Attempted Graft Transmission of Cytoplasmic Male Sterility in Sugar Beets (Beta vulgaris L.)[1]

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Graft-induced transmission of cytoplasmic male sterility (CMS) in Petunia has been demonstrated by Frankel (4)[4] who reported that fertile scions grafted to CMS stocks, though remaining phenotypically unaltered, produced some male-sterile progeny. Edwardson and Corbett (3) generally agreed with this result but suggested that sterility resulted from a transfer of factors across the graft union. In 1962, Frankel (5) published the results of more extensive experiments, corroborating his initial results. Failure to detect graft transmission of CMS in tobacco has been reported by Sand (9), who concluded that it was neither easily repeatable nor a general phenomenon in plants.

Use of CMS is the most practical means for large-scale hybridization in sugar beets. Therefore, basic knowledge concerning the genetic nature and the behavior of the cytoplasmic entities governing CMS is of considerable value for the sugar beet breeder.

The experiments reported herein were conducted for the purpose of detecting the possible transmission of CMS factors across a graft in sugar beets. Such an occurrence would not only be of academic interest, but would be of great practical value since it would provide a rapid and economical means of converting type 0 (non-restorer) sugar beet lines to the male-sterile state.

Materials and Methods

Ten populations of sugar beets were used in grafting experiments at Fort Collins, Colorado, and Logan, Utah. All grafts at both locations were made in the greenhouse during the years 1962-64.

Plants of five populations (Table 1) were grafted at two stages of growth at Fort Collins. Growth stages were 14 to 16

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[4] Numbers in parentheses refer to literature cited.

days after planting (cotyledon stage) and 26 to 36 days after planting (one to three-leaf stage). A stock and its scion were always the same age. Stocks were grown in small pots of sterilized soil, scions were grown in sand and removed at the time of grafting. Both stock and scion plants were split down through the crown with the split tapering out to one side at about 1/4 to 1/2 inch down the hypocotyl. The split faces of plant halves with intact roots were matched as nearly as possible, and, in the first group, were bound together with silk thread. In a second group, a spring-loaded hair clip was used to hold the cut faces of stock and scion together. After grafting, the scion root was put into the soil beside the stock root with little apparent transplanting shock. The constriction caused by the thread necessitated its removal about 20 days after grafting. The crown of the stock and root of the scion were cut and removed about 10 days later. The stock crown and scion root of clipped grafts were severed after about 15 days, but the clips were left in place for about 45 days.

Table 1.—Description of sugar beet populations used in grafting experiments.

Population	Genotype[1]	Description
Fort Collins, Colorado		
52-305 CMS	rrvvbb	CMS inbred used as stock and scion
52-307	RRyybb	type 0 inbred used as scion and stock
52-407	RRYYbb	" " " "
52-346	RRYYbb	" " " "
A62-2	rryyBB	" " " "
Logan, Utah		
SLC 03 CMS	rryyBB	CMS inbred used as stock and scion
SLC 03	rryyBB	type 0 inbred used as stock and scion
94625	RRyyBB	" " " "
92.592.1	RRyybb	" " " "
M 3579-5	rrYYbb	" " " "

[1] R = red; r = green hypocotyl; Y = red root in presence of R, yellow root in presence of r; y = white root; B = annual, b = biennial growth habit.

Three plants each from 52-407 and 52-346, grafted onto 52-305 CMS, had their stock crowns left intact but their scion roots severed. Hence, they were double-crown plants.

All grafted plants were kept in a high humidity chamber until the graft union was established. They were checked periodically for scion rooting and stock budding, although the latter was not a problem.

Four CMS plants grafted onto fertile stocks were included in the study to detect graft transmission of male fertility.

A few cleft grafts were also attempted, but none survived to the flowering stage.

