Making Use of a Male Sterile Cytoplasm of *Brassica campestris* in F_1 Seed Production

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A new male sterile cytoplasm was detected in *Brassica campestris* (OHKAWA, Y. 1981). In this paper, the inheritance of male sterility and the possibility of utilizing the male sterility of *B. campestris* for F_1 seed production in *B. campestris* and *B. napus* are briefly outlined.

In 1980, some plants of I-4 line were found to be partially male fertile in Tsukuba, Japan. I-4 line which is a kind of wild turnip in New Zealand was introduced to Japan by Dr. Hinata(Tohoku Univ.). For investigating the inheritance of male sterility, the reciprocal F_1 hybrids between I-4 line and other 33 cultivars or lines of *B. campestris* were examined. The thirty three cultivars or lines consisted of ssp. *rapifera*(17 lines), *pekinensis*(7), *oleifera*(4), *nipposinica*(2), *chinensis*(1), *trilocularis*(1) and of an unknown one. In some of the F_1 hybrids between I-4 line(♀) and the cultivars or lines(♂), male sterile plants were observed. As shown in Fig. 1, the male sterile plants had the following characteristics,

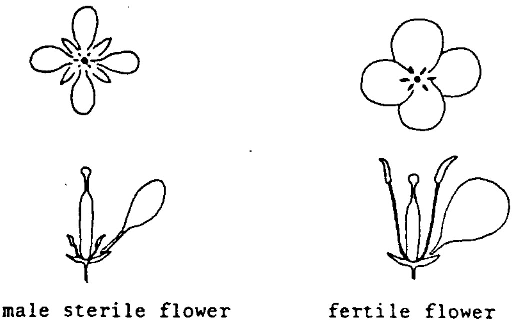

Fig. 1. Floral structure of male sterile and fertile plants.

(1) small anther with or without few pollen grains,
(2) short filaments,
(3) narrow petals.

The sterile flower had anthers without pollen grains, while a fully fertile flower had anthers with many pollen grains. A partially male fertile flower had an intermediate amount of pollen grains. As shown in the footnote of Table 1, there was a correlation between the degree of male sterility and the relative position of anther to stigma. Thus, the degree of male sterility was represented by the score of the relative position of anther to stigma.

Table 1. Degree of male sterility in hybrids and their parents.

Parents/ Hybrids	No. of Plants observed	*Relative Position of Anther/Stigma						
		1	2	3	4	5	6	mean
Maruba-santosai	15				1	3	11	5.67
I-4 x Maruba-santosai	18			11	5	2		3.50
Maruba-santosai x I-4	20					4	16	5.80
Shin-isogo-kyona	18				1	15	2	5.06
I-4 x Shin-isogo-kyona	17			4	8	5		4.05
Shin-isogo-kyona x I-4	19					7	12	5.63
Sensuji-kyona	20					3	17	5.85
I-4 x Sensuji-kyona	16	2	10	4				2.13
Sensuji-kyona x I-4	19				3	12	4	5.05
Shogoin-kabu	5				1	4		4.80
I-4 x Shogoin-kabu	19		4	9	5	1		3.16
Shogoin-kabu x I-4	12					2	10	5.85
I-4 x Yorii-kabu	15	3	8	3	1			2.13
Yorii-kabu x I-4	20					4	16	5.80
Hakatasuwari	7					5	2	5.29
I-4 x Hakatasuwari	19	1	13	3	1	1		2.37
Hakatasuwari x I-4	18					3	15	5.83
Kisobeni	20					14	6	5.30
I-4 x Kisobeni	20		9	8	3			2.70
Kisobeni x I-4	19					4	15	5.79
Hidabeni	12					8	4	5.33
I-4 x Hidabeni	19		8	3	7	1		3.05
Hidabeni x I-4	16				1	4	11	5.63
Waseimaichi	4					4		5.00
I-4 x Waseimaichi	15			5	4	6		4.07
Waseimaichi x I-4	18					15	3	5.17
Yonagoaka-kabu	5					1	4	5.80
I-4 x Yonagoaka-kabu	12			8	2	2		3.50
Yonagoaka-kabu x I-4	2					1	1	5.50

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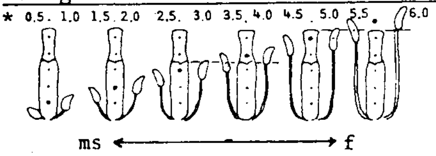

In 33 reciprocal crosses, there were male sterile plants in the F_1 hybrids between I-4 line(♀) and cultivars(♂), while all of their reciprocal F_1 hybrids(cultivars x I-4) had only fertile flowers(Table 1). Thus male sterility was considered to be cytoplasmically controlled. Since I-4 line had a male sterile cytoplasm and had fertility restorer gene(s) in its nucleus, it was partially male fertile. The ten cultivars listed in Table 1 were considered to have normal(non-male sterile) cytoplasms and not to have fertility restorer gene(s). These belonged to *B. campestris* ssp. *pekinensis* (1), *nipposinica*(2) and *rapifera*(7). Both of the reciprocal F_1 hybrids between the other 23 cultivars or lines and I-4 line were fertile. The fertility of the reciprocal hybrids was estimated to result from the fact that these 23 cultivars or lines had fertility restorer gene(s).

