ANALYSIS OF GENES CONTROLLING F_1 STERILITY IN RICE BY THE USE OF ISOGENIC LINES[1]

HIKO-ICHI OKA

National Institute of Genetics, Misima, 411 Japan

Manuscript received January 25, 1974

ABSTRACT

In order to look into the genetic basis of intervarietal F_1 sterility in rice (*Oryza sativa* L.), a series of backcrosses (up to B_{13}) was carried out using Taichung 65 (Japonica type) as the recurrent parent and several Indica varieties as donor parents. A number of "isogenic F_1-sterile lines" were isolated by test-crossing fertile F_2 plants obtained from the selfing of partly pollen-sterile backcross segregants. Crossing experiments with the isogenic lines confirmed the author's previous hypothesis that there are sets of duplicate gametic lethals (*s* genes) and that gametes carrying a double recessive combination ($s_1\ s_2$) of these deteriorate during development, though in the present hypothesis the genes are considered to affect the development of microspores only. Assuming that Taichung 65 has the genotype $s_1/s_1 + {}_2/+_2$ and a donor parent (like an isogenic F_1-sterile line derived from it) has $+_1/+_1\ s_2/s_2$, pollen grains with $+_1\ s_2$ have shown a higher fertilizing capacity in the genetic background of Taichung 65 than those with $s_1 +_2$, while those with $+_1 +_2$ have a lower fertilizing capacity. This certational advantage of alien genes was considered to be an internal mechanism that helped the development of F_1 sterility relationships among rice varieties. The isogenic F_1-sterile lines derived from different donor parents each had a set of *s* genes at different loci. Linkage relations were detected between the *s* loci and three gene markers.

HYBRID sterility is commonly found among distantly related taxa of plants and animals. The present work is an attempt to extract from a genotype a particular gene or genes responsible for the sterility and to transfer it to an isogenic genetic background. Rice cultivars (*Oryza sativa* L.) are differentiated into the so-called Indica and Japonica types, and the F_1 hybrids between distantly related varieties (not necessarily between the two types) exhibit pollen and embryo sac sterilities which vary in the percentage of normal gametes from about 5 to 95 percent depending upon the parental combination. However, no significant disturbances in chromosome pairing are observed in the meiosis of F_1 plants, and abortion of a part of micro- and megaspores starts at the stage of the first haploid mitosis (OKA 1957a; 1964). Usually, reciprocal crosses show no significant differences.

This F_1 sterility, once called "sexual affinity" on the assumption that it measures the degree of genetic difference between parents (TERAO and MIZUSHIMA 1939), attracted the interest of many rice geneticists. A number of papers on this problem have since been published as reviewed by the present author

[1] Contribution from National Institute of Genetics, Japan, No. 957.

(OKA 1964). Some workers were inclined to consider this sterility to be due to cryptic structural differences in chromosomes. On the other hand, the present author (1953; 1957a) has put forward a hypothesis that this sterility is controlled by sets of duplicate gametic lethals (called "gametic-development genes") a model for which is given as follows: Strains A and B have genotypes $s_1/s_1 +_2/+_2$ and $+_1/+_1 s_2/s_2$, respectively, where the s_1 and s_2 loci are independent. Their F_1 hybrid is 25 percent sterile since the presence of at least one + gene in the gamete is necessary for its normal development, hence the gametes with $s_1 s_2$ deteriorate.

This hypothesis was based on data obtained from experiments of (A × C) × B design where the F_1 hybrids of A × C and B × C were fertile and those of A × B were partly fertile; several such crosses were tested in which strains A and C were generally closely related and B was distantly related to A and C. Then, the (A × C) × B progeny segregated into 1 fertile : 1 partly fertile class. In one of such experiments, strains B and C had the glutinous (*wx*) gene while A was non-glutinous (*Wx*). The progeny segregated into four classes: (a) partly fertile *Wx/wx*, (b) partly fertile *wx/wx*, (c) fertile *Wx/wx*, and (d) fertile *wx/wx*; the number of plants in classes (a) and (d) was greater than the number in (b) and (c). Furthermore, in class (a), there were more glutinous pollen grains than non-glutinous ones. The F_2 plants from A × C were testcrossed with B, and it was concluded that in this case, strains A, B and C had genotypes s_1–*Wx* $+_2$, $+_1$–*wx* s_2, and $+_1$–*wx* $+_2$, respectively (– shows linkage). The recombination fraction between s_1 and *Wx* loci was estimated to be about 21 percent by different methods (OKA 1953; 1957a).

Notwithstanding this work, the genetic basis of the F_1 sterility has been an unsettled issue and was much discussed at the Symposium on Rice Genetics and Cytogenetics, 1963 (held by the International Rice Research Institute, cf. CHANDLER 1964). In order to look more closely into the nature of this sterility, the present author has initiated backcrossing experiments to isolate sterility factors in isogenic lines. As reported in this paper, the data obtained from the experiments have generally supported the hypothesis of duplicate gametic lethals.

MATERIALS AND METHODS

A pure line of a rice cultivar from Taiwan, Taichung 65 (Japonica type; abridged as T65) was used exclusively as the recurrent parent in backcrossing. Five Indica cultivars, 108 (Peh-ku from Taiwan), 144 (O-luen-chung from Taiwan), 435 (Pachchai-perumal from India), 706 (He-nan-tsao from China), and 727 (Chintsao from China), were used as donor parents. In order not to involve cytoplasmic differences, T65 was used as the female parent in the initial crosses. In each backcross generation, a few plants showing 40–75 percent pollen fertility were selected in each line by staining the pollen grains with iodine solution, and they were used as either the female or the male parent for the next backcrossing. Up to the B_5 generation, the selected partly pollen-fertile (called "semi-sterile" in this paper) segregants were used as the female parent (called "female backcrossing"), and after B_6 they were often used as the male parent ("male backcrossing").

