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CYTOPLASMIC-GENETIC MALE STERILITY IN CULTIVATED RICE, *ORYZA SATIVA* L.
II. THE INHERITANCE OF MALE STERILITY[1]

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Basing on the hybrid sterility of cultivated rice, *Oryza sativa*, Kato (1930) classified rice varieties into two sub-species, *Indica* and *Japonica*. Oka (1953, 1954) and Matsuo (1952) classified them into three groups. Moreover, Oka (1954, 1963) and Katsuo *et al* (1958) considered that hybrid sterility of the distantly related varieties did not contain cytoplasmic sterility.

But Shinjyo and Omura (1966) detected cytoplasmic-genetic male sterility in B_1 F_1 population: (Chinsurah Boro II×Taichung 65)×Taichung 65.

In this paper it is reported that trials were made to analyse the interaction of cytoplasm and nuclear genes in order to elucidate the inheritance of male sterility. The crossing results showed that the occurrence of male sterility in individual plants resulted from the interaction of a pair of genes in the nucleus and cytoplasm. Moreover, it also became clear that this sterility was male gametophytic. An outline of the results is reported in this paper.

MATERIALS AND METHODS

Besides the BT-1 line with sterile cytoplasm which was derived from Chinsurah Boro II, twenty-five *Japonica* and six *Indica* varieties of rice were used for crosses.

The BT-1 line was derived from the progeny of back-crosses as follows: When the F_1 hybrid, Chinsurah Boro II (♀)×Taichung 65, was back-crossed with Taichung 65 (♂), the B_1 F_1 population segregated into partially male-sterile and completely male-sterile plants in a ratio of 1 : 1. Then partially male-sterile plants in each hybrid generation were back-crossed repeatedly with Taichung 65 as the pollinater (recurrent parent). Completely male-fertile line was selected by selfing partially male-sterile B_6 F_1 plants. This was the BT-1 line which had presumably the same genetic background as that of Taichung 65.

For crossing, hot water emasculation method (at 43°C for 6 minutes) was employed. Hybrid seedlings were planted in a paddy field and the standard practice of cultivation was followed.

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Five young flowers expectedly blooming in the next morning were collected from each plant, were fixed in Carnoy's solution, and were preserved in 75% alcohol. The pollen grains were stained with iodine and potassium iodide solution for observation. Fertile pollen grains were globular in shape and were deeply stained, while the sterile pollens, though globular in shape, were smaller in size and were not so stained.

RESULTS

Experiment 1.

When the BT-1 line, completely male-fertile, which had the cytoplasm of Chinsurah Boro II, was crossed with Taichung 65 (♂), the F_1 hybrid showed a 50% pollen fertility though seed fertilities were more than 90%. The F_2 population segregated into completely male-fertile (BT-A line) and partially male-sterile plants (BT-B line) in a 1 : 1 ratio. When the BT-B line was crossed with Taichung 65 (♂), the progeny segregated into partially male-sterile and completely male-sterile plants (MsBT- C line) in a 1 : 1 ratio again, as shown in Table 1. Out of the three lines, MsBT- C as used female parent

Table 1. F_1 plants of BT-B×Taichung 65, [*ms*] *Rf rf*×[*ms*$^+$] *rf rf*, segregating into male-sterile and partially male-sterile classes.

Line and cross		Pollen fertility (%) 0	10	20	30	40	50	60	Number of plants	χ^2-test 1 : 1
BT-B×Taichung 65	1	49				1	49	1	100	0.040
	2	45					40	1	86	0.186
	3	65				1	60	1	127	0.071

Table 2. Pollen fertility of F_1 plants of MsBT-C×BT-A, [*ms*] *rf rf*×[*ms*] *Rf Rf*; and MsBT-C×BT-B, [*ms*] *rf rf*×[*ms*] *Rf rf*.

Line and cross		Pollen fertility (%) 30	40	50	60	70	Number of plants
MsBT-C×BT-A	1		1	80	2		83
	2		3	92	3		98
	3		2	69	1		72
	4		2	98	1		101
MsBT-C×BT-B	5		5	106	2		113
	6		1	96	3		100
	7		2	120	1		123

Table 3. Segregation of pollen-fertility in the selfed progeny of BT-B line, [*ms*] *Rf rf*.

Line		Pollen fertility (%) 40	50	60	70	80	90	100	Number of plants	χ^2-test 1 : 1
BT-B (Selfed)	1		71				2	72	145	0.062
	2	1	72	1			1	71	146	0.027

was crossed with BT-A and BT-B lines, respectively. In their F_1 generations, only partially male-sterile plants appeared, regardless of the degree of pollen fertility of the male parent, as shown in Table 2.