For determining the existence of segregation of male sterile plants in F_2 and BC_1, five F_2 and BC_1 populations out of ten cultivars having no fertility restorer gene(s) and 15 F_2 and BC_1 populations out of 23 cultivars having restorer gene(s) were developed(ohkawa, Y. 1982). In the F_2 and BC_1 populations of the hybrids resulting from crosses between eight out of the 15 cultivars or lines having restorer gene(s) and I-4 line, male sterile plants were segregated(Table 2), while those from seven out of 15 cultivars or lines did not segregate male sterile plants. In the F_2 and BC_1 populations resulting from crosses between three out of five cultivars having no restorer gene(s) and I-4 line, male sterile plants were segregated(table 2). And also hybrids of the residual two plants out of five cultivars segregated male sterile plants, though data are not shown in Table 2. In other words, male sterile plants were obtained from the F_2 or BC_1 populations of the hybrids between I-4 line and the cultivars having no fertility restorer gene(s) as well as from those of several cultivars having restorer gene(s).

As mentioned above, male sterile lines can be obtained in *Brassica campestris* by using I-4 line as a female parent. One third of the 33 cultivars or lines examined lacked fertility restorer gene(s). Thus, it appears to be easy to obtain a large number of male sterile lines if cultivars having no fertility restorer gene(s) are used as male parents. When, cultivars having restorer gene(s) are used, male sterile lines can be bred from the progeny of hybrids between some male sterile lines and the cultivars or between I-4 line and the cultivars as shown in Table 2.

For building up an F_1 seed production system using cytoplasmic male sterility in *Brassica campestris*, it is important to avoid self-incompatibility. One possible way to achieve this objective is to introduce self-compatibility from self-compatible varieties, for example in using brown sarson.

As described above, the male sterile plants in *B. campestris* had three floral characteristics; small anther with or without few

Table 2. Degree of Male Sterility in F_2 or BC_1 Populations between I-4 line and Other Cultivars in *Brassica campestris*.

Parent/	No. of Plants	Relative Position of Anther/Stigma						
F_2/BC_1	Observed	1	2	3	4	5	6	mean
Hakusai 4	11					1	10	5.91
(I-4 x Hakusai 4)F_2	62		3	9	26	22	2	4.18
(I-4 x H 4) x H 4	33		3	6	8	11	5	4.27
Hakusai 5	13						13	6.00
(I-4 x Hakusai 5)F_2	63		1	3	25	32	2	4.49
(I-4 x H 5) x H 5	18			3	3	10	2	4.61
Hakusai 7	13						13	6.00
(I-4 x H 7)F_2	58	1	4	10	21	21	1	4.03
(I-4 x H 7) x H 7	26			2	8	13	3	4.65
Hakusai 8	17						17	6.00
(I-4 x H 8)F_2	51		4	6	13	24	4	4.35
(I-4 x H 8) x H 8	35			6	14	11	4	4.37
Matsushimakekkuhakusai L2	1						1	6.00
(I-4 x Matsushima. L2)F_2	69		2	8	13	34	12	4.67
(I-4 x Matsu.) x Matsu.	37		1		5	22	9	5.03
Mana	12					7	5	5.42
(I-4 x Mana)F_2	72		9	22	29	11	1	3.63
(I-4 x Mana) x Mana	31		6	8	8	8	1	3.68
Pakuchoi	11						11	6.00
(I-4 x Pakuchoi)F_2	68		3	15	30	19	1	4.00
(I-4 x Paku.) x Paku.	40	1	23	14	2			2.42
Akane-kabu	16				5	11		4.69
(I-4 x Akane-kabu)F_2	14		2	3	3	6		3.93
(I-4 x Akane.) x Akane.	31				13	18		4.58
Maruba-santosai	14					10	4	5.29
(I-4 x Maruba.)F_2	70			11	34	22	3	4.24
(I-4 x Maruba.) x Maruba.	35		2	7	19	6	1	3.91
Shin-isogo-kyona	16				3	12	1	4.88
(I-4 x Shin.)F_2	52		2	3	29	18		4.21
(I-4 x Shin.) x Shin.	26		1	5	10	10		4.12
Wase-imaichi	18					8	10	5.56
(I-4 x Wase-imaichi)F_2	74	2	11	11	22	25	3	3.89
(I-4 x Wase.) x Wase.	40	4	26	8	1	1		2.23

pollen grains, short filaments and narrow petals. These characteristics were the same as those of male sterile plants in *Brassica napus*(Shiga and Baba 1971, 1973). If the male sterile cytoplasm of *B. campestris* is considetred to be identical with that of *B. napus*, or if the male sterile cytoplasm of *B. campestris* induces male sterility in *B. napus*, the cytoplasm of *B. campestris* could be used for developing F_1 seed production system in *B. napus*. Thus, attempts were made to determine whether the male sterile cytoplasm of *B. campestris* and that of *B. napus* were similar(Ohkawa, Y. 1981).