In the first experiment at Logan in 1962, three grafting methods were used with the annual populations SLC 03 and SLC 03 CMS: Coe's (2) seedling-insert method, Johnson's (7) seedling-seedstalk method, and cleft grafting of succulent seedstalks. Grafts were made of male-sterile scions onto fertile stocks, fertile scions onto male-sterile stocks, male-sterile scions onto male-sterile stocks, and fertile scions onto fertile stocks. The seedling insert method consisted of placing a wedge-shaped, 7 to 10-day-old seedling into a hole in the crown of a 4 to 5-day-old stock seedling and keeping the graft in a humid atmosphere. In the seedling-seedstalk method a bolting sugar beet with a 6 to 10-inch succulent seedstalk was used as the stock. The third or fourth basal seedstalk leaf and auxiliary bud were removed with a razor blade and an incision 1/4 to 3/4 inch long was made in the stalk in the region of the excised leaf. The scion consisted of a small seedling in the cotyledon or two-leaf stage of growth. A wedge-shaped cut was made through the hypocotyl, and the scion was carefully placed into the incision in the stalk and bound in place with coarse thread. Subsequently, the graft was completely covered with melted grafting wax. To prevent the stem from drying out before graft union occurred, all buds and all leaves, except one leaf above the graft, were removed from the seedstalk. Cleft seedstalk grafts were also wrapped with string and covered with melted grafting wax.

During the winter of 1963-64, several new grafts were made in a second experiment at Logan. This was done to test transmission by a new grafting technique and, at the same time, to incorporate other lines of possible genetic diversity into the study. New populations were 94625, M3579-5, and 92.592.1 (Table 1).

When plants to be used for stocks were two to three weeks old, the hypocotyl was split 1/4 inch down through the center of the crown with a razor blade. The seedling scion was trimmed to a wedge shape and inserted into the stock so that the crown tissue of the scion and stock were parallel. A woman's spring hair clip was placed across the graft and left until union occurred. Red or yellow hypocotyl color served as a marker to identify the scion. When green hypocotyl plants were used for both stock and scion, leaves of the scion were marked with India ink to identify the grafted segment.

All scion (G_0) plants were examined for male sterility and then self-pollinated or crossed to the annual type 0 pollinator SLC 03. The degree of fertility in the G_0 and subsequent genera-

tions was determined by microscopic examination of aceto-carmine-stained pollen collected from several newly opened flowers on the plant.

Results

Grafting successes are outlined in Table 2. The seedling-insert method was the only one which resulted in complete failure. This was caused by the dislodging of the scion by leaf growth from the apex of the stock and, to a lesser extent, by inability to maintain high humidity at the time of grafting. The cleft-seedstalk method was only succesful when very young succulent seedstalks of comparable size were used. Grafts of plants in the cotyledon stage held together with clips were the most successful.

Table 2.—Effect of method on grafting success in sugar beets; all populations considered.

Method	Age of plant (days)		Number made	Number successful	Percent successful
	Scion	Stock			
Seedling-insert	7-10	4-5	50	0	0
Seedling-seedstalk	7-10	132-140	322	40	12
Cleft-seedstalks	132-140	132-140	53	9	17
Cleft-seedling:					
Wrapped	7-10	12-14	35	3	9
Clipped	7-10	12-14	585	182	31
Approach-seedling:					
Wrapped	14-16	14-16	200	21	10
Wrapped	26-36	26-36	75	9	12
Clipped	14-16	14-16	175	49	28
Clipped	26-36	26-36	180	30	17

Fort Collins Experiment

The 105 male-fertile scions grafted on 52-305 CMS stocks were examined for male sterility (Table 3). There was no indication of male sterility: no sterile plants, branches within plants, flowers within branches, or anthers within flowers.

Self-pollinated seed was obtained from 98 of the 105 scions. In each of these 98 G_1 progeny lines, 5 to 30 plants were examined for pollen sterility among plants or within plants.

Hybrid seed was obtained from 13 of 35 attempted crosses as follows:

(52-307 on 52-305 CMS) × 52-346, F_1 6 plants
(52-307 on 52-305 CMS) × A62-2, F_1 1 plant
(52-407 on 52-305 CMS) × A62-2, F_1 6 plants

The hybrids were identifiable since 52-346 had a red root and A62-2 was annual. The inbreds used as scions (52-307 and 52-407) were highly self-fertile, partially accounting for the few hybridizations. Also, the flowering date of the male and female did not coincide in certain crosses. In each of these 13 F_1 hybrids, 1 to 20 plants were examined for male sterility. There was no indication of pollen sterility among or within plants.

The six plants in which the stock crown was left intact were brought to flower. The inflorescences from the stock were completely sterile; all those from the scion were fertile.

The four scions of 52-305 CMS on fertile stocks remained completely male sterile. These scions were pollinated by 52-346. Five plants from each of the resulting four F_1 hybrids were completely sterile.