This recurrent backcrossing was continued up to B_{13}. During this process, several semi-sterile B_7 plants were selfed in each line, and from their F_2 populations a number of fertile segregants were selected. They and their progeny lines (B_7F_3, all being fertile) were testcrossed by T65, and when all the F_1 plants from testcrosses showed partial pollen sterility, the

parental seed was selected as representing an "isogenic F_1-sterile line" of T65. More purified isogenics were obtained from $B_{13}F_2$ plants by the same method. So far, a total of 45 isogenic lines carrying sterility genes from the five different donor parents have been obtained. For the sake of brevity, however, data from the lines derived from donor parents 144 and 435, which are more complete than those for other lines, are primarily used in this paper; all other lines showed essentially the same behavior. Those lines are classified into Families A (derived from 435) and B (from 144).

Genic Models Tested

1) *Duplicate gametophytic lethal model*

As mentioned in the introduction, this model was previously adopted (OKA 1953; 1957a). It is assumed that there are two independent loci each carrying either *s* or + alleles. Letting T65 have $s_1/s_1\ +_2/+_2$, a donor parent as well as an isogenic F_1-sterile line derived from it is expected to have $+_1/+_1\ s_2/s_2$. The presence of either $+_1$ or $+_2$ or both in the gamete is necessary for its development, and gametes with $s_1\ s_2$ deteriorate at a certain stage of development. This results in 25 percent sterility of the F_1 hybrid, $s_1/+_1\ s_2/+_2$.

When the *s* genes affect the development of microspores only, the F_1 embryo sac genotypes will be $s_1\ +_2$, $+_1\ +_2$, $+_1\ s_2$, and $s_1\ s_2$. Backcrossing of an F_1 plant with the pollen of T65 ($s_1\ +_2$) produces four different genotypes: $s_1/s_2\ +_2/+_2$ (fertile), $s_1/+_1\ +_2/+_2$ (fertile), $s_1/+_1\ s_2/+_2$ (25% sterile), and $s_1/s_1\ s_2/+_2$ (50% sterile) in a 1:1:1:1 ratio. Of the two partly sterile genotypes, if the former (25% sterile) is used as the female parent for backcrossing with T65 (female backcrossing), the progeny segregates again into the same four genotypes. When the same genotype is used as the pollen parent for backcrossing with T65 (male backcrossing), its functional pollen grains are of three genotypes, $s_1\ +_2$, $+_1\ +_2$, and $+_1 s_2$, and may be subjected to certation. Then, three different zygotic genotypes, $s_1/s_1\ +_2/+_2$ (fertile), $s_1/+_1\ +_1/+_2$ (fertile), and $s_1/+_2\ s_2/+_2$ (25% sterile), will be produced in the progeny in a ratio modified by certation.

On the other hand, when the second partly sterile genotype ($s_1/s_1\ s_2/+_2$, 50% sterile) is used for female backcrossing, two different zygotic genotypes, $s_1/s_1\ +_2/+_2$ (fertile) and $s_1/s_1\ s_2/+_2$ (50% sterile), are produced in a 1:1 ratio. However, if this same genotype is used for male backcrossing, its functional pollen grains are $s_1\ +_2$ (same as of T65) only, and no semi-sterile segregants will be obtained. Thus, it is expected that there are two kinds of semi-sterile plants and in one of them, pollen sterility is transmitted only through the female parent.

2) *One locus sporo-gametophytic interaction model*

This model concerns the alleles *S* and *Sa*, and if *S* is present in the maternal tissue, gametes with *Sa* deteriorate at a certain stage of development. When strains *S/S* and *Sa/Sa* are crossed, the F_1 hybrid (*S/Sa*) is 50 percent sterile and produces gametes with *S* only, as gametes with *Sa* deteriorate. Then, backcrossing of the F_1 with *Sa/Sa* in both female and male directions produces semi-sterile plants (*S/Sa*) only, but selfing of a *S/Sa* hybrid produces fertile plants (*S/S*) only. A case in which this hypothesis appeared to hold was observed in a backcrossing experiment between an Indica strain (PTB 10 from India) and T65 (OKA 1964, p. 168).

When *S* is present in the maternal tissue, if microspores with *Sa* deteriorate but all megaspores develop normally, it is expected that female backcrossing of *S/Sa* and *Sa/Sa* will produce *S/Sa* (50% sterile) and *Sa/Sa* (fertile) plants in a 1:1 ratio. Selfing of a *S/Sa* hybrid also results in segregation of *S/S* (fertile) and *S/Sa* (50% sterile) plants in a 1:1 ratio, and the former segregant may be selected as an isogenic F_1-sterile plant since its testcross by *Sa/Sa* will produce semi-sterile hybrids. Male backcrossing of *S/Sa* with *Sa/Sa* is expected to produce *S/Sa* plants (50% sterile) only, so far as the penetrance of the genes is complete.

3) *One locus sporophytic sterility model*

This model concerns the alleles S^f, S^1, and S^2, and in heterozygotes S^1S^2 a part of the gametes deteriorate irrespective of their genotypes on account of an adverse sporophytic effect of allelic interaction in the maternal tissue, although all homozygotes as well as heterozygotes S^fS^1 and

S^1S^2 are fertile. Backcrossing of S^1S^2 with S^1S^1 in both female and male directions produces S^1S^1 (fertile) and S^1S^2 (partly fertile) plants in a 1:1 ratio. The same ratio is also expected from selfing of a S^1S^2 plant, and S^2S^2 segregants (fertile) may be selected as representing an isogenic F_1-sterile line since their testcross by S^1S^1 will produce semi-sterile hybrids. The genes may affect the development of both micro- and megaspores or microspores only, but this difference does not influence the above expectations. This model is similar to the one adopted for the "corky" hybrids in cotton (STEPHENS 1946, 1950.)