Table 3. Degree of male sterility in hybrids and their parents.

Parents/	No. of Plants	Relative Position of Anther/Stigma						
Hybrids	observed	1	2	3	4	5	6	mean
I-4 line	18				13	4	1	4.33
Isuzu-natane	37					1	36	5.97
I-4 x Isuzu-natane	22		3	6	8	5		3.68
Isuzu-natane x I-4	20					10	10	5.50
Bronowski	37					2	35	5.95
I-4 x Bronowski	19	1	1	11	4	2		3.26
Bronowski x I-4	21				2	18	1	4.95
Norin 16	19					6	13	5.68
I-4 x Norin 16	19				1	15	3	5.11
Norin 16 x I-4	19					8	11	5.58
Mutsu-natane	39					3	36	5.92
I-4 x Mutsu-natane	20				2	15	3	5.05
Mutsu-natane x I-4	7					3	4	5.57

To investigate this characteristic, the following crosses were made and the degree of male sterility of the hybrids was evaluated. Namely, the degree of male sterility of hybrids between I-4 line and the maintainers for the male sterility in *B. napus* (Isuzu-natane and Bronowski) and hybrids between I-4 line and the restorers in *B. napus* (Mutsu-natane and Norin 16) was evaluated. As shown in Table 3, male sterile plants were identified in the F_1 populations between I-4 line(♀) and the maintainers, while all of their reciprocal hybrids were fertile. Especially the plants of I-4 line that underwent five series of back-crossing with Isuzu-natane were male sterile(the data are not shown here). Thus, the male sterility induced by the male sterile cytoplasm of *B. campestris* could be maintained by the maintainers for the cytoplasmic male sterility of *B. napus*. In the case of the F_1 hybrids between I-4 line and the restorers, all hybrids were fertile, i.e., the male sterility of *B. campestris* was restored by the restorers for that of *B. napus*. As described above, Maruba-santosai, a cultivar belonging to *B. campestris* that did not have the restorer gene(s) for the male sterile cytoplasm in *B. campestris*, was considered to be a maintainer for the cytoplasmic male sterility of *B. campestris*. The hybrid plants of a male sterile line of *B. napus* that had undergone seven series of back-crossing with Maruba-santosai were male sterile(the data are not shown here). That is to say, the male sterility of *B. napus* could be maintained by the maintainer(s) for that of *B. campestris*.

From the results obtained, it appears that the male sterile cytoplasm of *B. campestris* is identical with that of *B. napus* with regard to male sterility. It also seems possible to obtain some male sterile lines of *B. napus* having the male sterile cytoplasm of *B. campestris* by back-crossing, because *B. napus* can be easily crossed with *B. campestris*.

It thus seems useful to utilize the male sterile cytoplasm of *B. napus* as well as that of *B. campestris* in the F_1 hybrid system of *B. napus*, since it is not advisable to use only one kind of male sterile cytoplasm for establishing a F_1 seed production system. In maize, T cytoplasm which is one of the male sterile cytoplasms, was specifically susceptible to T race of *Helminthosporium maydis*. The populations of the F_1 hybrid system with cytoplasmic male sterility, therefore, were thoroughly damaged as only the T cytoplasm was used for establishing the system. So, it seems useful to utilize two male sterile cytoplasms of different origin when establishig the F_1 hybrid system of *B. napus*.

Literature cited

OHKAWA, Y. 1981: A Cytoplasmic Male Sterility Observed in Brassica campestris L. Jap. J. Breed. 31 (Suppl. 2): 114-115 (in Japanese).

OHKAWA, Y. 1982: Seed Production by Using a Male Sterile Cytoplasm in B. campestris. Jap. J. Breed. 32 (Suppl. 2): 90-91 (in Japanese).

SHIGA, T. and S. BABA 1971: Cytoplasmic Male Sterility in Rape Plant(Brassica napus L). Jap. J. Breed. 21 (Suppl. 2): 16-17 (in Japanese).

SHIGA, T. and S. BABA 1973: Cytoplasmic Male Sterility in Oil Seed Rape, Brassica napus L, and Its Utilization to Breeding. Jap. J. Breed. 23: 187-197 (in Japanese with English summary).