Multiple Alleles at a New Locus Causing Hybrid Sterility between a Korean Indica Variety and a Japonica Variety in Rice (*Oryza sativa* L.)

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Allelic interaction causing gamete abortion in wide crosses of cultivated rice was earlier identified at locus *S-5* on Chromosome 6 and recently at locus *S-7* on Chromosome 7. At *S-5* locus Indica and Japonica rices have *S-5ⁱ* and *S-5^j*, respectively, and Japonicas such as Ketan Nangka have a neutral allele *S-5ⁿ*. The *S-5ⁱ/S-5^j* genotype produces semi-sterile panicles due to abortion of female gametes carrying *S-5^j*, but both *S-5ⁿ/S-5ⁱ* and *S-5ⁿ/S-5^j* genotypes do not. The *S-5ⁿ* is called "wide compatibility gene" (WCG), as one of parents possessing this allele does not show hybrid sterility in their *F*₁. The WCG has been used for breeding hybrid varieties between Indica and Japonica rice. The *S-5ⁿ* allele was incorporated from Ketan Nangka to Japonica lines such as Nekken 1 or 2, which do not show semi-sterility in their cross to many Indica type. However, Nekken 2 showed typical hybrid sterility when it was crossed to a Korean Indica line, Yeong Pung. Therefore, a new locus was suggested for the semi-sterility in that hybrid. By genetic analyses using isozyme markers, a new locus for the hybrid sterility between Nekken 2 and the Korean Indicas was located near *Cat-1* locus on Chromosome 6, and designated as *S-8* following our recent report on *S-7*. On the basis of allelic interaction causing female gamete abortion, three alleles were found at *S-8* as follows: a Japonica line Nekken 2 and Yeong Pung possess *S-8^{kn}* and *S-8^{yp}*, respectively, and their hybrid genotype of *S-8^{kn}/S-8^{yp}* shows semi-sterility as a portion of female gametes carrying *S-8^{kn}* was aborted. A Japonica tester variety, Akihikari was found to have a neutral allele, *S-8ⁿ* at this locus, and another neutral allele, *S-7ⁿ* at *S-7*. A possibility to cumulate three neutral alleles in a breeding line is suggested to solve hybrid sterility problem in wide crosses of rice.

KEY WORDS: *Oryza sativa*, hybrid sterility, gamete abortion, wide compatibility, wide cross.

Introduction

Hybrid sterility limits application of wide cross to rice breeding. It also lowers the productivity of hybrid rice. The sterility in *F*₁ hybrids between Indica and Japonica varieties has been attributed to an allelic interaction at locus *S-5*, where Indica and Japonica varieties have *S-5ⁱ* and *S-5^j*, respectively, and some Japonicas have a neutral allele, *S-5ⁿ*. The *S-5ⁱ/S-5^j* genotype produces semi-sterile panicles due to abortion of female gametes carrying the allele of *S-5^j*, but *S-5ⁿ/S-5ⁱ* and *S-5ⁿ/S-5^j* do not. The donor of *S-5ⁿ* is called wide-compatibility variety (WCV) (IKEHASHI and ARAKI, 1986). This allele has been incorporated into Indica or Japonica variety to overcome sterility problem in wide crosses and hybrid rice breeding (ARAKI et al. 1988, IKEHASHI, 1991).

So far, the *S-5ⁿ* allele seems to be effective for many of Indica-Japonica hybrids. In the expansion of varietal screening, however, some hybrids showed semi-sterility despite the

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$S-5^n$ allele in one of their parents (IKEHASHI *et al.*, 1991). Recently, for one case of semi-sterility in the hybrids between Aus varieties and some Javanicas, a new locus $S-7$ for female gamete abortion was identified between Rc (brown pericarp and seed coat) and $Est-9$ on Chromosome 7 (YANAGIHARA *et al.*, 1992b). Another case of sterility was shown in the hybrid between a Korean Indica line Yeong Pung and Nekken 2 which possesses $S-5^n$. Thus, a new locus was suggested for the semi-sterility. In this paper, we report a new locus $S-8$ for allelic interaction causing female gamete abortion, where two interacting alleles were found; $S-8^{kn}$ and $S-8^{yp}$ in Nekken 2 and Yeong Pung, respectively. A portion of female gametes carrying $S-8^{kn}$ were found to be aborted in $S-8^{kn}/S-8^{yp}$. A Japonica tester variety, Akihikari was found to have a neutral allele $S-8^n$ at this locus. Utilization of the new neutral allele at $S-8$ is suggested to solve the problem of hybrid sterilities.