4) *Complementary sporophytic sterility genes model*

This model concerns sporophytic sterility genes at two different loci, $S_1/+_1$ and $S_2/+_2$, and when both S_1 and S_2 are present in the maternal tissue, their complementary effect results in

TABLE 1

Examples of pollen-fertility distributions

Generation	Cross* direction	Parent, pollen fertility (%) (No. of lines)	No. of plants falling in percent fertility class 25	35	45	55	65	75	85	95	No. of plants† Ferti.	Semi-st.	Mean ferti. of semi-st. plants (%)
a. Family A—Donor parent 435:													
		%											%
B_1	Female	55 (F_1)	3	6	4	4	3	.	2	2	4	20	44
B_5	Female	74 (1)	.	.	.	10	4	4	.	14	14	18	62
B_7	Female	64,70 (2)	.	.	.	2	4	5	2	19	19	13	70
B_8	Female	72,81 (2)	.	1	2	7	4	4	.	15	15	18	59
B_7	Male	64–79 (7)	.	.	3	18	28	12	2	20	22	61	63
B_9–B_{12}	Male	55–79 (8)	.	.	2	16	16	8	6	15	19	44	63
B_7F_2	Self	59–81 (5)	.	.	8	12	18	16	7	75	77	59	65
b. Lines in which semi-sterility was transmitted only through the female parent (Family A):													
B_5	Female	41–68 (3)	.	2	7	11	.	.	1	25	26	20	50
B_8	Female	50–63 (3)	.	2	9	3	.	.	.	18	18	14	46
B_7F_2	Self	48–51 (3)	.	.	13	12	4	.	.	49	49	29	52
c. Family B—Donor parent 144:													
B_1	Female	57 (F_1)	2	3	2	1	3	4	4	4	7	16	55
B_7	Female	53–71 (3)	.	1	.	3	7	9	3	23	23	23	69
B_8	Female	72–77 (4)	3	7	17	14	2	.	1	37	18	43	46
B_7	Male	53–72 (8)	.	.	4	5	7	27	24	6	8	65	74
B_9–B_{12}	Male	35–76 (8)	.	14	13	11	1	2	1	5	6	41	46
B_7F_2	Self	48–80 (5)	.	3	4	18	18	13	1	75	75	57	61
d. Comparison of lines from parents with different pollen fertilities:													
Family A													
B_8	Male	56,56 (2)	.	.	.	4	4	5	1	5	6	13	66
B_8	Male	72,72 (2)	.	.	.	7	3	5	1	4	4	16	65
Family B													
B_8	Male	46,48 (2)	.	.	4	11	5	1	.	.	0	21	56
B_8	Male	68 (1)	.	.	2	3	4	.	.	.	0	9	57
B_8	Male	80 (1)	.	2	8	16	9	1	.	1	1	36	55
e. Crosses between isogenic lines:													
F_1	T65 × A1	Fertile	.	.	3	5	5	3	.	.	. .	16	60
F_1	T65 × B2	Fertile	.	1	6	7	1	.	.	.	. .	15	50
F_1	A1 × B2	Fertile	4	6	.	.	.	.	.	.	. .	10	31

* Female: Semi-sterile plant used as the female parent, Male: as the male parent.
† Plants with a pollen fertility higher than 87.5% were considered fertile.

partial abortion of the gametes. A major difference between this and the above model is that according to the model being considered here, S_1/S_1 S_2/S_2 plants which breed true for partial sterility should be obtained by the selfing of partly sterile segregants. This model is similar to the one adopted for F_1 weakness in *Oryza sativa* (OKA 1957b) and *O. glaberrima* (CHU and OKA 1972), and also for F_1 necrosis in wheat (TSUNEWAKI 1960).

EXPERIMENTAL RESULTS

1. *Segregation pattern for pollen fertility*

After the B_4 generation, partly pollen-fertile (called "semi-sterile") and fertile plants could be clearly distinguished, since abortive pollen grains were of the same type in each family (incompletely filled with starch, or small and almost empty, or very small and triangular in shape). The semi-sterile plants showed, however, a range of pollen fertilities from about 80 to 35 percent even within a line. The F_1 plants between T65 and an isogenic F_1-sterile line which must be genetically homogeneous also showed such a range (Table 1e). The mean pollen fertility for semi-sterile plants of a line differed from generation to generation and tended to be lower after the B_7 generation (Table 1a,c). It did not reflect the pollen fertility of the parent used for backcrossing (Table 1d).

Throughout the backcross generations after B_5, when a semi-sterile segregant (B_nF_1) was used as the female parent of backcrossing (female backcrossing), the progeny showed a 1:1 semi-sterile/fertile ratio (Table 2). In contrast, when a semi-sterile segregant was used as the pollen parent (male backcrossing), there were several times as many semi-sterile plants in the progeny as fertile ones (Table 2). This suggests that pollen grains with different genotypes are subject to certation. However, in certain lines (e.g., 7 of 11 B_7 lines in Family A and 2 of 9 B_7 lines in Family B, which had been maintained by female backcrossing until B_6), male backcrossing produced no semi-sterile segregants; female backcrossing produced fertile and semi-sterile plants in a 1:1 ratio, and the semi-sterile plants generally had lower pollen fertilities (about 50%) than those of other lines (examples in Table 1b). This indicates that the semi-sterile plants in those lines have a different genotype from that of the other semi-sterile plants whose pollen sterility was transmitted through the pollen parent. The occurrence of two such

TABLE 2

Numbers of fertile and semi-sterile segregants recorded in different generations

Family	Generation*	Cross†	No. of lines	No. of plants			Ratio observed
				Ferti.	Semi-st.	Total	
A	B_4–B_8	Female	14	161	145	306	1.11 : 1
	B_6–B_{13}	Male	28	71	189	260	1 : 2.66
	B5–$B_{13}F_2$	Self	34	428	364	792	1.18 : 1
B	B_4–B_8	Female	15	151	156	307	1 : 1.03
	B_6–B_{13}	Male	28	17	226	243	1 : 13.29
	B_5–$B_{13}F_2$	Self	36	493	471	964	1.05 : 1

* Data for different lines were pooled as they were homogeneous.
† Female or male backcrossing: A semi-sterile segregant was used as the female or male parent.

genotypes among semi-sterile segregants supports the hypothesis of duplicate gametophytic lethals (1st model).

