Inheritance Studies with a Pollen Fertility Restorer Sugarbeet Inbred[1]

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Introduction

Genes which partially restore pollen fertility in sugarbeets, *Beta vulgaris* L. are common; however, strong pollen fertility restoration genes are scarce. In an extensive study with curly-top resistant material, Owen (6)[3] observed only one line that he considered of strong restorer genotype. He noted that normal hermaphrodite beets crossed to cytoplasmic male steriles (CMS) frequently segregated male sterile, partial fertile, and fertile offspring, but there were no lines having 100% fertile progeny. He attributed this to the fact that these pollinator lines carried sterile rather than normal cytoplasm.

In 1958 progeny of a subline of US 201-20 showed a high degree of pollen fertility when crossed with CMS. Inheritance studies involving this pollen restorer (R_f) line have been carried out and are reported in this paper.

Materials and Methods

S_2 plants of US 201 R_f were crossed with SLC 03 CMS, a BC_6 annual male-sterile tester at Salt Lake City, Utah in 1959. Subsequent F_1, F_2 and BC_1 progenies were classified visually for pollen fertility.

Three S_3 lines of the same pollen restorer were again crossed to SLC 03 CMS in an effort to confirm the earlier findings. The fertility of the F_1, F_2, and BC_1 generations were checked visually and also microscopically by staining pollen with aceto-carmine. All generations were evaluated in a greenhouse maintained at approximately 21 C. χ^2 statistics were utilized to evaluate goodness of fit to genetic ratios.

In 1964 the S_4 R_f pollinator was crossed to 19 biennial CMS inbreds from diverse sources. F_1 progenies were overwintered at St. George, Utah and Salinas, California and were classified for fertility during the following summer. Because of the striking differences noted in the field, three hybrids, NB-1 CMS × R_f, CT9 CMS × R_f, and SLC 129 CMS × R_4 were grown as stecklings,

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[3]Numbers in parentheses refere to literature cited.

photothermally induced in a cold chamber at 5 C and classified for fertility in the greenhouse at 21 C during the winter of 1966. Four F_1, nine F_2 and five BC_1, R_f populations were evaluated for pollen fertility in the field at St. George in 1966.

Results

All F_1 plants of the first SLC 03 CMS × US 201 R_f, cross were vigorous fertile annuals (Table 1) and were remarkably uniform in growth habit and foliar characteristics in the greenhouse. Some plants produced great quantities of viable pollen, but there was striking variability from plant to plant and between different branches of the same plant. Careful observation showed that these plants were much more sensitive to environmental conditions than were normal hermaphrodite beets. When subjected to low temperatures or moisture stress, pollen production and dehiscence was poor.

Table 1.—Segregation of SLC 03 CMS × US 201 R_1 S_2 pollinator in the greenhouse at Salt Lake City, Utah in 1959.

	No. of plants				Goodness of fit to 3:1 ratio P*
	Male sterile	Partial fertile	Fertile	Total	
F_1	0	0	113	113	
F_2 Observed	41	43	65	149	
Expected (3:1)	37.25	111.75			.50-.30
BC_1 Observed	116	117	1	234	
Expected (1:1)	117	117			.90-.80

*Probability for caluculated χ^2 ratio.

Vigor was reduced in the F_2 generation but some individual plants appeared to be excellent pollen producers. Approximately 25% of the plants bore white empty anthers, which gave an excellent fit to a 3 fertile: 1 male sterile ratio (Table 1).

In the BC_1 generaion, growth was also vigorous and uniform, but pollen production was drastically suppressed as compared with the F_1. Only one plant was classified as completely fertile. Most of the partial fertiles bore badly shrunken yellow anthers with only a trace of viable pollen. Half of the offspring resembled the annual CMS parent. A χ^2 test of pollen producers versus white anther male steriles, gave excellent agreement with an expected 1:1 ratio for monogenic inheritance.

Data from the crosses made in 1963 did not confirm the results of the earlier SLC 03 CMS × US 201 R_f cross. (Table 2). Three lines of family RB 3105 gave segregation ratios indicative of monogenic inheritance. Conversely, family RB 3104 lines showed a better fit to a 9 fertile: 7 male sterile F_2 ratio and a 1:3

Table 2.—Segregation of SLC 03 CMS × S_3 families derived from US 201 R_1, in the greenhouse at Logan, Utah in 1964.

