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Genic Male Sterility in *Brassica rapa* ssp. *rapa* cv. 77B

By

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Summary：The mode of inheritance and morphology of a male sterile (ms) turnip (*Brassica rapa*) was studied. Morphology of the male sterile plants did not differ from that of a male fertile (mf) plant, except for the shorter stamens and smaller petals. The results of a cross between a ms plant and two cultivars of *B. rapa* indicated that male sterility is controlled by a single recessive gene without any cytoplasmic effect. Light microscopic observations showed no pollen grains on the inside of ms anthers, suggesting collapse of microsporogenesis at the tetrad stage or at an earlier stage in the ms plants. Analysis of the amino acid content from the 4-5 mm flower buds of mf and ms plants showed a remarkable increase (2.7-fold) in proline content in the former.

Key words：genic male sterility, turnip, *Brassica rapa* ssp. *rapa* cv. 77B

Introduction

The utilization of heterosis in *Brassica* crops has great potential for increasing yield[1-3]. Several genetic systems have been developed for the commercial production of hybrid seed, such as cytoplasmic male sterility (CMS), genic male sterility (GMS), and self-incompatibility (SI)[4]. The GMS system is not widely used because removal of 50% of segregating fertile plants in the sterile female line is required to produce seeds when a trait is controlled by a single recessive gene, which limits its utilization for the production of hybrid seed. However, it has great potential for hybrid seed production due to its particular characteristics : complete and stable male sterility, no negative cytoplasmic effects on yield, and ease of transfer of the male sterile gene(s) to diverse genetic backgrounds[5].

Brassica rapa is an allogamous species as a result of its self-incompatible system, and contains some of the most important arable vegetable subspecies such as Chinese cabbage (ssp. *pekinensis*), turnip (ssp. *rapa*), Mizuna (ssp. *japonica*), Taina (ssp. *chinensis*) and Yukina (ssp. *narinosa*). Some GMS in *B. rapa* has been found. One such case was described by Van der MERR (1987) for ssp. *pekinensis* as a dominant GMS. A recessive GMS line of ssp. *chinensis* was later selected and used in F_1 hybrid seed production of *Brassica* crops[7,8]. TAKAHATA *et al.* (1996) reported a second recessive GMS of ssp. *pekinensis*, the ms type, whose gene acts after microspore formation. However, GMS turnip has not yet been reported.

We discovered a GMS plant in a population of turnip cv. 77B. In this study, in order to characterize GMS of this cultivar, we examined the mode of inheritance, pollen morphology and tapetum development, as well as amino acid content of flower buds in a newly found male sterile line.

Materials and Methods

Five plants of *B. rapa* ssp. *rapa* cv. 77B were grown in an uncontrolled-environment greenhouse. A male sterile (ms) plant was found from this population. Ms plant was crossed with a male fertile (mf) plant of cv. 77B, and then their F_1 plants were selfed and backcrossed to ms plants to obtain F_2 and BC_1 progenies, respectively. From the segregating population, six flowers were arbitrarily selected from one individual in ms and mf, respectively, and then the sizes of stamen, petal, pistil and sepal were measured. Measurement of size was repeated three times using different individuals in ms and mf, respectively. Difference of the size of each organ between ms and mf was tested by paired *t*-tests. Also, ms plant was crossed

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with *B. rapa* ssp. *rapa* cv. Tsuda-kabu, and *B. rapa* ssp. *pekinensis* cv. Matsushima-shin-2gou-hakusai, respectively. Their F_1 plants were selfed and backcrossed to male sterile ms plants to obtain F_2 and BC_1 progenies, respectively. Tsuda-kabu, as the female plants, was crossed with F_1 plants produced by crossing a ms and a mf cv. 77B. Moreover, the F_1 progenies of 13 plants were selfed to obtain F_2 seed from the resulting plants.

At least ten flower buds of the ms and mf plants were fixed in FAA (50% ethanol, 5% glacial acetic acid, 3.7% formaldehyde) for 18–24h. Samples were rinsed several times in 50% ethanol, dehydrated through a graded ethanol series, and stained in 100% ethanol containing 1% safranin for easy orientation of the material. The buds were embedded in paraffin, then cut into 2–3 μm sections and stained with hematoxylene.

Frozen flower buds were triturated by pestles, and extracted three times with 80% ethanol. The extract was desiccated by a rotary evaporator, and then re-dissolved in 1000 μl of distilled water, serving as the samples for the HPLC analysis. The quantity of the resulting 38 amino acids in the samples was determined by an Amino Acid Analysis System (Shimadzu).

