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CLASSIFICATION OF MALE STERILE CYTOPLASMIC TYPES IN SUGAR BEET (*BETA VULGARIS* L.)[1)]

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Introduction

Most of the sugar beets cultivated in the world are hybrid varieties at diploid or triploid levels. Cytoplasmic male sterility (CMS) caused by the interaction between *S* cytoplasm and recessive nuclear genes is extensively used for hybrid seed production on a large scale. Therefore, there is the possibility of a risk caused by genetic vulnerability which may be brought about by the use of monoplasm as already indicated in the event of *T* cytoplasm in hybrid maize.

In this study, we explored the new sources of male sterile cytoplasms which originated from the mutants found in wild beets, *Beta maritima*. They are useful for the seed production of hybrid sugar beet varieties including the new types found recently.[2,8)]

Materials and Methods

Cytoplasmic male sterile lines and maintainers having *N* cytoplasm used in the experiment are listed in Table 1. The CMS lines of I-12CMS (R) to (8) possess different sources of cytoplasm but the nuclear genotype is equivalent to that of a sugar beet maintainer (type-O) line, I-12-61L. These materials were provided by courtesy of Dr. R. K. OLDEMEYER, the Great Western Sugar Co., TK76-CMS or TK81-CMS and TK76-O or TK81-O are CMS and its maintainer inbred line respectively, which were bred in Hokkaido National Agricultural Experiment Station. W162-6 is also introduced from the University of Wisconsin as a type-O strain of table beets. SP561001-0 is a red beet population possessing *N* cytoplasm and the marker gene for coloration. TA-2-O is an annual beet used as a type-O line and TK84-O(4*x*) is a tetraploid maintainer (type -O) line. They were used for the breeding work at Hokkaido National Agricultural Experiment Station.

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TABLE 1. List of lines or strains used in the crossing experiment

Strain or line	Description
TK76-O	Type-O line
TK76-CMS	CMS line isogenic to TK76-O
TK81-O	Type-O line
TK81-CMS	CMS line isogenic to TK81-O
I-12-61L	Type-O line for all of I-12CMS lines
I-12CMS(2)	Male sterile line having CMS derived from wild beet in Turkey
I-12CMS(3)	ditto in Pakistan
I-12CMS(4)	ditto in Turkey
I-12CMS(5)	ditto in Turkey
I-12CMS(7)	ditto in Manchuria
I-12CMS(8)	ditto in Turkey
I-12CMS(R)	Male sterile line having CMS derived from GW359 (sugar beet)
W162-6	Table beet, type-O line
SP561001-0	Red beet, N cytoplasm line
TK81-O(4x)	Tetraploid, type-O line
TA-2-O	Annual beet, type-O line

Male sterile types were grouped into four classes by visual and microscopic observations of anthers and pollens according to the demarcation standard used in previous papers[7,13] namely normal (N), semi-sterile type-a (SSa), semi-sterile type-b (SSb) and completely sterile (CS). Normal (N) and semi-sterile-a (SSa) plants were grouped together to form a male fertile type (MF) when the segregation modes are investigated.

Results

1. Polymorphism of organelle genomes in male sterile cytoplasms.

First, the circumstantial evidence for mitochondrial involvement in the inheritance of *S* type cytoplasmic male sterility in sugar beets was demonstrated from both restriction fragment analysis of mitochondrial (mt) DNAs and the variation in the number of small circular DNA species in their contour lengths from 0.28 to 0.4 μm.[3,9,14,15,16] Following this, chloroplast (ct) DNAs from the same material were classified into two groups though only a single HindIII site was different with *N* cytoplasm.[9,10,11] In addition, these data demonstrated a strict maternal inheritance of mt and ctDNAs. As well as this the cytoplasms derived from I-12CMS (2), (3), (4), (5), (7), (8) and (R) were classified into four groups including three new cytoplasms (*S-2, S-3* and *S-4*) and *S*, due to the molecular nature of both mt and ctDNAs as quoted in Table 2.[11,12] Thus, the polymorphism of