					χ^2 Probabilities			
		No. plants[1]			F_2	Ratios	BC_1	Ratios
Family	Gen.	F	MS	Total	3:1	9:7	1:1	1:3
RB 3104-7	F_1	32	0	32				
	F_2	25	12	37	.30-.50	.10-.20		
	BC_1	12	31	43			<.01	.50-.70
-13	F_1	30	0	30				
	F_2	41	23	64	.02-.05	.20-.30		
	BC_1	68	160	228			<.001	.05-.10
-16	F_1	31	0	31				
	F_2	26	22	48	<.001	.95-.98		
	BC_1	7	25	32			<.001	.50-.70
-17	F_1	38	0	38				
	F_2	70	36	106	.02-.05	.02-.05		
	BC_1	11	34	45			<.001	.90-.95
Total	F_1	131	0	131				
	F_2	162	93	255	<.001	.01-.02		
	BC_1	98	250	348			<.001	.10-.20
RB 3105-1	F_1	12	0	12				
	F_2	41	15	56	.80-.90	.01-.02		
	BC_1	17	14	31			.50-.70	<.001
-11	F_1	38	0	38				
	F_2	21	4	25	.30-.50	<.01		
	BC_1	16	19	35			.50-.70	<.01
-13	F_1	46	0	46				
	F_2	9	1	10	.20-.30	.02-.05		
	BC_1	23	27	50			.50-.70	<.001
Total	F_1	96	0	96				
	F_2	41	20	91	.30-.50	<.001		
	BC_1	56	60	116			.70-.80	<.001

[1] F = stainable pollen with aceto-carmine and MS = white or brown anther flowers with no stainable pollen.

BC_1 ratio. Considerable variation in dehiscence and the percent stainable pollen was again noted in this material. Some plants produced abundant pollen while others bore shrunken yellow non-dehicing anthers, and had less than 50% stainable pollen.

In field plantings, F_1 restorer hybrids involving 19 biennial CMS lines produced fertile and partial-fertile offspring, but no white anther male steriles (Table 3). Performance of the individual hybrids was quite similar for the two locations. All plants were vigorous and a majority of them shed pollen profusely. At St. George the hybrids ranged from 24% to 100% fertile plants while at Salinas the fertility range was 39% to 100%. The NB-1 CMS × R_f hybrid had the lowest fertility percentage of the lines

Table 3.—Pollen fertility readings of F_1 restorer hybrids at St. George, Utah and Salinas, California in 1965.

Hybrid	St. George, Utah				Salinas, Calif.			
	No. plants[1]			%	No. plants			%
	MS	PF	F	F	MS	PF	F	F
SL 211H3 × Rf	0	5	30	86	0	24	20	45
SLC 127 × Rf	0	0	22	100	0	2	7	78
SLC 129 × Rf	0	0	13	100	0	0	5	100
SLC 128 × Rf	0	7	18	72	0	1	5	83
AI-1 × Rf	0	11	17	61	0	3	16	84
AI-10 × Rf	0	1	2	67	0	1	4	80
CT9 mm × Rf	0	1	23	96	0	7	10	59
NB-1 × Rf	0	16	5	24	0	11	7	39
C515 × Rf	0	0	2	100	0	0	17	100
F54-22-H-14 × Rf	0	1	16	94	0	1	55	98
S3317-5 × Rf	0	1	37	97	0	2	27	93
F.C. 502 Rf	0	0	2	100	0	1	5	83
S3317-14 × Rf	0	0	3	100	0	1	22	96
F.C. 503 × Rf	0	2	6	75	0	0	37	100
308HO 1 × Rf	0	1	30	97	0	1	31	97
2937 × Rf	0	0	30	100	0	0	41	100
2938 × Rf	0	5	26	84	0	0	35	100
SLC 126 × Rf	0	0	64	100	0	1	59	98
SL 0130 × Rf	0	0	55	100	0	1	41	98
Total	0	51	401	—	0	57	444	—
Average	0	2.7	21.1	88.7	0	3.0	23.4	88.6

[1]MS = white or brown shrunken anthers with no pollen, PF = partial fertile plants having yellow anthers with none or little dehiscent pollen, F = yellow anthers with abundant dehiscent pollen.

studied. At Logan 76% of the offspring for this cross were partial-fertile plants with relatively little pollen dehiscence. This same line also showed a greater tendency to resist pollen restoration than the other hybrids evaluated at Salinas.