When the semi-sterile segregants (B_nF_1) were selfed, the F_2 ratios were always close to 1:1 semi-sterile/fertile (Table 2). The F_3 and F_4 lines from semi-sterile parental plants (61 lines in total) invariably showed the same pattern of segregation, and no true-breeding semi-sterile lines could be established. On the other hand, all progeny lines from fertile backcross segregants (53 lines in total) were completely fertile. This rules out the hypothesis of complementary sporophytic sterility genes (4th model).

Thus, a total of 119 fertile B_7F_2 plants were testcrossed by T65 (used as the female parent). A majority of them showed F_1 sterility with T65, but some produced fertile F_1 hybrids, and others segregated into fertile and semi-sterile plants (Table 4). The occurrence of fertile and semi-sterile segregants in the progeny of a single testcross implies the heterozygosity of the testcrossed fertile plants and rejects the one locus hypotheses (2nd and 3rd models). It then follows that the duplicate gametophytic lethal hypothesis (1st model) remains for further test.

The duplicate gametophytic lethal model does not essentially differ from the author's previous one (OKA 1953, 1957a), though in the previous model the *s* genes were considered to affect the development of both micro- and megaspores. Whether or not sterility genes affect embryo sac development can be judged from seed fertilities. In early backcross generations, seed fertility appeared to be associated with pollen fertility. As the generations proceeded, however, seed-sterile plants gradually decreased, and all B_5 plants were completely seed-fertile. Also, a quite different segregation pattern is expected when the *s* genes affect embryo sac development; the observed pattern has agreed with that expected when the genes affect microspore development only. Possibly, sterility genes conditioning the development of both micro- and megaspores had been involved in the initial crosses, but they might have been lost since pollen-sterile plants had been selected in each backcross generation.

The selfed progenies (B_7F_3) of fertile F_2 plants showing F_1 sterility with T65 were testcrossed again for reconfirmation of their F_1 sterility, and isogenic F_1-sterile lines were isolated from the seed of the fertile F_2 plants. They were completely self-fertile, but their F_1 hybrids with T65 showed partial pollen sterility. The isogenic lines did not differ from T65 in heading time, plant height, and in other metric characters.

2. *Estimation of the intensity of certation*

According to the duplicate gametophytic lethal model, the functional pollen grains of a semi-sterile plant with $s_1/+_1\ s_2/+_2$ are of three genotypes, $s_1\ +_2$, $+_1\ s_2$, and $+_1\ +_2$, and their backcrossing with T65 produces three zygotic genotypes, $s_1/s_1\ +_2/+_2$ (fertile), $s_1/+_1\ s_2/+_2$ (25% sterile), and $s_1/+_1\ +_2/+_2$ (fertile); T65 gametes are assumed to have $s_1\ +_2$. Letting the relative fertilizing capacities of the three pollen genotypes be $1:k_1:k_2$, the ratio of fertile:semi-sterile segregants in a male backcross progeny is expected to be $(1 + k_2):k_1$. The observed ratios, about 1:2.7 (71:189) in Family A and 1:13.3 (17:226) in Family B (Table 2), indicate that $k_1 > 1$.

TABLE 3

F_2 genotypes from T65 × an isogenic F_1-sterile line

No.	Genotype	Pollen fertility expected	Relative frequency*	Ferti./Semi-st. ratio from test-cross with: T65	Ferti./Semi-st. ratio from test-cross with: F_1-steri. line	Remarks
1	s_1/s_1 $+_2/+_2$	100%	1	1 : 0	0 : 1	= T65
2	$+_1/+_1$ s_2/s_2	100%	k_1	0 : 1	1 : 0	= F_1-steri. line
3	$s_1/+_1$ $+_2/+_2$	100%	$1 + k_2$	1 : 0	k_2 : 1	
4	$+_1/+_1$ $s_2/+_2$	100%	$k_1 + k_2$	(p : q)	1 : 0	
5	$+_1/+_1$ $+_2/+_2$	100%	k_2	1 : 0	1 : 0	
6	$s_1/+_1$ $s_2/+_2$	75%	$1 + k_1 + k_2$			= F_1
7	s_1/s_1 $s_2/+_2$	50%	1			
8	$s_1/+_1$ s_2/s_2	50%	k_1			

* Relative fertilizing capacity of pollen grains with $s_1 +_2$, $+_1 s_2$, and $+_1 +_2 = 1 : k_1 : k_2$.

On the other hand, an F_2 population between T65 ($s_1/s_1 +_2/+_2$) and an isogenic F_1-sterile line ($+_1/+_1 s_2/s_2$), or that from selfing of a semi-sterile B_nF_1 plant ($s_1+_1 s_2/+_2$), is expected to segregate into 8 different genotypes as given in Table 3; five of them (1–5) produce fertile plants, and the remaining three (6–8) produce semi-sterile plants. The ratio of fertile:semi-sterile plants will be $(2 + 2k_1 + 3k_2):(2 + 2k_1 + k_2)$. The nearly 1:1 ratios observed in F_2 populations (Table 2) indicate that $k_2 < 1$.