TABLE 2. Organelle genome diversity among normal and male sterile cytoplasms

Line	Cytoplasm	Cytoplasmic source	mtDNA												ctDNA	
			Plasmid-like DNAs (kbp)								Sma I		Hind III		Hind III	
			1.5	1.45	1.4	1.35	1.3	1.25	1.2	1.1	T#	U#	T	U	T	U
TK81-O	N	Japanese sugar beet variety	+		+		+				40	−	35	−	26	−
TK81-MS	S	do.	+		+						38	15	36	14	25	0
NK169-O	N	do.	+		+		+				40	0	35	0	26	0
NK169-MS	S	do.	+		+						38	15	36	14	25	0
I-12-61L	N	American sugar beet variety	+		+		+				40	0	35	0	26	0
I-12CMS (R)	S	do.	+		+						38	15	36	14	25	0
I-12CMS (2)	S-2	Wild beet from Turkey		+		+		+	+	+	34	7	38	9	27	2
I-12CMS (3)	S-3	Wild beet from Pakistan	+		+				+	+	37	11	38	9	27	2
I-12CMS (4)	S	Wild beet from Turkey	+		+						38	15	36	14	25	0
I-12CMS (5)	S	Wild beet from Turkey	+		+						38	15	36	14	25	0
I-12CMS (7)	S-4	Wild beet from Manchuria	+		+				+	+	36	8	38	9	27	2
I-12CMS (8)	S	Wild beet from Turkey	+		+						38	15	36	14	25	0

T: Total fragments, U: Cytoplasm-specific fragments which are not common with TK81-O.

organelle genomes was demonstrated by the biochemical method. In this study, we examined the same materials from the stand point of breeding by crossing experiments to identify the cytoplasm.

2. Crossing experiment using type-O plants.

It is known that a double recessive to Rf_1 and Rf_2 with N cytoplasm, namely N rf_1rf_1 rf_2rf_2, is most desirable as the pollen parent (type-O) in the production of a complete male sterile type (S rf_1rf_1 rf_2rf_2) when crossed with complete male sterile plants with S cytoplasm such as TK76-CMS and TK81-CMS. To investigate the nuclear genotype of male sterile isogenic lines, I-12CMS (2) to (8) and (R), complete male sterile plants from these lines were crossed with the pollen parents possessing the genotype, N rf_1rf_1 rf_2rf_2 from TK76-O. Phenotypic frequencies of male sterile types in F_2 generation are shown in Tables 3 and 4.

From the results which show that most of the F_2 plants indicated CS type (except for a few SSb type) in the progenies of crosses with CS plants from I-12CMS (4), (5) and (R) as well as in the crosses with TK76-CMS and TK81-CMS, it is estimated that these plants have a genetic constitution of S rf_1rf_1 rf_2rf_2. In contrast with this, all progenies of the crosses between CS type plants from I

TABLE 3. Frequencies of male sterile types in progenies of the crosses between CS plants having *S* cytoplasm and TK76-O (O-type)

Cross combi.	Male sterile type				Total
	N	SSa	SSb	CS	
I-12CMS(4) × TK76-O	0	0	8	161	169
I-12CMS(5) × 〃	0	0	0	32	32
I-12CMS(R) × 〃	0	0	7	232	239
TK76-CMS × 〃	0	0	0	51	51
TK81-CMS × 〃	0	0	5	68	73
Total	0	0	20	544	564

TABLE 4. Frequencies of male sterile types in progenies of the crosses between CS plants from I-12CMS (2) or (3) and plants randomly chosen from TK76-O

Cross combi.	Male sterile type				Total
	N	SSa	SSb	CS	
I-12CMS(2) × TK76-O: 2	0	0	27	1	28
〃 × 〃 :60	0	1	10	12	23
〃 × 〃 :71	0	0	8	9	17
Total	0	1	45	22	68
I-12CMS(3) × TK76-O: 2	35	36	2	0	73
〃 × 〃 : 3	12	8	0	0	20
〃 × 〃 :10	8	10	2	0	20
〃 × 〃 :12	1	2	0	8	11
〃 × 〃 :15	13	27	6	14	60
〃 × 〃 :71	10	9	1	0	20
Total	79	92	11	22	204

TK76-O (genotype: $S\ rf_1rf_1\ rf_2rf_2$).