Results and Discussion

The ms plants were not morphologically different from the mf plants except for the flowers. The flower of the ms plants was characterized by small and shriveled anthers that contained no fertile pollen. Figure 1 shows the ms and mf flowers and their constitutive organs, which differed in size (Table 1). Ms flowers had significantly ($p<0.01$) shorter stamens and sepals, and smaller petals than mf flowers. The pistil was longer in the ms flower, which was similar to that reported by TAKAHATA *et al.* (1996) for ms turnip.

All the F_1 plants obtained from the cross between ms and two cultivars (cv. Tsuda-kabu and Matsushima-shin-2gou-hakusai) were mf (Table 2). In addition to mf plants, ms plants were segregated in the F_1 of the cross, ms x cv. 77B. This indicates that the population of cv. 77B used as the male parent included heterozygous mf plants as well as homozygous mf plants. The segregation pattern in the F_2 and BC_1 generations is shown in Table 2. Segregation data on the F_2 population of ms x cv. Tsuda-kabu and ms x cv. Matsushima-shin-2gou-hakusai showed a 3 : 1 ratio (mf : ms). In BC_1 plants obtained from the cross, ms x (ms x cv. Tsuda-kabu) and ms x (ms x Matsushima-shin-2gou-hakusai), a 1 : 1 ratio of mf : ms was obtained. This suggests that male sterility is controlled by a ingle recessive gene. In order to examine the effect of cytoplasm, a cross using cv. Tsuda-kabu as the female parent and the mf progeny of ms x cv. 77B as the male parent, was carried out. All the F_1 plants showed mf. In F_2 families derived from 13 F_1 plants selected randomly, five lines were composed of only mf plants, and eight lines were segregated ms plants (Table 3). Segregation data of the eight lines was in agreement with an expected 3 : 1 (mf : ms) ratio. The presence of five homozygous and eight heterozygous mf plants in F_1 showed good agreement with an expected 1 : 1 ratio following the chi-square test ($\chi^2=0.35$, $0.8<P<0.9$), confirming that the ms turnip found in this study is controlled by a monogenic recessive gene.

Fig. 1 Morphological characterization of GMS in *B. rapa* ssp. *rapa* cv. 77B. (A) and (B) show the flower and constitutive organs of mf (left) and ms (right), respectively.

Table 1 Comparison of flower characteristics between male sterile and male fertile *B. rapa* lines.

Phenotype	Characters (mm)					
	Length of long stamen	Length of short stamen	Length of petal	Width of petal	Length of pistil	Length of sepal
MF[1)]	6.9±0.08	4.5±0.07	10.6±0.17	4.7±0.09	6.1±0.19	6.2±0.09
MS[2)]	4.3±0.07	2.6±0.06	9.5±0.08	4.0±0.08	6.9±0.1	4.8±0.06
t value	24.96**	20.33**	6.50**	6.30**	3.44**	12.84**

[1)] Male fertile, [2)] Male sterile

**: Significantly different at the 1% level

Table 2 Segregation of male sterility obtained in three F_1's of ms *B. rapa* x cultivars and their progenies.

Cross combination		Phenotype[1)] MF	Phenotype[1)] MS	χ^2	P
ms x cv. Tsuda-kabu	Obs.	11	0		
ms x cv. Matsushima[2)]	Obs.	24	0		
ms x cv. 77B	Obs.	58	45	1.64	0.2<P<0.3
	Exp. (1 : 1)	51.5	51.5		
(ms x cv. 77B) F_2	Obs.	52	16	0.08	0.7<P<0.8
	Exp. (3 : 1)	51	17		
(ms x cv. Tsuda-kabu) F_2	Obs.	29	8	0.23	0.6<P<0.7
	Exp. (3 : 1)	27.75	9.25		
(ms x cv. Matsushima) F_2	Obs.	44	17	0.27	0.6<P<0.7
	Exp. (3 : 1)	45.75	15.25		
ms x (ms x cv. 77B) F_1	Obs.	23	19	0.38	0.5<P<0.6
	Exp. (1 : 1)	21	21		
ms x (ms x cv. Tsuda-kabu) F_1	Obs.	25	22	0.19	0.6<P<0.7
	Exp. (1 : 1)	23.5	23.5		
ms x (ms x cv. Matsushima) F_1	Obs.	21	27	0.75	0.3<P<0.4
	Exp. (1 : 1)	24	24		

[1)] MF: male fertility, MS: male sterility
[2)] Matsushima: Matsushima shin-2gou hakusai

Table 3 Segregation of male sterile plants obtained in the F_2 generation of cv. Tsuda-kabu x (ms x cv. 77B).