Materials and Methods

Plant materials

Varieties tested and their marker genotypes are shown in Table 1. Yeong Pung is a Indica variety developed in Korea from a cross of Milyang 23 // Milyang 23 / IR2061-481. A Japonica line Nekken 2 is developed from a series of crosses, i.e., Akihikari///Ketan Nangka/Nihon-masari//Akihikari to incorporate $S-5^n$ from Ketan Nanka to Japonica type. Nekken 2 produces fertile hybrids when it is crossed to Indicas. But the hybrids between Yeong Pung and Nekken 2 showed a low level of spikelet fertility. Hybrids between Yeong Pung and Ketan Nangka or Akihikari also showed sterility, but Yeong Pung showed normal fertility in its cross to IR36, a typical Indica variety and to Ingra, an Aus variety from the Indian Subcontinent (Table 2).

Table 1. Tested varieties and their markers

Parental Varieties	Allele at marker loci and chromosome				
	Chrom. 6				Chrom. 7
	<i>Amp-3</i>	<i>Est-2</i>	<i>Cat-1</i>	<i>Pox-5</i>	<i>Est-9</i>
Akihikari	1	0	2	1	1
Nekken 2	1	0	2	1	1
Yeong Pung	2	1	1	2	2
Ketan Nangka	1	0	2	1	1

Table 2. Hybrid fertility in F_1 hybrids

Crosses	Mean %
Akihikari/Yeong Pung	13.6
Nekken 2/Yeong Pung	30.7
Yeong Pung/Nekken 2	48.2
Yeong Pung/Ketan Nengka	8.6
Yeong Pung/Ingra	90.3
Ingra/IR36	87.8

To detect distorted segregation of marker genes which may indicate a gamete abortion locus, isozyme markers were analyzed in three F_2 populations, i.e., Yeong Pung/Nekken 2, Yeong Pung/Ketan Nangka and Akihikari/Yeong Pung (Table 3). The progeny of Yeong Pung/2*Ketan Nangka was also analyzed for segregation of markers together with the F_2 populations. To determine linkages between isozyme genotypes and spikelet fertility a B_1F_1 population of Akihikari/Yeong Pung//Akihikari and an F_1 population from a three-way cross, i.e., Akihikari/Yeong Pung//Nekken 2 were grown at The National Agriculture Research Center, Tsukuba in 1992, each with 69 and 89 plants, respectively. The spikelet fertility was determined by counting fertile and sterile spikelets on the upper half of 3-4 panicles for each plant.

Genetic analysis using marker genes

According to the model of one-locus allelic interaction for F_1 semi-sterility (IKEHASHI and ARAKI, 1986), segregations of semi-sterile and fertile plants in the progeny of three way crosses are expected at the ratio of 1:1. For an instance, the progeny of Akihikari/Yeong Pung//Nekken 2 would be divided into two components; semi-sterile one expected by Yeong

Table 3. Segregation of marker genotypes in F_2 and B_1F_1

Locus	Marker genotypes ¹⁾			
	II	H	JJ	
Yeong Pung/Nekken 2				
<i>Cat-1</i>	60	69	18	**2)
<i>Pox-5</i>	52	72	13	**
<i>Est-9</i>	13	72	63	**
Yeong Pung/Ketan Nangka				
<i>Amp-3</i>	35	65	31	
<i>Cat-1</i>	52	62	17	**
<i>Est-9</i>	19	63	49	**
Yeong Pung/Ketan Nangka//Ketan Nangka				
<i>Amp-3</i>		64	57	
<i>Cat-1</i>		81	40	**
<i>Est-9</i>		39	82	**
Akihikari/Yeong Pung				
<i>Cat-1</i>	44	71	29	*
<i>Pox-5</i>	40	69	35	
<i>Amp-3</i>	61	70	13	**
<i>Est-2</i>	56	75	13	**
<i>Est-9</i>	36	74	34	

¹⁾II and JJ indicates homozygotes of Indica and Japonica allele, respectively, and H indicates heterozygote of the two.

²⁾* and ** shows significant deviation from Mendelian ratio at 5% and 1%, respectively.