-12CMS (2) and (3), and TK76-O showed a partial or full pollen fertility restoration. As shown in Table 3, a slight recovery of pollen types from CS to SSb occurred in the crossings with I-12CMS (2), while male fertile types (N or SSa) dominated in I-12CMS (3) × TK76-O though the segregations were heterogeneous depending on the genotype of TK76-O. From the results, a different nuclear gene or genes other than Rf_1 and Rf_2 for *S* cytoplasm seemed to be required for the pollen fertility restoration in the crosses involving I-12CMS (2) and (3) and the genes are derived from the genotype of TK76-O.

If we suppose the specific relation between the pollen fertility restoring gene or genes and the male sterile cytoplasms as demonstrated in sugar beet, maize and other crops,[1,6] the cytoplasmic types from I-12CMS (2) and (3), would be

candidates for the new cytoplasm different from *S* type.

3. Crossing experiments involving I-12CMS (3) and I-12CMS (7).

As mentioned before, both lines of I-12CMS (3) and (7) are isonucleargenic except for the cytoplasmic type. Therefore the different segregation modes of male sterile types imply a difference in cytoplasmic types.

SP561001-0 was bred true for the red coloration of root and leaves as a marker and it is supposed to be heterozygous for the genes related to the pollen fertility restoration. Three pollen parents were chosen randomly from a population of SP561001-0 and each plant was crossed with both CS type plants from I-12CMS (3) and (7). As shown in Table 5, the segregation modes of male sterile types differed between the two crosses involving I-12CMS (3) and (7), suggesting a difference of cytoplasmic type.

TABLE 5. Frequencies of male sterile types in F_2 progenies of the crosses between CS type plants from I-12CMS(3) or (7) and plants from SP561001-0 (*N* cytoplasm)

Cross combi.	Male sterile type in F_2				Total	Homogeneity χ^2 (d.f.=3)
	N	SSa	SSb	CS		
I-12CMS(3) × SP561001-0:a	37	44	16	31	128	11.946
I-12CMS(7) × SP561001-0:a	16	10	3	0	29	p=0.001−0.01
I-12CMS(3) × SP561001-0:b	51	59	4	0	114	8.791
I-12CMS(7) × SP561001-0:b	10	25	4	1	40	p=0.02−0.05
I-12CMS(3) × SP561001-0:c	10	12	2	16	40	9.628
I-12CMS(7) × SP561001-0:c	28	11	5	11	55	p=0.02−0.05

Following this, the mode of inheritance on the pollen fertility restoration was examined in the F_2 populations which derived from the pair-crossings between male fertile plants (N or SSa) in F_1s of the respective crosses. Throughout all of the crosses at least two major genes were responsible for pollen fertility restoration and the recessive allele in one locus was epistatic to the dominant allele in the other locus, showing a 9 : 3 : 4 ratio (Table 6).

It is already known that Rf_1 and Rf_2 interacting with *S* cytoplasm indicate the F_2 segregation to fit the 9 : 6 : 1 ratio for male fertile (N + SSa) : semi-sterile-b (SSb) : complete sterile (CS). Therefore it is reasonable that the Rf_a and Rf_b genes other than Rf_1 and Rf_2 are responsible for the new cytoplasmic types derived from I-12CMS (3) and (7).

However, the fitness of the observed numbers to the expected ratio, 9 : 3 : 4, in the crosses of I-12CMS (3) × SP561001-0 is inferior in comparison with those of the crosses, I-12CMS (7) × SP561001-0. It is supposed that the effects of modifying genes and/or environmental factors also affect the segregation ratio as

TABLE 6. F_2 segregtions of male sterile types in the crosses between CS type plants from I-12CMS(3) or (7) and SP561001-0 (*N* cytoplasm)

Cross	Cross type in F_1	Male sterile type			Total	Fitness (9:3:4)	
		N + SSa	SSb	CS		χ^2	p
I-12CMS(3) × SP561001-0	N × N	266 (273.9)	67 (91.3)	154 (121.8)	487	15.25	<0.001
	SSa × SSb	277 (328.5)	111 (109.5)	196 (146.0)	584	25.22	<0.001
I-12CMS(7) × SP561001-0	N × N	153 (159.8)	48 (53.3)	83 (71.0)	284	2.83	0.3-0.5
	SSa × SSb	202 (209)	78 (70)	92 (93)	372	1.24	0.5-0.7

Calculated number based on 9:3:4 ratio.

a feature of this cytoplasm. Thus the minor difference of cytoplasmic type was detected between I-12CMS (3) and (7).

