Genetical Studies on Genic Male Sterile Mutant Obtained through Breeding of Isogenic Translocation Line of Taichung 65 in Rice, *Oryza sativa* L.

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Male sterile mutants lacking pollen grains in anthers segregated in the $B_{10}F_2$ population of Taichung 65 carrying interchange chromosome 1-3a. Genetical studies were carried out using the male sterile plants to examine the mode of inheritance. Taichung 65 and its isogenic lines having marker genes or interchange chromosome 1-3a were crossed with the mutant. The results showed that the male sterility is controlled by a single recessive gene, and is allelic to the genic male-sterile gene, *ms-9*. The gene was independent of the break-point 1-3a, while it was linked to two marker genes, *Ur* (Undulate rachis) and *Cl* (Clustered spikelet) in the sixth linkage group. The order of the three genes was estimated to be *Cl-ms-9-Ur*. The length of the culm and upper two internodes in the male sterile plants was significantly reduced, compared with that of Taichung 65. Although similar male sterile mutants occurred in an early backcross generation B_3F_2 , it is unlikely that the male sterility was maintained until $B_{10}F_2$. Therefore, it appears that Taichung 65 is prone to undergo gene mutations.

KEY WORDS: *Oryza sativa* L., *japonica*, Taichung 65, genic male sterility, mutation

Introduction

Male sterility is important for the development of hybrid varieties, and cytoplasmic male sterility has been used in several crops, whereas genic male sterility was not considered to be as useful as cytoplasmic male sterility because male sterile individuals can not be propagated easily, although many genic male-sterile lines were artificially produced. However, genic male sterility has been reassessed, since photoperiod-sensitive (LI *et al.* 1988) and thermo-sensitive male sterility (SUN *et al.* 1989, MARUYAMA *et al.* 1989) was discovered in rice.

We have been engaged in the breeding of isogenic reciprocal translocation lines of a *japonica* rice variety, Taichung 65 by successive backcrossings using well known 75 reciprocal translocation homozygotes produced from several varieties by NISHIMURA (1961), IWATA (1970), SATO *et al.* (1975), OKA and SANO (YOSHIMURA *et al.* 1980, and SATO 1981). One line RT1-3a·T65 out of them segregated male sterile plants lacking pollen grains in the B_3F_2 population. However, such plants were not selected for further backcrossings. Similar male sterile plants segregated again in the $B_{10}F_2$. To examine the mode of inheritance and the cause of segregation in the male sterile plants, the present studies were carried out.

Materials and Methods

A reciprocal translocation homozygote RT1-3a that originated from a line AP23 (IWATA 1970) was used as a donor parent of interchange chromosomes, and Taichung 65 (abbreviated as T65) was used as the recurrent parent for breeding of the isogenic reciprocal transloca-

tion homozygote of T65, RT1-3a·T65. Male sterile plants lacking pollen grains in anthers appeared in the B₃F₂ generation, but they were not maintained. Similar male sterile plants segregated again in the B₁₀F₂. They were crossed with T65. A male sterile line T65·ms carrying normal chromosomes was selected from B₁₁F₂ and used for the following genetic studies.

F₂'s of T65·ms/T65, T65·ms/RT1-3a·T65 and T65·ms/RT1-3a, and F₁'s from a backcross, T65·ms//T65·ms/T65 were grown to analyse the genetic behaviour of male sterility. Since T65 was used as the recurrent paternal parent for the breeding of T65·ms and RT1-3a·T65, the cytoplasm of T65·ms originated from RT1-3a. To examine the role of the cytoplasm in the expression of male sterility, 17 F₂ families from the cross, T65//T65·ms/T65 were cultivated. F₂'s of reciprocal crosses between T65 and RT1-3a, and the three-way-cross F₁'s of T65·ms//RT1-3a/T65 were also cultivated to examine the number of genes concerned and the role of the cytoplasm at the same time.

Since it was determined that the current male sterility was genic and controlled by a single male-sterile gene, an allelism test was carried out between the present line and male-sterile lines given by KO and YAMAGATA (1987), using three-way crosses, male-sterile testers//T65·ms/T65.