Pung/Nekken 2 and fertile one by Akihikari/ Nekken 2, because the F_1 of Yeong Pung/ Nekken 2 was semi-sterile and that of Akihikari/Nekken 2 was not. In terms of genotypes the heterozygote of an allele from Yeong Pung and another from Nekken 2 is expected to show semi-sterility due to abortion of female gamete carrying one of the alleles, while the other heterozygote of an allele from Akihikari and another from Nekken 2 would show normal fertility as no female gamete abortion may occur in that genotype. If any genetic markers cosegregate with the semi-sterility, a locus for the semi-sterility can be indicated by such markers.

Four markers on Chromosome 6, i.e., *Amp-3*, *Est-2*, *Cat-1* and *Pox-5* and *Est-9* on Chromosome 7 (Table 1) were analyzed for progeny plants. Of the markers, *Amp-3* and *Est-2* are marker for S-5 locus (YANAGIHARA, *et al*, 1992a). *Est-9* is a marker for S-7 (YANAGIHARA *et al*, 1992b). *Cat-1* and *Pox-5*, which are linked with a recombination frequency of 22% are located on Chromosome 6 (WU *et al*, 1988). The isozyme analyses were conducted according to ISHIKAWA *et al* (1989) and GLASZMANN *et al* (1988).

Estimation of allelic difference at a new locus by gamete abortion

Allelic difference at a new locus was estimated following the model of allelic interaction at S-5, where $S-5^i/S-5^j$ causes abortion of female gametes carrying $S-5^j$, but $S-5^n/S-5^i$ and $S-5^n/S-5^j$ do not. For three given varieties, A, B and N, if a hybrid A/B shows semi-sterility, while both hybrids of N/A and N/B do not, the variety N may possess a neutral allele at respective loci. This test was applied to tested crosses in this experiment to estimate allelic difference at S-7 and S-8.

Results

Segregation distortion of markers in F_2 and B_1F_1 populations

Segregation distortions were found at *Est-9* in F_2 populations of Yeong Pung/Nekken 2 and Yeong Pung/Ketan Nangka and in B_1F_1 of Yeong Pung/2*Ketan Nangka (Table 3). This fact indicated that allelic interaction causing abortion of female gamete possessing Indica type allele at S-7 occurred, as *Est-9* is a marker for S-7 (YANAGIHARA *et al*, 1992b). Segregation distortions were also found at *Cat-1* and *Pox-5* in the F_2 population of Yeong Pung/Nekken 2, and at *Cat-1* in the F_2 of Yeong Pung/Ketan Nangka. The reduction of Japonica-type allele at *Cat-1* in Yeong Pung/2*Ketan Nangka confirmed that female gametes possessing Japonica type allele were partially aborted by an allelic interaction at a new locus near *Cat-1* and *Pox-5*.

In the cross of Akihikari/Yeong Pung, segregation distortion at *Amp-3* and *Est-2* was significant, indicating the allelic interaction at S-5. Segregations of the allele were normal for *Est-9* and *Pox-5*, but not so for *Cat-1* with a significance level of 5%.

In the crosses of Yeong Pung/Ketan Nangka and Yeong Pung/2*Ketan Nangka, there was normal segregation of *Amp-3*, suggesting that the semi-sterility is not ascribed to S-5.

Relation between marker genotypes and fertility levels

In the progeny of Akihikari/Yeong Pung//Akihikari, the level of spikelet fertility was

differentiated by marker genotypes at *Amp-3* and *Est-2*, which are markers for *S-5* (Table 4). Therefore, the semi-sterility in the F_1 of Yeong Pung/Akihikari must have been caused by the allelic interaction at *S-5*. In the two marker genotypes homozygotes showed a high level of spikelet fertility, while the heterozygotes between Indica and Japonica type alleles showed a lower fertility. Segregation distortion found in the F_2 of Akihikari/Yeong Pung for alleles at *Cat-1* was not observed in Akihikari/Yeong Pung//Akihikari. This fact suggested that the segregation distortion of *Cat-1* in the F_2 was not caused through female gamete.

In the progeny of Akihikari/Yeong Pung//Nekken 2, the level of spikelet fertility was significantly differentiated by marker genotypes at *Cat-1* and *Pox-5*, and also at *Est-9* (Table 5). The lowered fertility of the heterozygote at *Cat-1* and *Pox-5* was considered to be caused by an allelic interaction at a new locus near these markers. The lowered fertility of the heterozygote at *Est-9* can be explained by the effect of *S-7*. The fact that fertility level was not ascribed to *Amp-3* genotypes indicated that in this cross no allelic interaction was detectable for *S-5*.