The values of k_1 and k_2 were estimated by solving two simultaneous equations: $(1 + k_2)/k_1 =$ male-backcross ratio, and $(2 + 2k_1 + 3k_2)/(2 + 2k_1 + k_2) = F_2$ ratio. However, the estimates thus obtained fluctuated much with a small change in the F_2 ratio. The following k_1 and k_2 values were obtained from the data for B_7 to B_{12} lines which were considered to be most reliable (given below; a part of those given in Table 2), and were used for analysis of other series of data.

Family	Male-backcross ratio	F_2 ratio	k_1	k_2
A	68 : 181 (25 lines)	346 : 309 (30 lines)	4.54	0.71
B	16 : 193 (23 lines)	292 : 291 (22 lines)	12.63	0.05

In order to assess the genotypes of fertile F_2 plants and their frequencies, the data from testcrosses with T65 were reexamined. Further, a number of fertile F_2 plants from T65 × A1 (an isogenic F_1-sterile line of Family A) and T65 × B2 (Family B) were testcrossed by both T65 and the parental isogenic line (both used as the female parent). As shown in Table 3, the five F_2 genotypes for fertile plants (1–5) can be distinguished by the results of these testcrosses. However, the F_1 plants obtained from a testcross were only 3 to 10. Therefore, the probability that a testcrossed F_2 plant had a given genotype was calculated from the data by using Bayes' theorem (cf. DAVID 1951, p. 94). For instance, when an F_2 plant testcrossed by T65 produced no fertile and 3 semi-sterile plants, the F_2 plant must have either genotype (2) or (4), the relative frequencies of which are k_1 and $(k_1 + k_2)$, respectively (Table 3). If it had genotype (2), the testcross could produce no fertile plant. If it had genotype (4), the testcross progeny would segregate into fertile and semi-sterile plants in a ratio (p:q) which can be known

TABLE 4

Frequencies of different genotypes among fertile F_2 plants estimated from the results of testcrosses

(A) Testcrosses by T65

Observed *vs.* expected number	No. of F_2 plants with genotype* (1), (3) & (5) Test-cross F_1: Fertile	(4) Segregating	(2) Semi-sterile	Total no. of plants
Family A				
Visually observed	8	11	13	32
Estimated by Bayes' theorem	7.1	14.1	10.8	
Expected (k_1=4.54, k_2=0.71)	8.3	12.7	11.0	
Family B				
Visually observed	3	7	34	44
Estimated by Bayes' theorem	3.0	20.5	20.5	
Expected (k_1=12.63, k_2=0.05)	3.4	20.3	20.3	

(B) Testcrosses by T65 and parental isogenic line

Observed *vs.* expected number	No. of F_2 plants with genotype* (1)	(3)	(5)	(4)	(2)	Total no. of plants
Family A						
Visually observed	1	1	0	4	8	14
Estimated by Bayes' theorem	0.6	1.4	0.0	5.8	6.2	
Expected (k_1=4.54, k_2=0.71)	1.1	1.8	0.8	5.6	4.8	
Family B						
Visually observed	1	0	0	0	10	11
Estimated by Bayes' theorem	0.5	0.5	0.0	4.3	5.7	
Expected (k_1=12.63, k_2=0.05)	0.4	0.4	0.0	5.1	5.1	

* Given in Table 3.

from the data for segregating testcross progenies of the same family. Then, the probability that the given plant has genotype (2) will be $k_1/[k_1 + (k_1 + k_2)q^3] = P$, and that for genotype (4) will be $1 - P$. After this calculation was completed for all test-cross progenies, the p:q ratio was reestimated including the probabilities of non-segregating test-cross progenies to have genotype (4), and the same calculation was iterated. Thus, the frequencies of respective genotypes were estimated in terms of probabilities (Table 4).

As shown in Table 4, the estimated frequencies of five genotypes for fertile F_2 plants agreed well with those given by substituting the k_1 and k_2 values given above into the expectation formulas in Table 3, though the number of testcrossed plants was not large enough for precise comparisons. In both Families A and B, as predicted from the large k_1 values, genotype (2) (same as the F_1-sterile line) was much larger in number than genotype (1) (same as T65). As also predicted from the small k_2 values, plants with genotype (5) (producing fertile F_1 hybrids with both T65 and parental F_1-sterile line) were not found; a few plants with this genotype were obtained later from the selfed progenies of plants with genotypes (3) and (4). Even though the k_1 and k_2 estimates are not fiducial, it may be concluded that in the genetic background of T65, + s_2 pollen generally has a

certational advantage over $s_1 +_2$ pollen ($k_1 > 1$), while $+_1 +_2$ pollen has a lower fertilizing capacity ($k_2 < 1$).

As mentioned above, all experimental results could be explained by the hypothesis of duplicate gametic lethals except for the pollen fertilities of semi-sterile plants with $s_1/+_1\ s_2/+_2$ which fluctuated toward lower values. According to the genic model, the pollen fertility of this genotype must be 75 percent. Actually it ranged from about 75 to 35 percent in male backcross progenies after B_7 (Table 1c). Plants with $s_1/s_1\ s_2/+_2$ whose pollen fertility is expected to be 50 percent will not occur in male backcross progenies.

It is known that in the case of sporophytic sterility (duplicate recessive sterility genes causing F_2 sterility), the pollen fertility of plants with the same genotype varies over a wide range (OKA and DOIDA 1962). To account for the fluctuation of pollen fertility, some sporo-gametophytic interaction may be assumed. For instance, it may be assumed that the s_2 gene in the maternal tissue exerts an adverse effect on the development of microspores with $+_2$ resulting in reduction of normal pollen grains with $s_1 +_2$ and $+_1 +_2$. Within the scope of the present experiment, however, the reason for the fluctuation of pollen fertilities in certain genotypes remains unelucidated.