Plant no. of F_1 between cv. Tsuda-kabu and (ms x cv. 77B) F_1			No. of plants in F_2 MF[1)]	No. of plants in F_2 MS[2)]	χ^2 (3 : 1)	P
	F_1 – 1	Obs.	29	9	0.02	0.9<P<1.0
		Exp.	28.5	9.5		
	F_1 – 2	Obs.	46	13	0.14	0.9<P<1.0
		Exp.	44.25	14.75		
	F_1 - 3	Obs.	43	10	0.58	0.7<P<0.8
		Exp.	39.75	13.25		
	F_1 - 4	Obs.	25	7	0.09	0.9<P<1.0
		Exp.	24	8		
	F_1 - 5	Obs.	15	0		
cv. Tsuda-kabu	F_1 - 6	Obs.	47	19	0.24	0.8<P<0.9
x		Exp.	49.5	16.5		
(ms x cv. 77B)	F_1 - 7	Obs.	32	9	0.11	0.9<P<1.0
		Exp.	30.75	10.25		
	F_1 - 8	Obs.	63	0		
	F_1 - 9	Obs.	63	0		
	F_1 - 10	Obs.	33	11	0.00	1.0
		Exp.	33	11		
	F_1 - 11	Obs.	64	0		
	F_1 - 12	Obs.	24	0		
	F_1 - 13	Obs.	44	12		
		Exp.	42	14	0.20	0.9<P<1.0
Total of F_1 - 1-4, 6, 7, 10,13		Obs.	299	90	0.37	0.8<P<0.9
		Exp.	291.75	97.25		

[1)] MF: male fertility, [2)] MS: male sterility

Light microscopic observations revealed that pollen and tapetum development of the ms plant was different from that of the mf plant at an early microspore stage (Fig. 2). In the mf anther, microspores released from tetrads contained densely cytoplasmic cells with a nucleus and cell wall (Fig. 2A). The tapetum degenerated gradually, and then completely disappeared at the late microspore stage (Fig 2B and 2C). In ms anthers, on the other hand, microsporogenesis—even tetrad formation—could not be observed on the inside of flower buds 2–3 mm in length (Fig. 2D). The substance inside pollen sacs was crushed and adhered (Fig. 2E), and no pollen grains were observed (Fig. 2F). In most histologically described male sterility, microsporogenesis breaks down at the tetrad stage and sometimes at the uninucleate vacuolate microspore stage[10)]. In *B. rapa*, breakdown of micro-

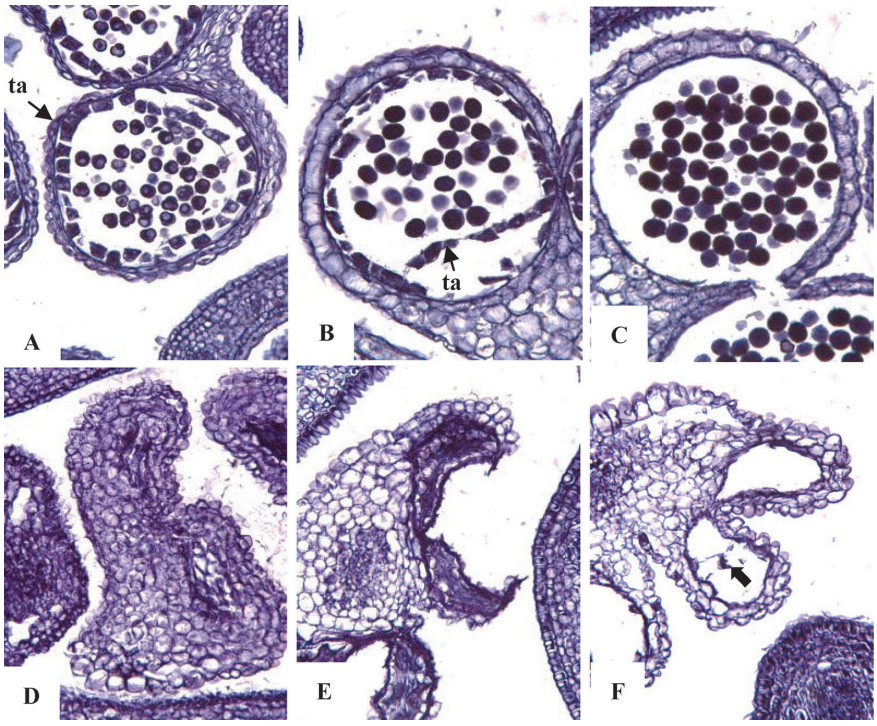

Fig. 2 Cytological characterization of GMS in *B. rapa* ssp. *rapa* cv. 77B. (A)–(C) and (D)–(F) show microsporogenesis of mf and ms plant, respectively. Bud size is as follows ; (A) and (D) : 2–3 mm, (B) and (E) : 4–5 mm, (C) and (F) : 6–7 mm. ta : tapetum. Arrow indicates the concrescence of the substance in the pollen sac. — : Bar : =10 μm

