3	4	0	0	1	4	1	3	7	0	55.8	24
B 4923-3	80	2	3	2	0	1	1	0	2	2	4	3	56.0	20
B 4923-1	90	6	7	0	1	0	3	0	3	2	7	2	46.8	31
B 4924-3	1	7	1	0	3	0	0	0	1	2	5	0	41.0	19
B 4924-9	1	10	4	4	2	0	0	0	1	6	11	0	47.8	36
B 4924-4	90	3	2	1	2	0	0	2	1	1	5	0	48.2	17
B 4924-6	99	14	1	1	2	0	1	2	2	2	4	2	36.1	31

for low fertility (1 to 10%) again exhibited the lowest fertility, but they did not exhibit the magnitude of difference of their selected parents. B4923-1 and B4924-6 were expected to have the highest fertility. They were, however, among the progenies of their respective populations with the lowest percent of stainable pollen. Differences between the parent populations were still evidenced by their progenies in that B4921 tended to have more male-sterile segregates and lower fertility on the average than did the two other populations. There was not only variation from plant to plant within a given line, but there was considerable variation from branch to branch on the same plant. These data demonstrate that there is a great amount of variability in selfed lines of partial male-fertile sugarbeets.

It would appear that one could not obtain a stable inbred line that would yield 100% partial male-fertile plants regardless of the degree of fertility they exhibited. Since 10%, 50%, and 90% fertile parent selections yield approximately the same distribution in the next self generation, the variation could be attributed to environmental effects. However, interactions

of modifier genes, physiological disturbances or a combination of all of these factors could be alternate explanations.

Partial male-fertile F_1 sugarbeet hybrids show considerable variation in fertility as the plant ages. As illustrated in Table 2, there is a general tendency for the first flowers that open on

Table 2.—Variation among branches of individual partial-fertile F_1 plants in the greenhouse at Logan, Utah in 1964.

	Pollen readings (% fertile)					
Plant	1	2	3	4	5	6
B 4149-1	MS	10	60	60	60	30
B 4152-1	Trace	40	90	50	----	----
B 4152-2	20	90	60	- -	----	----
B 4157-1	MS	30	10	- -	----	----
B 4162-1	Trace	20	Trace	20	10	----
B 4162-2	25	Trace	30	70	----	----

the terminal branch of the florescence to be low in fertility. In most instances the later forming branches show increased fertility, then taper off to a lower fertility reading as the plant completes flowering development. There were exceptions, however, as shown by B4162-2 where the last branch on a plant was the most fertile. These results demonstrated that plants should be well developed, with over 50% of the flowers open, to be certain of the fertility classification of a plant.

Inheritance of a Partial Male-Fertile Sugarbeet

The monogerm F_1 plant (9136) of SLC 133 CMS $\times$ SLC 130 selected for study developed an eight-branch seedstalk. There were 113 flowers on the terminal branch and an average of 52 flowers on each of the other 7 branches (Figure 1). Eleven percent of the 479 total flowers had yellow anthers and some pollen dehiscence. One flower contained two brown shrunken anthers and three yellow anthers. Another had one yellow and four brown shrivelled anthers. All of the remaining flowers were male sterile, each having brown shrunken anthers devoid of pollen.

Plant 9136 produced 43 seeds; 41 were from fertile flowers, one from a male-sterile flower, and one from a flower having 3 yellow and 2 brown shrunken anthers. Nine flowers having all yellow anthers and the flower with only one yellow anther failed to set seed.

The more fertile flowers on this plant were concentrated in the area of the 6th to 20th flower from the basal end of each lateral branch. Since flowers in this area mature before those at the tips, one could attribute the fertility to a particular fertility-inducing substance or nutrient effect which was not available to the later developing flowers. This reasoning, how-