F₂'s of crosses between T65·ms and isogenic linkage testers of T65 (Table 1) were planted to examine the linkage relations between the present male-sterile gene and marker genes. Nomenclature of the linkage groups followed the report of the Committee on Gene Symbolization, Nomenclature and Linkage (KINOSHITA 1990).

Pleiotropic effects of male sterility on several agronomic traits were also examined based on the comparison between T65, and male sterile and normal segregants selected from B₁F₁'s of T65·ms//T65·ms/T65, using 42 plants in each.

Experimental plants were grown in the first and second cropping seasons of 1986 to 1989

Table 1. Linkage testers used

Linkage tester	Marker genes involved (name)	Number of backcrosses ¹⁾
T65· <i>Cl</i> , <i>lg</i>	<i>Cl</i> (Clustered spikelet) <i>lg</i> (liguleless)	8
T65· <i>Ur</i>	<i>Ur</i> (Undulate rachis)	8
T65· <i>lg</i> , <i>gl-1</i>	<i>gl-1</i> (glabrous leaf and hull), <i>lg</i>	13
T65· <i>A</i>	<i>A</i> (Anthocyanin activator)	11
T65· <i>Pl</i>	<i>Pl</i> (Purple leaf), <i>A</i>	9
T65· <i>d-1</i>	<i>d-1</i> (daikoku dwarf)	10
T65· <i>nl-1</i>	<i>nl-1</i> (neck leaf-1)	11
T65· <i>la</i>	<i>la</i> (lazy growth habit)	9
T65· <i>dl</i>	<i>dl</i> (drooping leaf)	9
T65· <i>gh-1</i> , <i>Hg</i>	<i>gh-1</i> (gold hull and internode-1) <i>Hg</i> (Hairy glume)	8

¹⁾ Backcrossed with Taichung 65 (T65).

in the experimental field of the College of Agriculture, University of the Ryukyus. Seedlings at about the 4.5-leaf stage were transplanted at a spacing of 15×20cm with a single plant per hill. Fertilizers (1.5kg/a each of N, P₂O₅ and K₂O) were applied at a ratio of 5:3:2 as basal, first and second top dressings, respectively.

Segregation of male sterility was examined based on the spikelet fertility. Characteristics of anthers and pollen fertility were also observed in crosses involving interchange homozygotes.

Recombination values between male sterility and marker genes were calculated by the maximum likelihood method.

Results

To examine the genetic behaviour of male sterility, T65·ms was crossed with T65 and its isogenic lines with markers. Each F₁ plant was fertile. Their F₂ plants segregated in a 3 normal:1 male sterile ratio, and the backcrossed plants, T65·ms//T65·ms/T65 segregated in a ratio, 1 normal:1 male sterile (Table 2), suggesting that a recessive gene controlled the segregation of male sterility. Since the F₂'s of T65·ms/RT1-3a·T65 segregated in a ratio, 3 normal:3 semisterile:2 male sterile, the gene was estimated to be independent of the breakpoint 1-3a. The F₂'s of T65·ms/RT1-3a segregated also in the ratio, 3 normal:3 semisterile:2 male sterile. To examine the relation of the genes concerned with the segregation of male sterility in two crosses between T65·ms and T65, and T65·ms and RT1-3a, the segregations of male sterility were observed in three-way-cross F₁'s, T65·ms//RT1-3a/T65, and F₂'s of reciprocal crosses between T65 and RT1-3a. No individuals with male sterility were observed in any of the crosses. It was, thus, assumed that the same gene controlled the segregation of male sterility, and that it arose by mutation. Incidentally, normal and semisterile individuals segregated nearly evenly in the respective crosses.

As mentioned above, T65·ms carries the cytoplasm derived from RT1-3a. To examine the

Table 2. Mode of inheritance of male sterility

Cross-combination	Segregation mode			Number of plants	χ^2 value		
	Normal	Semisterile	Sterile		3:1	1:1	3:3:2 ¹⁾
F ₂ :T65·ms/T65	755	0	249	1001	0.096		
F ₂ :T65·ms/Linkage testers	2589	0	887	3476	0.497		
B ₁ F ₁ :T65·ms//T65·ms/T65	172	0	170	342		0.012	
F ₂ :T65·ms/RT1-3a·T65	98	91	60	249			0.371
F ₂ :T65·ms/RT1-3a	146	159	89	394			1.794
Three-way-cross F ₁ :T65·ms//RT1-3a/T65	197	152	0	349		5.802*	
F ₂ :T65/RT1-3a	284	291	0	575		0.085	
F ₂ :RT1-3a/T65	185	196	0	380		0.318	

*: Significant at the 5% level.