3. *Comparison of different sets of* s *genes*

Seven isogenic F_1-sterile lines (isolated from B_7F_2 plants) were selected for further experiments. Two of them (A1 and A2) had *s* genes derived from donor parent 435 (Family A), three (B1, B2 and B3) from 144 (Family B), and the remaining two (C1 and C2) from 727 (Family C); those belonging to the same family were descendants from different B_3 plants.

In order to compare the *s* loci of these lines, diallel crosses (8×8) were made among them including T65. It was learned from the results that all crosses within the same family produced fertile F_1 hybrids, and those between different families produced semi-sterile F_1 hybrids. The pollen fertilities of the F_1 plants between different families were as low as 20 to 35 percent indicating the effect of two sets of *s* genes (Table 1e). Furthermore, five isogenic F_1-sterile lines each carrying a set of *s* loci from a different donor parent (108, 144, 435, 706, or 727) were obtained from $B_{13}F_2$ plants. Diallel crosses among them reconfirmed the above relation except that the lines from 108 and 706 appeared to have the same *s* loci.

The results of these intercrossing experiments thus indicated that lines derived from the same donor parent had a set of $+_1$ and s_2 genes at the same two loci, while those from different donor parents each had a set of the genes in most cases at different loci. It then follows that T65 has at least four different sets of *s* loci. The gametic genotype of T65 may then be written as $(s_1 +_2)_A$, $(s_1 +_2)_B$, etc., where A, B, etc. represent the sets at different loci. This is in agreement with the author's earlier postulation that there are a number of sets of duplicate gametic lethals among rice varieties (OKA 1956). Perhaps, many "gametic-development genes" are needed for gametogenesis.

4. *Linkage relations of the* s *loci*

In parallel with the breeding of F_1-sterile lines, isogenic lines of T65 carrying

TABLE 5

F_2 ratios observed in crosses between isogenic F_1-sterile lines (A1 and B2) and isogenic lines with marker genes

Cross	Markers character[1]	Linkage group	No. of F_2 plants AA	Aa	aa	Total	Expected ratio	Chi-square
A1 × *d_2*	"Ebisu" dwarf	2	53		13	68	3 : 1	0.99ns
A1 × *lg*	Liguleless	2	65		8	73	3 : 1	7.67**
A1 × *la*	Lazy habit	8	53		17	70	3 : 1	0.02ns
A1 × *nl*	Neck leaf	9	57		15	72	3 : 1	0.50ns
A1 × *bc*	Brittle culm	11	59		10	69	3 : 1	4.06*
B2 × *wx*	Glutinous endosperm	1	19	28	11	58	1 : 2 : 1	2.28 (P=0.15)
B2 × *d_2*	"Ebisu" dwarf	2	43		17	60	3 : 1	0.35ns
B2 × *lg*	Liguleless	2	45		18	63	3 : 1	0.93ns
B2 × *la*	Lazy habit	8	49		15	64	3 : 1	0.08ns
B2 × *bc*	Brittle culm	11	52		17	69	3 : 1	0.00ns

[1] cf. TAKAHASHI (1964).
** Significant at 1% level, * at 5% level.
ns: not significant.

different marker genes were established by backcrossing (B_7 to B_{16}); the donor parents each carrying a marker were obtained through the kindness of DR. M. TAKAHASHI of the Hokkaido University, Japan. In order to find out to which linkage groups the *s* loci belong, six of these isogenic marker lines were each crossed with F_1-sterile lines A1 and B2.

The F_2 ratios in crosses between ordinary rice varieties often deviate from theoretical ones, but those observed in crosses of isogenic lines are close to the theoretical ones (TSAI and OKA 1965). Therefore, if a significant deviation is found in the present crosses, it may be attributed to the effect of the *s* genes.

As given in Table 5, the F_2 populations observed showed deviations from 1:2:1 or 3:1 ratio in three crosses, i.e., A1 × $T65^{lg}$ (liguleless, linkage group 2), A1 × $T65^{bc}$ (brittle culm, linkage group 11), and B2 × $T65^{wx}$ (glutinous endosperm, linkage group 1; in this cross, the deviation from 1:2:1 did not reach the 5% level of significance, but the numbers of fertile and semi-sterile heterozygotes showed significant deviations as shown in Table 7). The isogenic marker lines should have the same combination of *s* genes as of T65 (s_1/s_1 $+_2/+_2$); the isogenic F_1-sterile lines have $+_1/+_1$ s_2/s_2 at different loci according to the donor-parent family.

When s_1 or $+_2$ is linked with gene *a* expressing a character, the frequencies of various F_2 genotypes expected are shown in Table 6. As is observed in the table, *aa* plants will become less than 1/4 of the total number when *a* is linked with s_1 or $+_2$ (so far as $k_1 > 1$ and $k_2 < 1$). When *a* is linked with s_1, furthermore, there will be more semi-sterile *Aa* plants than fertile *Aa* plants.

A predominance of semi-sterile heterozygotes over fertile ones was found in the F_2 of B2 × $T65^{wx}$ (Table 7). The *wx* gene (linkage group 1) may then be regarded as linked with the s_1 gene causing F_1 sterility in this cross (Family B).