Greenhouse readings on NB-1 CMS × R_f, CT 9 CMS × R_f and SLC 129 CMS × R_f hybrids confirmed the field readings that NB-1 CMS was a better emasculator than other CMS lines (Table 4). SLC 129 CMS and CT 9 CMS hybrids averaged over 80%

Table 4.—Fertility of F_1 restorer hybrids grown in the greenhouse at Logan, Utah in 1966.

Description	No. plants by upper class limits (% fertile)[1]											Total no. plants	Avg. % fertility
	MS	T[2]	10	20	30	40	50	60	70	80	90		
SLC 129 CMS × Rf	0	0	0	0	1	0	0	4	4	4	28	41	83
CT 9 CMS × Rf	0	0	0	3	1	1	1	0	2	7	37	52	81
NB-1 CMS × Rf	6	25	39	10	0	6	1	1	1	2	0	93	14

[1] Fertility determined by percent aceto-carmine-stained pollen at anthesis of flowers on main stem of seed stalk.

[2] T = trace, 1 or 2 stainable pollen grains per thousand.

fertility, whereas NB-1 CMS progeny tended to group around the 10% fertility class. Six plants of this cross had white shriveled anthers devoid of stainable pollen and appeared to be similar to their NB-1 CMS parent.

In the 1966 field planting, four F_1 hybrids produced 100% pollen-producing plants with varied degrees of fertility (Table 5). Consistently there were more pollen-producing plants than white anther steriles in the F_2 and vice versa for the BC_1 generation. This suggests that a complementary type of gene action is governing the inheritance of pollen restoration. The SLC 128 CMS hybrid gave an excellent fit to a 9 fertile: 7 male-sterile ratio in the F_2 and a 1 fertile : 3 male-sterile ratio in the BC_1. Segregation of SLC 129 CMS × R_f was similar to that of the SLC 128 × R_f cross, but the F_2 fit was not as satisfactory. Segregation of CT 9 CMS × R_f and Al-1 CMS × R_f crosses gave a better fit to a 3:1 ratio than to a 9:7 ratio in the F_2. However, the backcross data did not support the premise that there was only a single gene responsible for pollen restoration in these crosses.

Table 5.—Pollen fertility of F_1, F_2, and BC_1 generation restorer hybrids determined in St. George field planting in 1966.

Hybrid	Gen.	No. plants[1] F	No. plants[1] MS	χ^2 Probabilities	
SLC 129 CMS × Rf	F_1	130	0		
CT 9 × CMS × Rf	F_1	102	0		
NB-1 CMS × Rf	F_1	131	0		
2938 CMS × Rf	F_1	191	0		
				(3:1)	(9:7)
SLC 129 CMS × Rf	F_2	915	643	<.001	.02-.05
SLC 128 CMS × Rf	F_2	32	24	<.01	.80-.90
CT 9 CMS × Rf	F_2	46	19	.30-.50	.01-.02
AI-1 CMS × Rf	F_2	65	15	.10-.20	<.001
NB-1 CMS × Rf	F_2	428	163	.10-.20	<.001
308HO 1 × Rf	F_2	275	72	.05-.10	<.001
211H3 × Rf	F_2	62	40	<.001	.30-.50
S 3317-5 × Rf	F_2	351	61	<.001	<.001
S 3317-14 × Rf	F_2	54	25	.10-.20	.02-.05
2937 × Rf	F_2	565	70	<.001	<.001
2938 CMS × Rf	F_2	438	129	.20-.30	<.001
F. C. 503 CMS × Rf	F_2	102	35	.80-.90	<.001
				(1:1)	(1:3)
SLC 129 CMS × Rf	b_1	56	186	<.001	.50-.70
SLC 128 CMS × Rf	b_1	6	21	<.01	.70-.80
CT 9 CMS × Rf	b_1	49	105	<.001	.05-.10
AI-1 CMS × Rf	b_1	88	231	<.001	.20-.30

[1] Visual observation. MS = white-anther plants and F = yellow anther plants with varied degrees of pollen dehiscence.