¹⁾ Ratio when a male sterile gene is independent of the breakpoint 1-3a.

role of the cytoplasm in the expression of the present male sterility, 17 selfed families of B₁F₁ plants, T65//T65·ms/T65 were grown (Table 3). Normal and male sterile individuals segregated into a 3:1 ratio in ten families out of 17, and normal ones only appeared in the other seven. Consequently, it was assumed that the currently observed male sterility was not cytoplasmic but genic.

An allelism test was carried out between the present male sterile line and those given by KO and YAMAGATA (1987) using three-way-cross F₁'s, male sterile testers//T65·ms/T65 (Table 4). When male sterile lines in a three-way cross have male sterile alleles of the same locus, it is assumed that normal and male sterile individuals will segregate in a 1:1 ratio, whereas the normal ones will only appear if such genes occupy different loci. Normal and male sterile individuals appeared evenly in the cross including the male sterile line E1 carrying the male sterile gene *ms-9*, while no male sterile individuals were observed in the other ten. It was, thus, concluded that the present male sterile gene is an allele of the *ms-9* locus.

Chi-square values for independence (d.f.=1) between *ms-9* and 11 marker genes are shown in Table 5. The values between *ms-9* and two genes, *Cl* (Clustered spikelet) and *Ur* (Undulate rachis) belonging to the sixth linkage group were significant at the 0.1% level, while those between *ms-9* and other nine genes belonging to other groups were all nonsignificant.

Table 3. Segregation of male sterility in B₁F₂ families of the cross, T65//T65·ms/T65

F ₂ family	Segregation mode		Number of plants	χ^2 value 3:1
	Normal	Sterile		
1	81	27	108	0.000
2	81	26	107	0.028
3	80	25	105	0.079
4	84	24	108	0.444
5	79	27	106	0.013
6	82	24	106	0.314
7	82	26	108	0.049
8	82	24	106	0.314
9	83	25	108	0.197
10	79	29	108	0.098
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11	105	0	105	
12	105	0	105	
13	120	0	120	
14	103	0	103	
15	108	0	108	
16	105	0	105	
17	120	0	120	

The gene *ms-9* is, thus, estimated to be the gene in the sixth group. Recombination values between *ms-9* and the two genes, *Cl* and *Ur* were calculated to be 3.6 and 19.2%, respectively. Observed numbers fitted well to the expected ones obtained based on their recombination values (Table 6).

Table 4. Allelism test of male sterile genes in crosses, male sterile testers//T65.ms/T65

Male sterile tester	Male sterile gene	Segregation mode		Number of plants	χ^2 value 1:1
		Normal	Sterile		
K1	<i>ms-7</i>	13	0	13	0.077
K2	<i>ms-8</i>	13	0	13	
E1	<i>ms-9</i>	7	6	13	
T1	<i>ms-10</i>	13	0	13	
T2	<i>ms-11</i>	13	0	13	
T3	<i>ms-12</i>	5	0	5	
S32	<i>ms-13</i>	13	0	13	
S40	<i>ms-14</i>	13	0	13	
S55	<i>ms-15</i>	13	0	13	
S59	<i>ms-16</i>	13	0	13	
S81	<i>ms-17</i>	13	0	13	

Table 5. Chi-square test for independence between *ms-9* and 11 marker genes

Linkage group	Marker gene	χ^2 value (d.f. = 1)
1	<i>A</i>	0.000
3	<i>gh-1</i>	0.081
	<i>dl</i>	0.016
	<i>Hg</i>	0.288
4	<i>lg</i>	0.411
	<i>Pl</i>	1.995
5	<i>d-1</i>	0.500
	<i>nl-1</i>	0.000
6	<i>Cl</i>	325.099***
	<i>Ur</i>	116.777***
11	<i>la</i>	0.189