Determination of loci by evaluating recombination frequencies

According to the data in Table 4, where assumed recombinants were underlined, the recombination frequency between *S-5* and *Amp-3* was estimated to be 21.7%, and that between *S-5* and *Est-2* was 21.7%. As the recombination frequency between *Amp-3* and *Est-2* was 10.2%, the three loci were mapped as shown in Fig. 1.

In the progeny of Akihikari/Yeong Pung//Nekken 2, the new locus indicated by *Cat-1* and *Pox-5* can be hereafter named *S-8* (*t*). Then, according to the data in Table 5, where assumed recombinants were underlined, the recombination frequency between *S-8* (*t*) and *Cat-1*

Table 4. Distribution of spikelet fertility classified by marker genotype in Akihikari/Yeong Pung//Akihikari

Genotype	Number of plants in spikelet fertility (%)										Total	Mean %
	10	20	30	40	50	60	70	80	90	100		
<i>Amp-3</i> ¹ / <i>Amp-3</i> ¹	0	0	<u>2</u>	0	<u>2</u>	<u>2</u>	3	5	6	10	30	76.1**
<i>Amp-3</i> ¹ / <i>Amp-3</i> ²	1	0	5	6	4	10	4	<u>7</u>	<u>2</u>	0	39	52.5
<i>Est-2</i> ⁰ / <i>Est-2</i> ⁰	0	0	<u>1</u>	<u>3</u>	<u>1</u>	<u>3</u>	4	4	6	9	31	73.4**
<i>Est-2</i> ⁰ / <i>Est-2</i> ¹	1	0	5	1	11	9	4	<u>1</u>	<u>2</u>	<u>4</u>	38	55.8
<i>Cat-1</i> ² / <i>Cat-1</i> ²	0	0	1	1	1	7	4	7	4	6	31	71.0
<i>Cat-1</i> ¹ / <i>Cat-1</i> ²	1	0	5	3	4	5	4	5	4	7	38	61.0
<i>Pox-5</i> ¹ / <i>Pox-5</i> ¹	1	0	1	2	1	6	3	5	6	8	33	66.3
<i>Pox-5</i> ¹ / <i>Pox-5</i> ²	0	0	2	2	1	6	7	6	5	7	36	64.0
<i>Est-9</i> ¹ / <i>Est-9</i> ¹	1	2	1	2	2	5	4	7	3	5	32	65.0
<i>Est-9</i> ¹ / <i>Est-9</i> ²	1	0	5	2	3	7	4	5	5	5	37	64.0

** show significant deviation from Mendelian ratio at 5% and 1%, respectively.

was estimated to be 11.2%, and that between *S-8(t)* and *Pox-5* was 27.1%. As the recombination frequency between *Pox-5* and *Cat-1* was 21.3%, the three loci were mapped as shown in Fig. 1. In addition, the recombination frequency between *Est-9* and *S-7* was estimated to be 24.7% and also shown in Fig.1. Previously estimated recombination frequencies between *Est-9* and *S-7* were 32.7% in one cross and 20.4~24.4% in another (YANAGIHARA *et al.*, 1992b).

Determination of allelic difference at *S-7* and *S-8*

Allelic difference at *S-7* and *S-8* was determined on the basis of hybrid sterilities in related crosses. Generally, for three given varieties, A, B and N, if a hybrid A/B shows gamete abortion at a locus *S-X* by allelic interaction between *S-X^a* and *S-X^b* while both N/A and N/B do not, the variety N may possess a neutral allele *S-Xⁿ*.

The hybrid of Yeong Pung/Ketan Nangka showed an allelic interaction causing semi-sterility at *S-7* (Table 3). On the other hand, the cross of Yeong Pung/Akihikari showed semi-sterility attributable only to *S-5* (Table 4), indicating that this cross do not show allelic interaction at *S-7*, like as *F₁* hybrid of Akihikari/Ketan Nangka (IKEHASHI and ARAKI, 1986). Therefore, Akihikari is considered to possess a neutral allele at *S-7*.

Similarly, the allele of IR 36 at *S-7* can be determined. *F₁* hybrid of Ketan Nangka/Ingra showed semi-sterility due to allelic interaction at *S-7* (YANAGIHARA *et al.*, 1992b). *F₁* hybrid of Ingra/IR36 showed normal fertility (Table 2) and *F₁* hybrid of Ketan Nangka/IR 36 did so (IKEHASHI and ARAKI, 1986). Therefore, IR 36 may possess a neutral allele at *S-7*.