The three lines were each selfed and the pollen fertilities of the progenies were observed. The BT-A line produced only completely male-fertile plants, while the BT-B segregated into completely male-fertile and partially male-sterile plants in a 1 : 1 ratio (Table 3). Another line MsBT-C produced completely male-sterile plants.

On the other hand, in the reciprocal crossing where Taichung 65 was used as female and BT-1 as male, the F_1 hybrid was completely male-fertile. The F_2 generation showed no segregation for pollen-fertility and all the plants were completely male-fertile. Their seed fertility was also high. From this F_2 population, eleven plants were picked up at random and were crossed with MsBT-C line (♀). The pollen fertilities of F_1 hybrids of the eleven combinations were observed. Three of them showed a partially male-sterility, two showed completely male-sterility, and the remaining six segregated into partially male-sterile and completely male-sterile plants in a 1 : 1 ratio. The male parent of the cross combinations showing partially male-sterility was named as TB-X line; that of combinations showing complete male-sterility, TB-Z line; and that of combinations segregating into partially male-sterility and complete male-sterility, TB-Y line, as given in Table 4. When TB-X, TB-Y and TB-Z lines were selfed, respectively, all of the progenies showed complete male-fertility.

Considering the results up to this point, the difference in pollen fertility of the three lines, BT-A, BT-B and MsBT-C, similarly having the sterile cytoplasm, might be due to difference in the combination of a pair of pollen-fertility restoring genes in the nuclei. In TB-X, TB-Y and TB-Z which possessed the cytoplasm of Taichung 65, no pollen degeneration occurred that it was not related to the nuclear genotypes. These facts made it clear that the cytoplasm was an important factor in determining pollen fertility

Table 4. Pollen fertilities of F_1 plants of MsBT-C×TB-X, $[ms]\ rf\ rf \times [ms^+]\ Rf\ Rf$; MsBT-C×TB-Y, $[ms]\ rf\ rf \times [ms^+]\ Rf\ rf$; and MsBT-C×TB-Z, $[ms]\ rf\ rf \times [ms^+]\ rf\ rf$.

Line and cross		Pollen fertility (%) 0	10	20	30	40	50	60	Number of plants	χ^2-test 1 : 1
MsBT-C×TB-X	1					1	89	1	91	
	2					2	86	2	90	
	3					1	90	1	92	
MsBT-C×TB-Y	4	56				1	47	1	105	0.467
	5	44				2	41	3	90	0.045
	6	55				2	41	2	105	1.000
	7	49				1	30	2	82	3.122
	8	36				1	30	2	69	0.130
	9	54				2	42	3	101	0.485
MsBT-C×TB-Z	10	105							105	
	11	108							108	

Table 5. Genotypes of the six lines and their fertilities.

Line	Genotype*	Pollen fertility	Seed fertility
		%	%
BT-A	[*ms*] *Rf Rf*	99.8	94.1
BT-B	[*ms*] *Rf rf*	49.6	93.5
MsBT-C	[*ms*] *rf rf*	0.0	0.01
TB-X	[ms^+] *Rf Rf*	99.9	94.1
TB-Y	[ms^+] *Rf rf*	99.9	92.5
TB-Z	[ms^+] *rf rf*	99.8	91.6

*[*ms*] ....Sterile cytoplasm of Chinsurah Boro II.
[ms^+] ...Normal cytoplasm of Taichung 65.
Rf and *rf*....Pollen-fertility restoring gene in nucleus.

as well as nuclear gene. The cytoplasm of Chinsurah Boro II could be considered as sterile cytoplasm [*ms*] and that of Taichung 65 as normal cytoplasm [ms^+], and the gene was symboled by *Rf*.

The plasmatypes and the nuclear genotypes of these six lines were thus assumed to be as that shown in Table 5. BT-A, BT-B and MsBT-C lines were considered as [*ms*] *Rf Rf*, [*ms*] *Rf rf* and [*ms*] *rf rf*, respectively. On the other hand, the lines TB-X, TB-Y and TB-Z were considered as [ms^+] *Rf Rf*, [ms^+] *Rf rf* and [ms^+] *rf rf*, respectively.

These experimental results suggested that the genotype of BT-1 line as well as of its female parent Chinsurah Boro II was [*ms*] *Rf Rf*, and that of Taichung 65 was [ms^+] *rf rf*. Therefore, the pollen and seed fertilities of the two varieties as well as of BT-1 line were expected to be completely male-fertile; the pollen fertility was more than 98% and seed fertility was more than 90%. These results also showed that the above-mentioned hypothesis proved to be true.