***: Significant at the 0.1% level.

Pleiotropic effects of this gene on several agronomic characters were examined in T65, and heterozygotes for the gene and male sterile individuals (Table 7). Heterozygotes showed similar traits to those of T65. However, in the male sterile individuals the length of the culm and upper two internodes was considerably reduced, whereas that of the leaf blade, leaf sheath, ear, and lower three internodes, as well as the number of ears and elongated internodes, was similar to the values recorded in of T65. The lower degree of panicle emergence (DPE; distance from lamina joint of flag leaf to neck node of panicle) in the male sterile individuals than that of T65 was ascribed to the shortening of their uppermost internode length (In 1), although the length of the first leaf sheath (LS 1) was similar to that of T65.

Discussion

The present male sterility was determined to be genic and controlled by an allele at the *ms-9* locus (Tables 2, 3 and 4). The gene appeared spontaneously, as RT1-3a and T65 do not have any male sterile genes (Table 2). Since T65.ms produced anthers which did not contain any pollen grains at the blooming stage, as in case of the male sterile line E1 carrying the gene *ms-9*, this gene is estimated to be very similar to *ms-9*.

Although similar male sterile plants to T65.ms were observed in an early backcrossing generation B₃F₂, it is difficult to consider that they induced the appearance of T65.ms, because subsequent 7 backcrossings were successively made, by selecting semisterile individuals or interchange heterozygotes, the *ms-9* gene was independent of the breakpoint 1-3a (Table 2), and the probability of the gene to be selected till the B₁₀F₂ generation was very low (about 0.4%). It was, thus, considered that a mutation had occurred in a late backcrossing generation, suggesting that RT1-3a.T65 or T65 is prone to undergo gene mutations. We observed an other mutant (a gold-hull and internode line showing a weak growth habit) out of around 24,000 plants in the cross experiments using isogenic lines of T65 carrying marker genes in the last two years (unpublished). It is likely that T65 harbours some factor(s) which accelerate gene mutation, as reported by TSAI (1990).

The length of the culm and upper two internodes in the male sterile plants was significantly reduced compared with that of T65, as shown in Table 7. Similar results were

Table 6. Linkage relations between *ms-9* and marker genes, *Cl* or *Ur*

Gene pair	Phase	Segregation mode				Number of plants	Recombination value (%)	χ^2 value (d.f. = 3)
		A_,B_	A_,bb	aa,B_	aa,bb			
<i>ms-9-Cl</i>	Coupl.	261	9	4	90	364	3.6 ± 1.0	2.429
		(266.6)	(6.4)	(6.4)	(84.6)			
<i>ms-9-Ur</i>	Coupl.	266	30	40	66	402	19.2 ± 2.2	1.436
		(266.6)	(34.9)	(34.9)	(65.6)			

A and B: Dominant. a and b: Recessive.

Number in parenthesis indicates the expected number based on the recombination values.

Table 7. Comparison of some agronomic traits between T65, and heterozygotes (H) and male sterile plants (MS)

Line	No. of ear	Ear length	Culm length	Internode length					No. of elongated internodes	Leaf blade length			Leaf sheath length			
				In 1	In 2	In 3	In 4	In 5		LB 1	LB 2	LB 3	LS 1	LS 2	LS 3	
T65	7.4	23.8 cm	114.3 cm	48.6 cm	28.5 cm	20.0 cm	12.0 cm	4.3 cm	5.14	14.1 cm	37.9 cm	55.2 cm	68.3 cm	34.0 cm	27.0 cm	30.2 cm
H	7.0	24.2	112.7	47.8	29.2	19.3	11.7	4.3	5.04	13.2*	39.4	55.7	68.4	33.7	27.4	30.5
MS	6.7*	24.2	99.6***	38.1***	26.0***	19.0*	11.2	4.5	5.12	3.1***	38.5	54.0	65.8*	34.8	27.5	29.5*

* and ***: Significant at the 5 and 0.1% levels, respectively.