The reduction of Japonica-type allele at *S-8* in Yeong Pung/2*Ketan Nangka suggested that female gametes possessing Japonica type allele were aborted by an allelic interaction at *S-8*. Therefore, two interacting alleles, *S-8^{kn}* in Ketan Nangka and *S-8^{yp}* in Yeong Pung can be designated. At *S-8* a portion of female gametes carrying *S-8^{kn}* was aborted in *S-8^{kn}/S-8^{yp}*.

Table 5. Distribution of spikelet fertility classified by marker genotype in Akihikari/Yeong Pung//Nekken 2

Genotype	Number of plants in spikelet fertility (%)										Total	Mean %
	10	20	30	40	50	60	70	80	90	100		
<i>Cat-1²/Cat-1²</i>	0	0	0	<u>1</u>	<u>1</u>	<u>1</u>	6	10	15	5	39	77.5**
<i>Cat-1¹/Cat-1²</i>	2	6	1	4	5	11	14	<u>5</u>	<u>2</u>	0	50	50.5
<i>Pox-5¹/Pox-5¹</i>	0	<u>1</u>	0	<u>2</u>	<u>3</u>	<u>3</u>	8	8	11	3	39	69.8**
<i>Pox-5¹/Pox-5²</i>	2	5	1	3	3	9	12	<u>7</u>	<u>6</u>	<u>2</u>	50	56.6
<i>Amp-3²/Amp-3²</i>	1	3	0	1	3	3	9	11	6	2	39	63.6
<i>Amp-3¹/Amp-3²</i>	1	3	1	4	3	9	11	4	11	3	50	61.4
<i>Est-9¹/Est-9¹</i>	0	0	0	0	<u>3</u>	<u>2</u>	11	10	7	3	36	72.2**
<i>Est-9¹/Est-9²</i>	2	6	1	5	3	10	9	<u>5</u>	<u>10</u>	<u>2</u>	53	55.7

** shows significant deviation from Mendelian ratio at 1%.

The same allelic interaction was detected in Akihikari/Yeong Pung//Nekken 2 (Table 5). It was expected as Ketan Nangka is donor of $S-8^{kn}$ to Nekken 2.

While Yeong Pung/Ketan Nangka showed an allelic interaction for gamete abortion at $S-8$, B_1F_1 of Akihikari/Yeong Pung//Akihikari did not, showing normal segregation at $Pox-5$ and $Cat-1$. F_1 hybrids of Akihikari/Ketan Nangka were fertile (IKEHASHI and ARAKI, 1986). Therefore, Akihikari is considered to possess a neutral allele at $S-8^n$ (Table 6).

F_1 hybrid of Yeong Pung/Ingra showed normal fertility, indicating that Ingra's allele is

neutral or identical to Yeong Pung's. As F_1 hybrid of Ingra/IR 36 was fertile, the allele of IR 36 should be neutral or identical to Ingra's (Table 2). While IR 36/Ketan Nangka gave fertile hybrid, IR 36 should have an allele neutral or identical to Ketan Nangka's. Therefore, only one conclusion that IR 36 has a neutral allele at $S-8$ can satisfy all of above assumptions.

Discussion

A neutral allele $S-5^n$ (WCG) was identified at a gamete abortion locus on Chromosome 6, where female gametes carrying $S-5^j$ are aborted in heterozygote $S-5^j/S-5^j$ (IKEHASHI and ARAKI, 1986). It has been widely applied to solve hybrid sterility in Indica-Japonica crosses (IKEHASHI, 1991).

Fig. 1. Linkage map for the markers on Chromosome 6 and 7.

Table 6. Estimated allelic differentiation

Parental Varieties	Loci and Chromosomes		
	Chrom. 6		Chrom. 7
	S-5	S-8	S-7
Akihikari	j	n	n
IR 36	i	n	n
Yeong Pung	i	yp	ai
Ingra	n*	yp or n*	ai
Ketan Nangka	n	kn	kn
Nekken 2	n	kn	kn

*Ingra/Ketan Nangka showed no segregation distortion at ux which is a marker for $S-5$ (IKEHASHI *et al.* 1991). F_1 hybrids of IR36/Ketan Nangka, Ingra/IR36 and Ingra/Yeong Pung are fertile, but the markers for $S-8$ were not tested in Ingra/Ketan Nangka.