Experiment 2.

To confirm whether the principles obtained from Experiment 1 also hold for other varieties, twenty-one *Japonica* varieties were crossed with BT-1 line (♀), respectively. Pollen fertilities in their F_1 hybrids were around 50% and seed fertilities were higher than 95%. The segregation ratio of completely male-fertile to partially male-sterile plants was a 1 : 1, as shown in Table 6. The segregation ratio seen in Experiment 2 was the same as in Experiment 1 where BT-B line was selfed (Table 2). Thus, the mode of inheritance seen in Experiment 1 was confirmed in Experiment 2.

Therefore, the plasmatypes and the nuclear genotypes of these twenty-one *Japonica* varieties may presumably be the same as those of Taichung 65, [ms^+] *rf rf*. For confirmation, the twenty-one varieties were analysed by the test of plasmatype and nuclear genotype (Shinjyo, unpublished). The results showed that all of them had the genotype [ms^+] *rf rf*. These also verify the pollen-fertility variations shown in Table 6.

Table 6. F_2 segregation for pollen fertility.

Cross combination*	Pollen fertility (%) 30	40	50	60	70	80	90	100	χ^2-test 1 : 1
BT-1×Aikoku 5		2	41	2			2	35	0.780
BT-1×Eikō			37				2	35	0.000
BT-1×Fuziminori		1	39	2			2	40	0.000
BT-1×Fuzisaka 5		4	30	5			3	42	0.428
BT-1×Hamayū		2	39	2			2	34	0.620
BT-1×Hayanōrin		1	29	2			2	39	0.048
BT-1×Hōnenwase		2	29	2			6	32	0.390
BT-1×Hōyoku		3	30	2			6	39	1.250
BT-1×Koshihikari		2	39	2			9	30	0.342
BT-1×Manriyo		2	40	1			1	35	0.620
BT-1×Miyoshi		5	40	5			4	40	0.383
BT-1×Nan-ei		2	39	4			5	35	0.294
BT-1×Naruho		1	40	1			4	35	0.111
BT-1×Nōrin 15		2	45	2			3	45	0.010
BT-1×Nōrin 17		3	40	4			5	30	1.956
BT-1×Nōrin 20		1	43	1			4	44	0.097
BT-1×Nōrin 22		3	29	3			5	40	1.250
BT-1×Nōrin 24		1	39	2			6	32	0.200
BT-1×Oirase		1	40	1			2	45	0.281
BT-1×Toyohikari		1	40	2			3	38	0.106
BT-1×Yutakawase		4	30	4			6	31	0.013
Total			870				858		0.359

*BT-1....[*ms*] *Rf Rf*.
Male parents....[*ms*+] *rf rf*.

Experiment 3.

Six *Indica* varieties were used in order to test whether or not the pollen-fertility restoring gene from Chinsurah Boro II and those from these varieties differed in action.

Using MsBT-C line which possessed the sterile cytoplosm, as the female parent, the six *Indica* varieties (♂) were crossed, respectively. All the F_1 hybrids were partially male-sterile. F_1 hybrids of these six combinations were crossed with *Japonica* varieties (♂); the B_1 F_1 hybrids segregated into partially male-sterile and completely male-sterile plants in a 1 : 1 ratio. Moreover, when the partially male-sterile hybrids, used as the female parent, were repeatedly back-crossed with the *Japonica* varieties, the generations succesively segregated into the 1 : 1 ratios. The segregation pattern as observed in the B_4 F_1 population was shown in Table 7. When the pollen-fertility restoring gene was introduced into the sterile cytoplasm, the heterozygotes showed partially male-sterile. This was similar to that observed in the F_1 hybrid of BT-B×Taichung 65 in Experiment 1. No difference in pollen-fertility restoration was seen between the genes originating from Chinsurah Boro II and the other six varieties.

Table 7. Segregation of B_4 F_1 plants, {([*ms*] *rf rf*×*Rf Rf*)×[*ms*+] *rf rf*}×...., of different cross-combinations into male-sterile and partially sterile classes.