In sum, three new cytoplasmic types were proved from the experiments. They are named as *S-2, S-3* and *S-4* corresponding to the isogenic lines, I -12CMS (2), I-12CMS (3) and I-12CMS (7) respectively.

4. Classification of male sterile types due to pollen fertility restoration

TA-2-O is an annual beet used for the selection of type-O plants together with the isogenic male sterile line, TA-2-CMS. Complete sterile plants from TK76-CMS (*S* cytoplasm), I-12CMS (2) (*S-2* cytoplasm), I-12CMS (3) (*S-3* cytoplasm) and I-12CMS (7) (*S-4* cytoplasm) were crossed with the type-O lines (*N* cytoplasm).

As shown in Table 7, *S*, *S-2* and *S-4* male sterile cytoplasms were clearly distinguished from each other due to the degree of pollen fertility restoration in F_1 s of the crossings with this differentiating line, TA-2-O, while the strict type

TABLE 7. Classification of male sterile cytoplasm types by the crosses with a differentiating line, TA-2-O

Cross combi.	Cytoplasmic type (group)	Male sterile type				Total
		N	SSa	SSb	CS	
TK81-CMS × TA-2-O	*S* (S_A)	0	0	0	66	66
I-12CMS(R) × TA-2-O		0	0	0	95	95
I-12CMS(2) × TA-2-O	*S-2*(S_A)	0	0	158	2	160
I-12CMS(2) × W162-6		0	0	0	24	24
I-12CMS(7) × TA-2-O	*S-4*(S_B)	129	217	9	0	355
I-12CMS(7) × W162-6		0	0	0	26	26

-O plant such as W162-6 brought about 100% CS type plants throughout the crosses.

A tetraploid line, TK84-O is also a type-O line for TK84-MS (4*x*). Triploid progenies were made by means of crossing with diploid lines TK76-MS, I-12 CMS (2), (3), (4) and (7). As shown in Table 8, triploid plants having *S* or *S-2* type cytoplasm showed only complete male sterility, while pollen fertile plants were segregated in the progenies of the crossings with male sterile plants having *S-3* or *S-4* type cytoplasms. At the triploid level, there is a possibility that the chromosomal aberrations bring a high degree of pollen sterility. As shown in Table 9, the pollen fertility of N type plants reduced significantly in comparison with that in the diploid population. However, pollen fertility restoration occurred in the progenies of the cross combinations having *S-3* or *S-4* cytoplasm both at 2*x* and 3*x* levels.

Thus the above mentioned four male sterile cytoplasms were divided mainly into the two groups, S_A (*S* and *S-2* cytoplasms) and S_B (*S-3* and *S-4* cytoplasms)

TABLE 8. Classification of male sterile cytoplasm types by the crosses with a tetraploid type-O line, TK84-O(4×)

Cross combi.	Cytoplasmic type	Male sterile type				Total
		N	SSa	SSb	CS	
S_A group ;						
TK76-CMS × TK84-O(4×)	*S*	0	0	0	66	66
I-12CMS(R) × 〃	*S*	0	0	0	93	93
I-12CMS(4) × 〃	*S*	0	0	0	42	42
I-12CMS(2) × 〃	*S-2*	0	0	0	48	48
S_B group ;						
I-12CMS(3) × TK84-O	*S-3*	9	18	14	5	46
I-12CMS(7) × 〃	*S-4*	21	38	16	6	81

TABLE 9. Pollen fertility(%) of the segregants at different ploidy levels

Ploidy		Male sterile type		
		N	SSa	SSb
2×	Mean	93.0±6.81	43.8±15.50	5.1±4.05
	Range of var.	73−100	20−66	1−16
	No. of plants	78	13	19
3*x*	Mean	82.4±8.33	51.0±12.29	14.7±5.87
	Range of var.	71−79	25−71	4−29
	No. of plants	30	57	30

depending on the pollen fertility restoration in the crossings with the two testers, TA-2-O (2*x*) and TK84-O (4*x*)