Since then, two exceptional cases were found. A case of semi-sterility was found in the crosses between Aus varieties of the Indian Subcontinent and Javanica varieties possessing $S-5^n$. Later a new locus $S-7$ was identified on Chromosome 7 (YANAGIHARA *et al*, 1992b). Another case is semi-sterility found in the cross between Nekken 2 which possesses $S-5^n$ from Ketan Nangka and a Korean Indica variety Yeong Pung. In this study a new locus for this semi-sterility is identified as $S-8$ on Chromosome 6.

Four kinds of alleles were identified at $S-7$; $S-7^{ai}$ allele for Ingra, $S-7^{cp}$ for CP-SLO, $S-7^{kn}$ for Ketan Nangka and a neutral allele $S-7^n$ for Banten and N 22 (YANAGIHARA *et al*, 1992b). Gametes possessing $S-7^{ai}$ from Ingra were aborted in the heterozygote of $S-7^{ai}/S-7^{kn}$. In this study gametes possessing Indica type allele, $Est-9^2$ was reduced in Yeong Pung/Ketan Nangka and Yeong Pung/2 * Ketan Nangka, while F_1 of Yeong Pung/Ingra showed normal fertility (Table 2). As $Est-9$ is linked to $S-7$, Yeong Pung may also possess $S-7^{ai}$.

In this study three alleles were found at $S-8$. A Japonica line Nekken 2 and Yeong Pung possess $S-8^{kn}$ and $S-8^{yp}$, respectively. The data suggested that in the genotype of $S-8^{kn}/S-8^{yp}$ showing semi-sterility, a portion of the female gametes carrying $S-8^{kn}$ was aborted. A Japonica standard variety, Akihikari was found to have neutral alleles $S-7^n$ and $S-8^n$. Yeong Pung was an Indica variety developed in Korea from a cross of Milyang 23 // Milyang 23 / IR2061-481. Milyang 23 is considered to be typical Indica rice as it did not show sterility in its cross to Nekken 2. Therefore, $S-7^{ia}$ and $S-8^{yp}$ may have been introduced to Yeong Pung via IR 2061-481 parent. It is of interest that Nekken 2 retained two alleles from Ketan Nangka, i.e., $S-7^{kp}$ and $S-8^{kp}$ though it was developed from a cross in which Ketan Nangka was backcrossed by Japonicas three times.

The hybrid sterility in different varietal combinations can be explained by allelic interactions at different loci, at each of which several alleles seem to be differentiated. So far a neutral allele was identified at all the three loci for gamete abortion. Many varieties including Ketan Nangka and its derivatives like Nekken 2 are known to possess $S-5^n$. A Japonica tester variety was found to have neutral alleles $S-7^n$ and $S-8^n$, as well as IR 36. Therefore, breeding of a new line which may possess all the neutral alleles seems to be possible. Yeong Pung will be a good tester for such neutral alleles.

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イネの遠縁交配の雑種不稔を支配する新しい遺伝子座とその複対立遺伝子

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日本型稲とインド型稲の雑種における穂の半稔性は, 多くの場合, 第 6 染色体上の S-5 座における対立遺伝子間の相互作用によることが指摘されてきた. すなわち, 日本型稲のもつ S-5ⁱ と IR 36 などのインド型品種のもつ S-5ⁱ のヘテロ接合体上で, S-5ⁱ をもつ雌性配偶子が致死することに起因する. 一方, Javanica 型品種から不稔緩和遺伝子 S-5ⁿ が発見され, S-5ⁿ/S-5ⁱ および S-5ⁿ/S-5ⁱ の遺伝子型では雑種不稔が生じないことが判明した. S-5ⁿ を利用して日印交配によるハイブリッド品種育成が可能となった.

しかし, 雑種不稔の検定の範囲が拡大するにつれて, 例外が認められた. 第 1 に, インド亜大陸の Aus 群品種の中には, S-5ⁿ を持ちながら, S-5ⁿ を持つ Javanica 品種との雑種で不稔を示す品種がみられた. その不稔の原因となる座位は, 第 7 染色体上の赤米遺伝子 (*Rc*) および *Est-9* と連鎖していることが明らかにされた (YANAGIHARA, *et al.*, 1992b). 第 2 の事例として, 熱研 2 号は, Ketan Nangka (KN) に由来する S-5ⁿ を持つ日本型品種で, 普通のインド型品種とは不稔を示さないが, 韓国のインド型品種「円豊, Yeong Pung」との交雑では顕著な不稔を示した (Table 2). ここでは円豊と熱研 2 号あるいは KN を用いた交雑集団を用いて連鎖分析により決定された新しい雑種不稔遺伝子座を報告する.