TABLE 6

Expectation of frequencies of fertile and semi-sterile F_2 plants with different combinations of genes A/a *linked with* s *gene loci*

(A) When a is linked with s_1, F_1 genotype $= \frac{s_1\text{-}a}{+_1\text{-}A}\ \frac{+_2}{s_2}$

Genotype	Frequency
AA fertile	$2k_1 + 2k_2 + (1 - 4k_1 - 3k_2)p^* + (2k_1 + k_2)p^2$
Aa fertile	$1 + k_2 + (4k_1 + 2k_2)p + (-4k_1 - 2k_2)p^2$
aa fertile	$1 + (-1 + k_2)p + (2k_1 + k_2)p^2$
AA semi-sterile	$(1 + 2k_1 + k_2)p + (-2k_1 - k_2)p^2$
Aa semi-sterile	$1 + 2k_1 + k_2 + (-4k_1 - 2k_2)p + (4k_1 + 2k_2)p^2$
aa semi-sterile	$1 + (-1 + 2k_1 + k_2)p + (-2k_1 - k_2)p^2$
Total	$4 + 4k_1 + 4k_2$

(B) When a is linked with $+_2$, F_1 genotype $= \frac{s_1}{+_1}\ \frac{+_2\text{-}a}{s_2\text{-A}}$

Genotype	Frequency
AA fertile	$k_1 + (-k_1 + k_2)p + (2 + k_2)p^2$
Aa fertile	$k_1 + k_2 + (4 + 2k_2)p + (-4 - 2k_2)p^2$
aa fertile	$2 + 2k_2 + (-4 + k_1 - 3k_2)p + (2 + k_2)p^2$
AA semi-sterile	$k_1 + (2 - k_1 + k_2)p + (-2 - k_2)p^2$
Aa semi-sterile	$2 + k_1 + k_2 + (-4 - 2k_2)p + (4 + 2k_2)p^2$
aa semi-sterile	$(2 + k_1 + k_2)p + (-2 - k_2)p^2$
Total	$4 + 4k_1 + 4k_2$

* p = recombination fraction.

The observed frequencies of various F_2 genotypes (Table 7) were compared with the expectation formulas in Table 6 (assuming $k_1 = 12.63$ and $k_2 = 0.05$), and the recombination fraction between *wx* and *s* loci was estimated to be 32.8 percent by maximum likelihood technique.

In A1 × T65^{lg} and A1 × T65^{bc} crosses (Family A), as the same F_1-sterile line showed deficiency of recessive homozygotes for two independent markers, it was

TABLE 7

Observed and expected numbers of fertile and semi-sterile F_2 plants with different genotypes for the glutinous gene (wx) *in* B2 × T65^{wx} *(Family B)*

Pollen fertility	Glutinous gene	Observed no.	Expected no.*
Fertile	*Wx / Wx*	12	12.50
Fertile	*Wx / wx*	8†	12.94
Fertile	*wx / wx*	6	3.62
Semi-sterile	*Wx / Wx*	7	6.26
Semi-sterile	*Wx / wx*	20†	16.06
Semi-sterile	*wx / wx*	5	6.62
Total		58	$\chi^2 = 4.92^{ns}$

* Obtained by substituting $k_1 = 12.63$, $k_2 = 0.05$, and $p = 0.328$ into the formulas in Table 6(A).

† The ratio of fertile:semi-sterile *Wx/wx* plants, 8:20, significantly differs from 1.05:1 found for Family B (Table 2).

inferred that the s_1 and $+_2$ genes of T65 causing F_1 sterility in these crosses were linked with *bc* and *lg*, or *lg* and *bc*, respectively. Assuming *bc*–s_1 and *lg*–$+_2$ linkages, the maximum likelihood estimates of recombination fraction were 16.0 and 14.9 percent, respectively (also assuming that $k_1 = 4.54$ and $k_2 = 0.71$). Assuming *lg*–s_1 and *bc*–$+_2$ linkages, however, the *lg*–s_1 recombination fraction had a minus value and could not be duly estimated. It may then be inferred that the s_1 and $+_2$ loci are linked with *bc* (linkage group 11) and *lg* (linkage group 2), respectively. The $+_2$ locus appeared to be independent of d_2 which also belongs to linkage group 2 but is located 38 units distant from *lg* (TAKAHASHI 1964). The result of this linkage experiment may serve as an indication of the independence of the two *s* gene loci.

DISCUSSION

In many plant groups, the F_1 sterility has been attributed to structural differences in chromosomes or genomic differences which were often "cryptic" or unanalyzable. The intervarietal F_1 sterility in *Oryza sativa* can not be chromosomal; the F_1 hybrids of rice varieties having $n = 12$ chromosomes show no significant disturbances in meiotic pairing and disjunction (CHANDLER 1964, pp. 145–189). This view was confirmed by ENGLE, CHANG and RAMIREZ (1969). Yet it is possible that the *s* loci represent small rearrangements of chromosomal segments that behave in the same manner as genes; a possibility discussed by STEBBINS (1958, p. 178). Under this assumption, however, questions may arise as to why larger and cytologically detectable rearrangements are not found in rice hybrids.

In the present experiment, the data fitted the duplicate gametophytic lethal model best. It was also pointed out that there could be many similar sets of such genes. This hypothesis does not differ from the author's previous one (1953, 1957a) except for the assumption that microspores are affected but megaspores are not affected by the genes. In the previous hypothesis, the development of both micro- and megaspores were considered to be controlled by the same genes. Possibly, there are both types of genes and those controlling microspore development have been selected in the present backcrossing experiment. In addition, a case was found previously in which the one-locus sporo-gametophytic interaction model appeared to account for the data, although the data were insufficient for critical testing of the hypothesis (OKA 1964, p. 168). It may be inferred that there are different genic systems controlling the F_1 sterility and the present duplicate gametic gene hypothesis represents one of them.

The F_1 sterility dealt with in this study is not the only barrier between distantly related varieties of *O. sativa*. Cytoplasmic male sterility is also found in particular crosses (SHINJYO 1969). In addition to F_1 weakness and partial hybrid breakdown (OKA 1957b; CHU, MORISHIMA and OKA 1969), sporophytic F_2 sterility is frequently observed (OKA and DOIDA 1962). It differs from the gametophytic F_1 sterility in that partly fertile plants occur in the F_2 and later generations, and true-breeding semi-sterile lines can be isolated in the progeny.