Several of the other hybrids gave a better fit to a monogenic F_2 ratio than to the complementary two gene model. Three hybrids, 2938 CMS × R_f, 308HOI × R_f, and Al-1 CMS × R_f gave excellent fit to a 13:3 F_2 ratio. The observed segregation ratio in the backcross for Al-1 CMS × R_f, however, was <.001 probability of the 3 fertile : 1 male sterile expected ratio. The BC_1 generation of the other two hybrids was not evaluated.

Two crosses, S 3317-5 CMS × R_f and 2937 CMS × R_f failed to satisfactorily fit any of the expected ratios for 1, 2, or 3 factor pairs. In all cases failure to fit the complementary 9 fertile : 7 male-sterile ratio was due to an excess of plants in the fertile class.

Discussion

Several attempts (1, 2, 3, 5, 8, 9) have been made to improve upon the original model that Owen (6) proposed for the inheritance of CMS. This is because the breeding behavior of some CMS × hermaphrodite crosses could not be explained on the basis of the original premise. The isolation of a line of sugarbeets having strong pollen recovery genes made possible another approach to this problem.

In our study, the consistent segregation of more fertile plants in the F_2 and more CMS plants in the BC_1 tends to support Owen's (6) original hypothesis that two complementary chromosmal factors govern male sterility or fertility. However, only in the case of the annual population RB 3104, and 3 biennial crosses, SL 211H3 × R_f, SLC 129 CMS × R_f, and SLC 128 CMS × R_f, did we observe a good statistical fit to the expected 9:7 F_2 and 1:3 fertile : male sterile BC_1 ratios.

The reason for the plant-to-plant variation which was observed is not readily explainable. Some of it can be attributed to environmental influences such as moisture stress and low temperature. Environmental variation is demonstrated by the differences observed between the same hybrids grown in the greenhouse and in the field. Conversely, the similarity of readings made at Salinas and St. George on several hybrids shows that environment is not the complete explanation for the observed variation. Pfahler (7) noted assortive mating between four homozygous lines of corn in that pollen carrying the white endosperm allele (y) was 5-33% less effective than pollen with the yellow endosperm allele (Y). Inasmuch as an excess of fertile (pollen producing) plants was observed consistently for the F_2 and BC_1 generations of several hybrids (Table 5), there may be assortive mating in sugarbeets, wherein pollen carrying the fertile allele(s) is more effective than pollen with the male-sterile allele(s).

Our data supports that of other scientists (2, 4, 5, 8) that much of the inter-plant variability in pollen fertility of a segregating generation from a given cross is under chromosomal genetic control. Progeny of NB-1 CMS crossed with Type O pollinators such as SLC 129 or the reciprocal cross of SLC 129 CMS × NB-1 pollinator gives 100% male-sterile offspring. Only when both male steriles are crossed to the same restorer line, as was done in this study, did they show differences in the interaction of genetic factors. NB-1 tended to resist pollen restoration more than any of the other lines studied, which suggests that this line is a superior emasculator.

We have noted, as have others (4, 8), that non-type O lines show more variation due to environment and minor modifying genes than do the Type O pollinators. The same was true for pollen restorer crosses. The hybrids were considerably more variable in fertility than the selfed generations of the hermaphrodite pollen restorer parent.

It is apparent that extremely refined experiments will be necessary to further delineate the inheritance and interactions of cytoplasmic male sterility and pollen-restorer genes in sugarbeets.

Summary

1. An inbred carrying strong pollen restorer genes has been isolated from the variety US 201.
2. Studies of inheritance tend to confirm Owen's original premise that cytoplasmic male sterility is governed by complementary genetic factors. However, interaction between minor modifying genes and cytoplasms, and the influence of environment resulted in poor fits to classical genetic ratios.
3. NB-1 CMS is a superior emasculator tending to resist pollen fertility restoration when crossed with the 201 R_f inbred.
4. Extremely refined experiments will be necessary to further delineate the inheritance and interactions of cytoplasmic male sterility and pollen restorer genes in sugarbeets.

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