¹⁾ Degree of panicle emergence (distance from lamina joint of flag leaf to node of panicle).

reported by SHINJO (1975) in an experiment using an isogenic cytoplasmic male-sterile line of T65 with a male sterile cytoplasm derived from an *indica* variety Chinsurah Bollo II. However, the degree of the reduction on the length was considerably different between them; that of the former was larger than of the latter. The difference in the reduction degree between these two male-sterile lines was greater for the upper internode. This fact may be due to the difference in the degeneration stage of pollen grains and the difference in the pollen grain condition; pollen grains of the latter degenerated at the one-nucleus stage and filled with cytoplasm, while the degeneration in the former was considered to start before the meiosis, since a few pollen mother cells were observed and no pollen grains were seen at the blooming stage. In the heterozygotes for the restoring gene *Rf-1*, the length of the culm and upper two internodes was also reduced (SHINJO 1975), while the heterozygotes for the present gene showed the same characteristics as those of T65 (Table 7). The difference in pleiotropic effects between them is attributed to the difference in pollen fertility; the pollen fertility of the former was about 50% due to gametophytic cytoplasmic male sterility, whereas the latter was fertile. These findings suggest that the degree of pollen sterility and the conditions of sterile pollen grains are related to the degree of the reduction of the internode length developed during spermatogenesis. This assumption is supported by the fact that semisterile plants induced by interchange heterozygosity showed a similar reduction of the length of the upper two internodes, although the culm length was similar to that of normal individuals (SATO 1986). Incidentally, it was reported that in other genic male sterile plants used for male sterile testers in this study the culm length was reduced (Ko and YAMAGATA 1987).

It was estimated that four genes, *C* (Chromogen for anthocyanin color), *fs-1* (fine stripes in leaf margin), *Cl* and *Ur* belonging to the sixth linkage group were linked in the order (SATO 1976). Although a linkage

Fig. 1. Linkage map of the sixth linkage group involving *ms-9*. 1) and 2): Quoted from SATO (1976), and NAGAO and TAKAHASHI (1963), respectively.

study has not been carried out between the two genes, *Cl* and *Ur* yet, it is assumed that a similar recombination value to that of *fs-1-Ur* (27.5% NAGAO and TAKAHASHI 1963) will be obtained between *Cl* and *Ur*, for *fs-1* and *Cl* were closely linked, with a value of 0.8% (SATO 1976).

Since the linkage intensity between *ms-9* and two marker genes, *Cl* and *Ur* was obtained as 3.6 and 19.2% respectively, it is estimated the *ms-9* gene is located between the genes, *Cl* and *Ur*, as shown in Fig. 1.

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イネ品種台中 65 号の同質相互転座系統の育成過程に出現した雄性不稔性の遺伝

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転座染色体 1-3a を持つ台中 65 号の同質相互転座系統を育成する過程 ($B_{10}F_2$) において花粉のない葯を持つ雄性不稔個体が分離した。台中 65 号, 標識遺伝子を持つその同質遺伝子系統, 同質相互転座系統 RT1-3a・T65 ならびに元の相互転座系統 RT1-3a を用いた交雑実験の結果から, 本雄性不稔性は遺伝子雄性不稔性であり, 単純劣性の核内遺伝子の関与していることが明らかとなった。おそらく同遺伝子は自然突然変異により生じたものと考えてよい。また, 既報の雄性不稔遺伝子の 1 つである *ms-9* とは同座であることが同定された。*ms-9* は転座点 1-3a とは独立であり, 第 6 連鎖群の遺伝子である *Ur* (枝梗湾曲) および *Cl* (小穂叢生) とはそれぞれ 19.2% および 3.6% の組換え価で以って連鎖していた。また, それら 3 遺伝子を含む位置関係は *Cl* — *ms-9* — *Ur* であると推定された。雄性不稔植物の稈長と上位 2 節間長は台中 65 号のそれに比べ短く, 穂首の抽出度も小さかった。

戻し交雑初期世代 (B_3F_2) において本雄性不稔植物と類似した無花粉の葯を持つ雄性不稔植物を分離したが, その雄性不稔遺伝子が $B_{10}F_2$ まで保存されていたとは考え難いので, 新たに *ms-9* で突然変異を生じたものと推測される。このことから台中 65 号は突然変異を生じ易い品種であると考えられた。