Discussion

First it is known that the demarcation of male sterile cytoplasms due to the crossing experiments coincided with the polymorphism which was predicted from the molecular nature of both mt and ctDNAs. However, the distinctive molecular features are not always correlated with the degree of pollen fertility restoration caused by the crossings with TA-2-O, as shown in the case of *S-2* cytoplasm line, I-12CMS (2). Specifically, only a weak degree of pollen fertility restoration occurred under *S-2* cytoplasm in spite of a marked difference in small circular DNA species and restriction enzyme pattern of mtDNA. On the other hand, a slight difference of cytoplasmic types was obtained from both plant and molecular natures as shown in the *S-3* and *S-4* cytoplasms. In the previous report, one of the authors produced a cytoplasmic mutation such as *Si-1, Si-2, Si -3* and *Si-4* (Kinoshita 1976, 1977, 1980). Among them, a segregation ratio, 9 : 3 : 4 due to the two nuclear genes was recognized in the crosses involving γ-20 mutant having *Si-2* cytoplasm. Identification both of nuclear genes and cytoplasms are necessary in future between *Si-2* and *S-3* or *S-4* cytoplasms.

From the stand point of breeding, it would be advantageous for the reciprocal recurrent selection to use the reciprocal maintainer-restorer relationship which was found in the cross experiments involving S_A and S_B groups. A new cytoplasm, *S-2,* belongs to S_A together with *S* cytoplasm which have been only used for the hybrid seed production in sugar beet. From the stand point of genetic vulnerability, it is desirable to add the new cytoplasm for establishing multiplasm system. It is known that *S-3* and *S-4* cytoplasms belong to S_B group. Therefore, multiplasms were prepared in both S_A and S_B groups. As shown in Fig. 1,

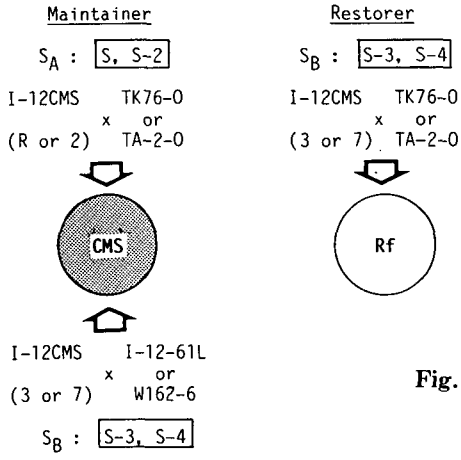

Fig. 1. Reciprocal maintainer-restorer relationship used in hybrid sugar-beet breeding.

isogenic lines with S_A and S_B cytoplasms are effectively used for the reciprocal recurrent selection due to the reciprocal maintainer-restorer relationships between them. Thus it is expected that the hybrid breeding of sugar beet will progress by the use of these materials.

Summary

In order to examine the relationship between the polymorphism found in mtDNAs and the diversity of cytoplasmic types, crossing experiments were carried out using the male sterile isogenic lines, I-12CMS (2) to (8) and (R) which were supplied by Dr. R. K. OLDEMEYER.

It was indicated that the pollen fertility restoring gene or genes other than Rf_1 and Rf_2 for *S* cytoplasm are responsible for cytoplasms derived from I-12CMS (2), (3) and (4) bringing out a partial or full fertility restoration even in the crossings with type-O plants (S rf_1rf_1 rf_2rf_2). These cytoplasmic types were denoted as *S-2*, *S-3* and *S-4* respectively.

As for the different cytoplasmic types, it was demonstrated that a differentiating line, TA-2-O and TK84-O (4*x*) clearly classified into the two groups, S_A (*S* and *S-2*) and S_B (*S-3* and *S-4*) by the pollen fertility restoration in the progenies. A reciprocal maintainer-restorer relationship was established in the cross combinations involving S_A and S_B groups and this relation can be used for reciprocal recurrent selection.

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