Cross-combination*	Pollen fertility in B_4 F_1		χ^2-test 1 : 1
	Male-sterile	Partially sterile	
(MsBT-C×T-24)×Jp-1^4	45	44	0.011
(MsBT-C×T-26)×Jp-54^4	44	49	0.269
(MsBT-C×T-28)×Jp-40^4	43	42	0.017
(MsBT-C×Am-15)×Jp-1^4	49	54	0.243
(MsBT-C×G-143)×Jp-3^4	15	15	0.000
(MsBT-C×G-452)×Jp-3^4	19	21	0.100

* T-24...Salak
T-26...Lati Soil
T-28...Bandang Putih
Am-15...Patnai 23
G-143...Liu-tou-tu
G-452...Tadukan
Jp-1...Taichung 65
Jp-3...Taichung moch 46
Jp-40...Norin 24
Jp-54...Akibae

DISCUSSION

When MsBT-C line was pollinated by the five lines, BT-A, BT-B, TB-X, TB-Y and TB-Z, which were completely male-fertile or partially male-sterile, the F_1 hybrids showed monogenic segregation ratios. Selfings of these six lines also proved that pollen-fertility restoring gene should be present in the nucleus. Moreover, these results were showed in Experiment 2 and 3.

From the results so far obtained the genotype of BT-B line may be regarded as [*ms*] *Rf rf* but the F_1 hybrid of MsBT-C×BT-B was partially male-sterile as that of MsBT-C×BT-A (Table 2). The selfing of BT-B line segregated into completely male-fertile and partially male-sterile plants in a 1 : 1 ratio (Table 3). It was thus assumed that in BT-B line, [*ms*] *Rf rf,* the pollen having *rf* degenerated at a certain stage of development due to its interaction with sterile cytoplasm and did not participate in fertilization. For the same reason the pollen fertility of BT-B line may be partially male-sterile (50%). The normal function of the female gametes was proved by selfing and in the segregation into partially male-sterile and male-sterile classes in a 1 : 1 ratio in the F_1 hybrid of BT-B×Taichung 65. Therefore, the present male sterility could be considered as gametophytic. Such examples as hereby found were also observed by Buchert (1961) in the USDA type of sterile corn (Duvick 1965).

Jones *et al* (1937, 1944) and Maunder *et al* (1959) reported that the mode of inheritance of male sterility in onion and in sorghum, respectively, was results from interaction of nuclear gene and cytoplasm. Both [*ms*] *Rf rf* and [*ms*] *Rf Rf* plants showed complete male-fertility, while [*ms*] *rf rf* plant was male sterile. Accordingly, male-fertile and -sterile plants showed a 3 : 1 ratio in a F_2 generation. In these cases, the male sterility was sporophytic.

In many crops, male sterility has been reported; the mode of inheritance was classified by Sears (1947) into three groups, (A) Cytoplasmic-genetic, (B) Cytoplasmic, and (C) genetic. The present case belongs to the group A.

In Experiment 3, the *Rf* gene of variety Tadukan which was used by Kitamura (1962a, 1962b) was introduced to *Japonica* varieties. Partially male-sterile and completely male-sterile plants segregated in a 1 : 1 ratio in the present study. In Kitamura's reports, no abortion was seen in both male and female gametes but due to interruption of anther dehiscence the flowers became sterile. This completely differed from the present case.

The author found in the present work an interactional male-sterility between nuclear gene and cytoplasm. The recessive homozygote showed completely male sterility. It can well be utilized as the female parent for breeding hybrid rice. In this case, the three genotype plants, [ms^+] *Rf Rf*, [*ms*] *Rf Rf* and [*ms*] *Rf rf* may be used as pollen parent.

SUMMARY

A case of cytoplasmic male sterility controlled by a restoration gene *Rf* was found in cultivated rice, *Oryza sativa* L. The sterile cytoplasm [*ms*] and the restoration gene *Rf* were derived from Chinsurah Boro II, an *Indica* variety, and the experiments were made by the isogenic lines having genetic background of Taichung 65, a *Japonica* variety.

When a plant with sterile cytoplasm [*ms*] had *Rf Rf*, it was completely male-fertile; when it had *Rf rf*, partially male-fertile (ca. 50%); and when it had *rf rf*, completely male-sterile. Plants with normal cytoplasm [ms^+] would be male-fertile regardless of the genotype for restoration genes. The F_1 plants of [*ms*] *rf rf* × [ms^+] *Rf rf* segregated into partially male-sterile and completely male-sterile classes in a 1 : 1 ratio, while the selfed progeny of [ms^+] *Rf rf* was completely male-fertile. The restoration gene was thus found to be of gametophytic type.

The F_1 plants of [*ms*] *rf rf* × *Rf Rf* had a 90% or higher seed set, though they were partially male-sterile. This source may be used for breeding "hybrid rice".

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