These hybrid abortions observed in crosses between sativa varieties were in most cases attributable to duplicate or complementary genes (OKA 1964). It may be inferred that gene duplication has resulted from chromosome doubling in the remote ancestry of the genus. This view is favorably supported by the secondarily balanced polyploidy hypothesis for the genus early advocated by SAKAI (1935), NANDI (1936) and HU (1962). It was found further that many Asian strains of *O. perennis* Moench, the wild progenitor of *O. sativa*, produced fertile F_1 hybrids in crosses with different sativa varieties which were inter-sterile (HINATA and OKA 1962). This phenomenon can be reasonably explained by assuming that the perennis strains have double dominant genotypes ($+_1 +_2$) and recessive mutations at one of the duplicated loci have brought about the F_1-sterility relationships as presently found among sativa varieties. The data on intermediate wild-cultivated strains collected from Jeypore Tract, India, suggested that the F_1 sterilities among sativa varieties might have developed with the domestication of the plants after their Indica-Japonica differentiation in other morphological and physiological traits took place (OKA and CHANG 1962).

It may be of interest to note in this context that in the genetic background of T65, pollen grains with a different combination of *s* genes from that of T65 had higher fertilizing capacity, but those with a double dominant combination had lower fertilizing capacity than the pollen grain of T65. This pattern of certation could have been an internal mechanism which helped to increase the frequency of alien genes in hybrid progenies and to develop F_1-sterility relationships among sativa varieties.

LITERATURE CITED

CHANDLER, R. F., 1964 *Rice Genetics and Cytogenetics*. Elsevier, Amsterdam. 274 pp.

CHU, Y. E., H. MORISHIMA and H. I. OKA, 1969 Reproductive barriers distributed in cultivated rice species and their wild relatives. Japan. J. Genet. **44**: 207–223.

CHU, Y. E. and H. I. OKA, 1972 The distribution and effects of genes causing F_1 weakness in *Oryza breviligulata* and *O. glaberrima*. Genetics **70**: 163–173.

DAVID, F. N., 1951 *Probability Theory for Statistical Methods*. Cambridge University Press, London, England. 230 pp.

ENGLE, L. M., T. T. CHANG and D. A. RAMIREZ, 1969 The cytogenetics of sterility in F_1 hybrids of Indica × Indica and Indica × Japonica varieties of rice (*Oryza sativa* L.) Philippine Agriculturist **53**: 289–307.

HINATA, K., and H. I. OKA, 1962 A survey of hybrid sterility relationships in the Asian forms of *Oryza perennis* and *O. sativa*. Japan. J. Genet. **37**: 314–328.

HU, C. H., 1962 Studies on meiosis in Oryza species, with special reference to secondary association. Cytologia **27**: 285–295.

NANDI, H. K., 1936 The chromosome morphology, secondary association and origin of cultivated rice. J. Genet. **33**: 316–336.

OKA, H. I., 1953 The mechanisms of sterility in the intervarietal hybrids. Phylogenetic differsentiation of cultivated rice. VI. (In Japanese with English summary) Japan. J. Breed. **2**: 217–224. ——, 1956 Polygenic nature of "gametic-development genes" controlling inter-varietal hybrid sterility in rice. Phylogenetic differentiation of cultivated rice. XII. (In Japanese with English summary) Japan. J. Breed. **6**: 51–55. ——, 1957a Genic analysis for the sterility of hybrids between distantly related varieties of cultivated rice.

J. Genet. **55**: 397–409. ——, 1957b Complementary lethal genes in rice . Phylogenetic differentiation of cultivated rice. XV. Japan. J. Genet. **32**: 83–87. ——, 1964 Considerations on the genetic basis of intervarietal sterility in *Oryza sativa*. Pp. 158–174. In: *Rice Genetics and Cytogenetics*. Edited by R. F. CHANDLER. Elsevier, Amsterdam.

OKA, H. I. and W. T. CHANG, 1952 Rice varieties intermediate between wild and cultivated forms and the origin of the Japonica type. Bot. Bull. Acad. Sinica **3**: 109–131.

OKA, H. I. and Y. DOIDA, 1962 Analysis of the genetic basis of hybrid breakdown in rice. Phylogenetic differentiation of cultivated rice. XX. Japan. J. Genet. **37**: 24–35.

SAKAI, K., 1935 Chromosome study of *Oryza sativa L.* I. The secondary association of the meiotic chromosomes. Japan. J. Genet. **11**: 145–156.

SHINJYO, C., 1969 Cytoplasmic-genetic male sterility in cultivated rice, *Oryza sativa* L. II. The inheritance of male sterility. Japan. J. Genet. **44**: 149–156.

STEBBINS, G. L., 1958 The inviability, weakness, and sterility of interspecific hybrids. Advan. Genet. **9**: 147–215.

STEPHENS, S. G., 1946 The genetics of "corky." 1. The New World alleles and their possible role as an interspecific isolating mechanism. J. Genet. **47**: 150–161. ——, 1950 The genetics of "corky." II. Further studies on its genetic basis in relation to the general problem of interspecific isolating mechanisms. J. Genet. **50**: 9–20.

TAKAHASHI, M., 1964 Linkage groups and gene schemes of some striking morphological characters in Japanese rice. Pp. 215–236. In: *Rice Genetics and Cytogenetics*. Edited by R. F. CHANDLER. Elsevier, Amsterdam.

TERAO, H. and U. MIZUSHIMA, 1939 Some consideration on the classification of *Oryza sativa* L. into two subspecies, so-called "Japonica" and "Indica." Japan. J. Bot. **10**: 213–258.

TSAI, K. H. and H. I. OKA, 1965 Genetic studies of yielding capacity and adaptability in crop plants. I. Characters of isogenic lines in rice. Bot. Bull. Acad. Sinica **6**: 19–31.

TSUNEWAKI, K., 1960 Monosomic and conventional gene analysis in common wheat. III. Lethality. Japan. J. Genet. **35**: 71–75.

Corresponding editor: O. E. NELSON