まず円豊を含む F₂ および B₁F₁ において標識遺伝子の分離を調査した (Table 3). 円豊/熱研 2 号の F₂ では, S-7 の標識遺伝子 *Est-9* および第 6 染色体の標識遺伝子 *Pox-5* および *Cat-1* の分離が異常であった. 円豊/KN の F₂ でも同様に *Est-9* および *Cat-1* の分離が異常であった. 円豊/KN/KN でも, *Est-9*, *Pox-5* および *Cat-1* の分離が異常であり, とくに *Cat-1* の KN 由来の対放遺伝子の減少が確認された (Table 3). S-5 の標識遺伝子 *Amp-3* については, 円豊/KN の F₂ および円豊/KN/KN では, 異常分離が認められなかった. アキヒカリ/円豊では, *Amp-3* および *Est-2* の分離の異常は有意であったが, *Est-9* および *Pox-5* の分離は正常であった.

以上の結果から, S-5 座に関しては, S-5ⁿ を持つ熱研 2 号および KN に対し円豊を交配した場合に, 配偶子致死がみられず, アキヒカリ/円豊の交配でのみ, S-5 座に関しては, 円豊/熱研 2 号, 円豊/KN および円豊/KN/KN を通じて, S-7 の標識遺伝子 *Est-9* の異常分離が観察された. また, この 3 組合せを通じて, 新たに染色体 6 の *Cat-1* の近くに雌性配偶子の致死に関する遺伝子座があることが推定された.

次に, B₁F₁ アキヒカリ/円豊/アキヒカリあるいはアキヒカリ/円豊/熱研 2 号の F₁ 集団において標識遺伝子型別の稔性分布の差異を調査した. その結果, アキヒカリ/円豊/アキヒカリでは S-5 の標識遺伝子 *Est-2* および *Amp-3* においてのみ遺伝子型別の稔性の分布に有意な差異がみられた (Table 4). 円豊/アキヒカリ F₂ で見られた *Cat-1* 座の異常分離は, B₁F₁ では見られなかった. アキヒカリ/円豊/熱研 2 号では S-5 の標識遺伝子 *Est-2* および *Amp-3* の遺伝子型別の稔性の分布に有意な差異は認められず, S-7 の標識遺伝子 *Est-9* および第 6 染色体の標識遺伝子 *Pox-5* および *Cat-1* の遺伝子型の差異と稔性の分離との間に有意な関係が認められた (Table 5). この結果から, 新しい不稔遺伝子座は第 6 染色体上の標識遺伝子 *Pox-5* と *Cat-1* の近傍に座上していることが示された. 標識遺伝子とこの新しい不稔遺伝子座との組換え価は, 稔性分布が連続的で組換え個体の判定が困難であったが, Table 4 および 5 に基づいて一応試算した (Fig. 1). 稲の遠縁雑種における不稔遺伝子座としてはすでに S-7 まで報告されているので, この新しい不稔遺伝子座は S-8 と呼称することにした.

この新しい遺伝子座では, 熱研 2 号と KN は S-8^{kn} を, 円豊は S-8^{yp} の対立遺伝子を持ち, S-8^{kn}/S-8^{yp} の遺伝子型では, S-8^{kn} を持つ雌性配偶子が致死となり, 半不稔を示す. 一方, 日本型の品種アキヒカリは, 円豊との交配では, S-5 座においては, S-5ⁱ/S-5ⁱ による不稔を示すが, S-8 座の標識遺伝子の分離は正常であり, KN との交配でも正常稔性を示すから, 中立遺伝子 S-8ⁿ を持つことが推定された. また *Est-9* を標識とする S-7 座で, KN/円豊の雑種は配偶子致死を示すが, アキヒカリは, 円豊との雑種で S-7 の近傍での分離の歪みを示さず, KN との雑種でも正常稔性を示すから, S-7ⁿ をもつものと推定された. S-5, S-7 および S-8 それぞれにおいて, 中立遺伝子が示されたことから, 3 種の中立遺伝子を集積した品種の育成の可能性が示された.