Table 3.—Fertility of G_0 (scion) generation grafts made on sugar beets at Fort Collins, Colorado, in 1962-63.

Scion and stock	Number plants	
	MS	F
52-307 on 52-305 CMS	0	40
52-407 on 52-305 CMS	0	37
54-346 on 52-305 CMS	0	23
A62-2 on 52-305 CMS	0	5
52-305 CMS on 52-307	1	0
52-305 CMS on 52-407	2	0
52-305 CMS on 52-346	1	0

Logan Experiment No. 1

The fertility of G_0 (scion), G_1 and G_2 generation grafts of SLC 03 and SLC 03 CMS made at Logan in 1962 is shown in Table 4. Repeated examinations of grafted plants failed to reveal any phenotypic alterations in the fertility of the scions. All branches of a fertile scion and all flowers on the different branches had yellow dehiscing anthers.

The fertility of the parental pollinator line (SLC 03) ranged from 50% to 90% with an average of 80% aceto-carmine-stained pollen. By comparison, fertile scions, regardless of the stock they were grafted onto, were also about 80% fertile. In the G_1 (first selfed) generation the plants varied from 10% to 95% stainable pollen, with an average of 52%. In comparison with the scions, the G_1 lines appeared to be considerably lower in fertility. However, the readings of pollinator grafted onto pollinator were similar to pollinator grafted onto male sterile. In one fertile on CMS graft, four G_1 plants were classified as male sterile when anthers were first observed cytologically. However, at a later date they were reclassified at 50%, 50%, 35%, and

30% fertile. No plant showed the opposite tendency, i.e., pollen-producing plants to become less fertile with age. SLC 03 annual grafts in the G_2 generation had a wide range (10% to 100%) in stainable pollen with a high average fertility (84%); however, no male-sterile segregates were obtained. Intra and inter-plant phenotypic variation in sterility was not observed.

Seed production of the SLC 03 grafted lines showed trends parallel to pollen-fertility observations. There was little difference between the range in seed production of selfed-parental plants and later generation progeny, but the G_1 plants tended to average less than their respective G_0 forebearers. Again, however, the same differences were manifest in the progeny of fertile on fertile as for fertile on male-sterile grafts.

The scions of the two other graft combinations shown in Table 4, i.e., male sterile on male sterile and male sterile on fertile, were also autonomous in that scions remained sterile in both cases.

Table 4.—Fertility of SLC 03 and SLC 03 CMS grafts in the G_0 (scion), G_1, and G_2, generations of sugar beets at Logan, Utah, 1962-63.

Scion and stock	Geno-type	Number lines	Number plants		Percent fertility[1]	
			MS	F	Range	Avg
SLC 03 on 03 CMS	G_0	—	0	13	50-90	78
	G_1	13	0	738	10-95	52
	G_2	13	0	1923	10-100	84
SLC 03 on SLC 03	G_1	1	0	6	65-90	83
	G_1	1	0	114	10-80	48
	G_2	4	0	618	10-100	81
SLC 03 CMS on SLC 03	G_0	—	11	0	———	—
SLC 03 CMS on SLC 03 CMS	G_0	—	25	0	———	—

[1] Fertility determined by microscopic examination of aceto-carmine-stained pollen.

Fertility readings of grafted segments crossed with their parental lines are shown in Table 5. Five lines of a male-sterile scion on a male-sterile stock were crossed with SLC 03 pollinator and produced 100% male-sterile progeny. Male-sterile scions on fertile stocks crossed to SLC 03 produced all male-sterile progeny. Likewise, seven lines of grafts of fertile scions on male-sterile stocks crossed to the SLC 03 CMS parent produced 100% steriles. Crosses between scions of reciprocal SLC 03/SLC 03 CMS grafts also yielded male-sterile offspring. There were no differences between these backcross progenies and the offspring from a cross of the ungrafted male-sterile SLC 03 and its type 0 pollinator as shown in the last line of the table.

Table 5.—Fertility readings of SLC 03 grafted segments crossed to parental lines of sugar beets.