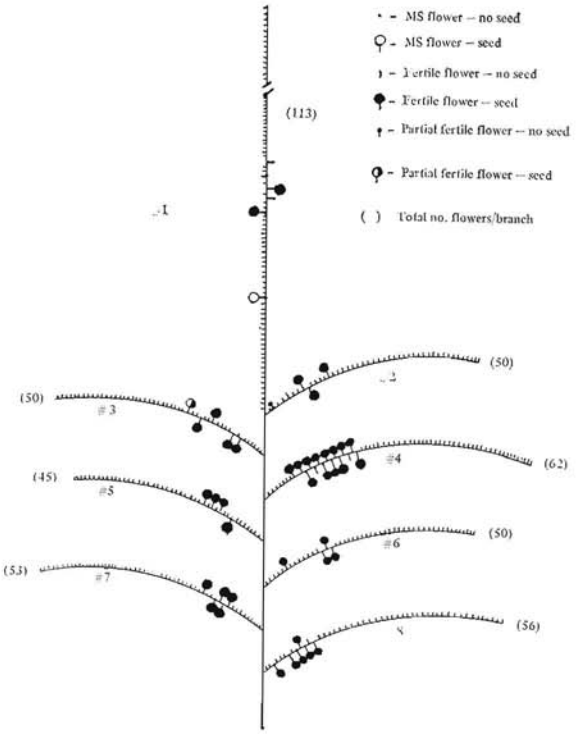

Figure 1.—Schematic diagram of the fertility of each flower on the eight branches of plant 9136 (SLC 133 CMS X SLC 130).

ever, does not explain why the first eight or nine basal flowers on a branch are male sterile, nor why male-sterile flowers are found interspersed among those that produce viable pollen. It may be that the intra-plant variation was caused primarily by irregular segregation of plasmon units as has been suggested by Michaelis (11). This conclusion would contradict Gabelman's (6) view that plasmon particle distribution is similar to that of the chromosomes during mitosis.

F_2 plants developed from the male-sterile flower (1-25), and the 3/5 partial-fertile flower (3-20) that formed seed, were completely male sterile (Table 3). In addition, 16 of the plants from yellow-anthered flowers were male sterile. The other 24 plants ranged from 5 to 75% stainable pollen, with an average of 36% fertility. Anthers in the flowers of these F_2 plants varied from shrunken, brown, and empty to plump and yellow with considerable inter- and intra-flower variation on each plant.

In accord with Owen's (14) original hypothesis, these results could be explained if the pollen parent was heterozygous for

one gene resulting in a ratio of one fertile: one male sterile. The actual F_2 segregation gave a better fit to a 9:7 genetic ratio, suggesting that partial male fertility was controlled by two complementary genetic factors. In the F_3 one would expect segregation of three fertile: one male sterile or nine fertile: seven male-sterile plants. Progenies for four F_3 plants had far too many male-sterile segregates to confirm either hypothesis (Table 4A).

Four F_2 male-sterile segregates were crossed with SLC 129 pollinator. Three of these F_1 progenies were 100% male sterile as expected when a CMS plant is crossed to a type O pollinator (Table 4B). The other population segregated one partial male

Table 3.—F_2 segregation of a single F_1 plant from the cross SLC 133 CMS × SLC 130.

Branch and flower no.	Parent* flower	Plant** fertility	Branch and flower no.	Parent flower	Plant fertility
1-25	MS	MS	4-22	F	75%
1-43	F	20%	4-23	F	40%
1-48	F	MS	5-9	F	MS
2-10	F	40%	5-12	F	40%
2-11	F	20%	5-14	F	5%
2-16	F	40%	5-16	F	MS
3-7	F	20%	6-6	F	50%
3-9	F	40%	6-15	F	60%
3-14	F	50%	6-16	F	70%
3-17	F	20%	6-17	F	40%
3-20	3/5 F	MS	7-10	F	35%
4-8	F	MS	7-11	F	50%
4-10	F	20%	7-12	F	MS
4-11	F	MS	7-13	F	50%
4-12	F	5%	7-16	F	20%
4-14	F	10%			
4-15	F	MS	8-3	F	MS
4-16	F	MS	8-3	F	40%
4-17	F	MS	8-9	F	MS
4-18	F	MS	8-10	F	MS
4-19	F	MS	8-11	F	MS
4-20	F	MS	8-13	F	
	Partial fertile	Male sterile	P		
Observed	24	18			
Expected	23.6	18.4	.90	(9:7 ratio)	

* MS = all 5 anthers shrunken, brown, no pollen; F = all anthers plump, yellow, some pollen dehiscence.

** Highest percentage stainable pollen for each plant read 3 times in January and February 1965.

fertile: three male sterile, which indicated that even though the male-sterile plants were similar in appearance, they differed in genotype.

Table 4.—Pollen fertility of F_3 progenies from partial-fertile plants and F_2 male-sterile segregates × Type O pollinators.