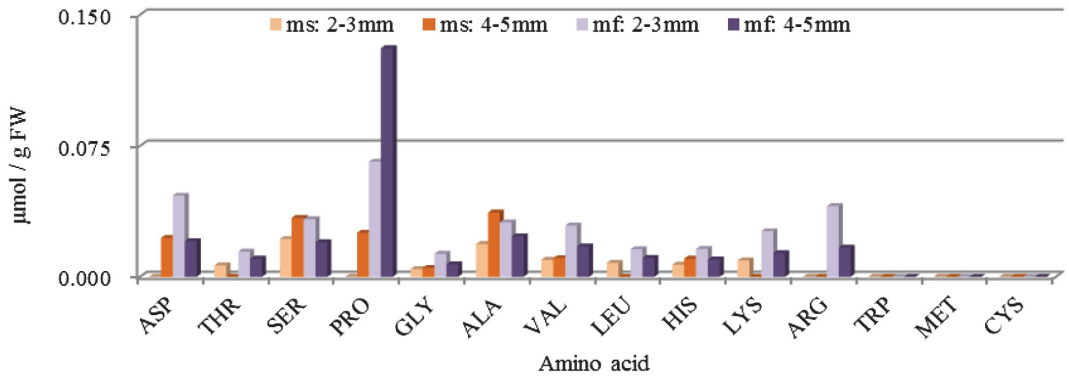

Fig. 3 Fifteen amino acid content in bud (2–3 mm and 4–5 mm) of ms and mf plant of *B. rapa* ssp. *rapa* cv. 77B, respectively. ASP : aspartic acid/aspartate, THR : threonine, SER : serine, PRO : proline, GLY : glycine, ALA : alanine, VAL : valine, LEU : leucine, HIS : histidine, LYS : lysine ARG : arginine, TRP : tryptophan, MET : methionine, CYS : cysteine

sporogenesis at the tetrad stage in male sterility was reported in var. brown sarson and yellow sarson[11-13]. Cytological observation in ms cv. 77B in this study also suggests the collapse of microsporogenesis at the tetrad stage, or at an earlier stage. Indeed, male sterile stamens only had vestigia of anthers (Fig. 1B).

Analysis of amino acid content from flower buds of mf and ms plants showed a remarkable increase in proline content in the former (Fig. 3). Proline content in flower buds (4–5 mm) of mf and ms plants accounted for 46.7% and 17.5%, respectively of the amino acid pool. This shows that proline content in flower buds is 2.7-fold higher in mf plants than in ms plants. Our data agrees with the result of WU and MURRY (1985), who showed that the levels of proline in the anther of a fertile line was 2–3 fold higher than that of a CMS line of *petunia*. Proline may function as the solute protectant in developing pollen preserving the grain against unfavorable environmental conditions[14,15].

We found a ms plant in a population of turnip cv. 77B,

which is a European turnip cultivar resistant to clubroot[16]. To date, in order to breed a new clubroot-resistant turnip in Japan, cv. 77B has been used as the male parent for crossing[17]. This means that this ms gene was introduced into a part of Japanese turnip. On the other hand, turnip differentiated into several hundreds of local varieties in each district after it was introduced in Japan[18,19]. In the future, another type of male sterility may be found from the gene pool of Japanese turnip varieties. We found some Japanese cultivars showing ms traits, and their characterization is in progress.

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Brassica rapa ssp. *rapa* cv. 77B における核遺伝子型雄性不稔性

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要約：カブ（*Brassica rapa* ssp. *rapa*）品種 77B で見いだされた雄性不稔性について，花の形態および遺伝様式を調査した。雄性不稔個体は可稔個体と比較して雄蕊が短く，花弁が小さかった。雄性不稔個体を 77B の可稔個体および異なる 2 品種と交配し，その後代の分離比を調査した結果，本雄性不稔性は劣性の一遺伝子座によって支配されていることが示された。雄性不稔性への細胞質による影響はみられなかった。また，雄性不稔および可稔個体の蕾をパラフィン切片法で薄切し，光学顕微鏡で観察した結果，雄性不稔個体における四分子期あるいはより早い時期における四分子形成の崩壊が示唆された。さらに，蕾（4–5 mm）のアミノ酸解析から雄性不稔個体と比べ可稔個体でプロリン含量の著しい増加（2.7–fold）が示された。

キーワード：核遺伝子型雄性不稔性，カブ，*Brassica rapa* ssp. *rapa* cv. 77B

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