Cross	Number lines	Number plants	
		MS	F
SLC 03 CMS scion / SLC 03 CMS × SLC 03	5	120	0
SLC 03 CMS scion / SLC 03 × SLC 03	3	96	0
SLC 03 CMS × SLC 03 scion / SLC 03 CMS	7	567	0
SLC 03 CMS scion / SLC 03 × SLC 03 scion / SLC 03 CMS	3	14	0
SLC 03 CMS × SLC 03	2	84	0

Logan Experiment No. 2

In the scion and G_1 generations, all the grafts made in 1963-64 showed autonomy with the exception of two G_1 plants from SLC 03 on SLC 03 CMS (Table 6). The data of this experiment confirm results of previous work, indicating that CMS was not readily transferred across a vegetative graft in sugar beets.

Table 6.—Fertility of G_0 (scion), G_1 generation seedling grafts of sugar beets made in 1963-64.

Scion on stock	Generation	Number lines	Number plants		Percent fertility[1]	
			MS	F	Range	Avg
M 3579-5 on SLC 03 CMS	G_0		0	39	60-90	88
	G_1	4	0	91	10-98	88
94625 on SLC 03 CMS	G_0		0	9	90	90
	G_1	8	0	263	30-98	86
SLC 03 on SLC 03 CMS	G_0		0	47	20-90	85
	G_1	45	2	1256	10-90	78
92.592.1 on SLC 03 CMS	G_1	--	0	21	90	90
94625 on SLC 03	G_0		0	2	90	90
	G_1	2	0	44	70-98	88
SLC 03 on SLC 03	G_0		0	34	70-90	87
	G_1	11	0	359	30-99	81
SLC 03 CMS on SLC 03	G_0		15	0	---	---

[1] Fertility determined by microscopic examination of aceto-carmine-stained pollen.

Discussion

With refinements and practice in the grafting techniques, it is likely that most seedling grafts would be successful.

All of the graft combinations in this study maintained phenotypic autonomy for at least eight months. In Frankel's work with Petunia (5), some of the scions and stocks remained unaltered for up to 3½ years. It was among the self-pollinated and F_1 progenies of Petunia grafts that some plants were male sterile, the proportion of male-sterile progeny appearing to be genotypically controlled.

With the exception of two plants, there was no indication of sterility in any of the selfed G_1 or hybrid progenies of sugar beets in these studies. The two G_1 male-sterile exceptions in a population of 1,256 plants probably were due to mutation or to misclassification resulting from environmental effects.

Considerable variation in the degree of pollen fertility among plants of related lines was noted in the Logan studies. This was attributed mainly to temperature and other possible environmental factors. Such variability also has been observed in extensive studies by Rohrbach (8), who suggested nutrition and photoperiod influenced the degree of fertility. Negative results of graft transmission and observation of variation due to temperature has also been reported by Cleij (1).

When male-sterile plants were used for scions, they also remained autonomous after grafting. Crosses of scions of SLC 03 CMS-SLC 03 grafts to parental lines gave the same results as did the cross between the ungrafted checks.

Gabelman (6), working with corn, concluded that the CMS factor was particulate and that reproduction and distribution of the particle was quite similar to that of chromosomes. He doubted whether a virus would be so dependent on chromosome division for its reproduction and distribution. Edwardson and Corbett (3), however, suggested that CMS in Petunia is analogous to a disease resulting from a virus infection. Regarding the "virus infection" hypothesis, Frankel (5) pointed out that a genotype-cytotype interaction must be responsible for the activation or production of the "virus." He found an absence of genotype-cytotype interaction in his material but he observed genotypical control in CMS induction.

Assuming the CMS factor in sugar beets is particulate, one could conclude that it is not a normal virus, since it is axiomatic that viruses are transmitted by grafting between individual plants of a type which they can infect systemically. In grafting studies with other male-sterile and fertile sugar beet populations not reported herein, the curly top virus was readily transmitted across the graft union.

Cleij (1) was unable to transmit male sterility to fertile plants by aphids or by rubbing leaves with sap.

Based on these studies, it would appear that transmission of CMS across a graft union in sugar beets is not a common occurrence as noted in Petunia (2, 3, 4). Hence, it is doubtful that grafting will become an effective means of converting type 0 populations to a CMS condition.

Summary

Transmission of a cytoplasmic male sterility (CMS) or fertility factor(s) through a graft union was attempted in sugar beets. All graft combinations maintained phenotypic autonomy. Among the self-pollinated G_1, backcross and hybrid progenies of the scions, there was no indication of transmission of a factor for male sterility or fertility. The transmission of a factor for CMS through a graft union does not appear to be a common occurrence in sugar beets, and it is somewhat doubtful that this technique will become an effective means of producing CMS populations.

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