Self or crossed progeny	No. plants		
	PF	MS	Total
A. F_3 Progenies:			
5906	10	34	44
5912	27	42	69
5930	5	18	23
5932	6	26	32
B. Male sterile × SLC 129 progenies:			
5905	5	17	22
5914	0	89	89
5917	0	15	15
5918	0	6	6
C. Male sterile × SLC 03 progenies:			
5913	4	32	36
5921	5	0	5
5924	11	16	27
5931	64	30	94
5933	1*	36	37
5936	11	0	11
5937	3	3	6

* On this plant one small branch bearing 5 flowers showed yellow anthers and dehiscent pollen. All other flowers were male sterile.

Seven white-anther male-sterile F_2 segregates that were crossed to SLC O3 annual type O pollinator were evaluated for fertility in a single bay of a 24°C greenhouse. All male-sterile plants were phenotypically similar. None of them produced pollen or seed on a branch of the inflorescence that was bagged to certify that each plant remained male sterile throughout the study. By using a 3X magnifier, anthers from three of the most fertile-appearing flowers and three of the least fertile-appearing flowers on each F_1 plant were selected to determine the range in pollen fertility. Five miscroscopic fields of 750X (average of 150 pollen grains) were counted for each sample. Considerable inter- and intra-flower variation was observed in these plants. Segregation for fertility is shown in Table 4C.

A genetic hypothesis that would fit all of the data is hard to visualize. Since all male steriles were produced on a single plant, they should carry the same sterile cytoplasm. Furthermore, the SLC O3 pollinator should be homozygous for genes conditioning sterility since this line was in the S_{10} generation of inbreeding.

If the conventional (14) double recessive *Sxxzz* represents the genotype of the CMS parents and *Nxxzz* that of the type O pollinator, we would expect only male sterile offspring. Only crosses 5933 and 5913 approach this expectation.

All 37 plants of 5933 were originally scored as male sterile. However, a few pollen-bearing flowers developed 2 weeks later on a small side branch of one plant. This could possibly have

been a mutation or an accumulation of fertility-promoting substances or the loss of fertility-inhibiting substances in the plant. Alternatively, it could be attributed to a change from sterile (S) to normal (N) cytoplasm without altering the X and Z genetic factors as suggested by Cleij (2). The four plants of cross 5913 scored as partial male fertiles had less than 5% stainable pollen and seed was not produced on any of these plants. Variation in pollen fertility for the CMS × SLC O3 progenies following clipping and regrowth of seedstalks during a 10-month period are shown in Table 5. There was an average of nine readings made on each plant.

Plants classified as completely male sterile remained male sterile throughout the 10 months with the exception of 1 plant in population 5933 and 15 plants in 5931. The later were male

Table 5.—Change in pollen fertility readings of CMS × SLC 03 progenies following clipping and regrowth of seedstalks during a 10-month period.

Population	Avg. no. readings per plant	No. of plants				
		Always MS	1st reading PF MS thereafter	Variable PF or MS	Always PF	Total
5913	10	32	4	0	0	36
5921	7	0	5	0	0	5
5924	9	16	3	8	0	27
5931	9	15	19	60	0	94
5933	10	36	0	1*	0	37
5936	11	0	5	6	0	11
5937	10	3	3	0	0	6
Total	9	102	50	64	0	216

* Third reading 5 flowers on one branch were partial fertile.

sterile for two or three readings made in January through March, but exhibited some randomly scattered partial male-fertile flowers and a trace to 40% stainable pollen for one or more readings during the summer months. Many of the plants which were originally partial male fertile showed 100% male sterility for all subsequent readings. None of the plants originally scored as partial male fertiles remained such for each seedstalk regrowth. Some seedstalks bore only male sterile flowers and at other times showed partial male fertility on the same plant. However, in every case the percentage of fertile pollen was less than that observed for the first reading made in January. No doubt some, but not all, of the variability was conditioned by environmental factors. Data from previous studies has shown that some apparent male sterile plants could become more fertile as the light intensity increased during the late spring and summer months. However, it was not expected that partial male fertile plants would become less fertile during this period. Unpublished research conducted in our lab under different temperature, light intensity, nutrient, and water stress

conditions has failed to show significant differences between treatments.

The data demonstrate that all white-anther male sterile sugarbeets are not of the same genotype *(Sxxzz)*. There are genetic modifying factors which interact under present poorly-understood conditions resulting in varied degrees of pollen fertility. Although the actual inheritance pattern is inconclusive, the data show that partial male sterility inheritance in the sugarbeet is complex. This same conclusion has been reached in studies on partial male sterility in corn (1,4) and sorghum (11). It is apparent that critical studies utilizing clonal or isogenic material under highly controlled environmental conditions will be required to elucidate the complex inheritance of partial male sterility in sugarbeets.

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