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Two new loci for hybrid sterility in cultivated rice (*Oryza sativa* L.)

Abstract Female gamete abortion in Indica-Japonica crosses of rice was earlier identified to be due to an allelic interaction at the S-5 locus on chromosome 6. Recently, in other crosses of rice, similar allelic interactions were found at loci designated as S-7 and S-8, located on chromosomes 7 and 6 respectively. All of them are independent of each other. At the S-5 locus, Indica and Japonica rice have S-5^i and S-5^j alleles respectively and Javanicas, such as Ketan Nangka, have a neutral allele S-5^n. The S-$5^i/S$-5^j genotype is semi-sterile due to partial abortion of female gametes carrying S-5^j, but both the S-$5^n/S$-5^i and S-$5^n/S$-5^j genotypes are fertile. The S-5^n allele is thus a "wide-compatibility gene" (WCG), and parents homozygous for this allele are called wide-compatible varieties (WCV). Such parents when crossed with Indica or Japonica varieties do not show F_1 hybrid sterility. Wide-compatible parents have been used to overcome sterility barriers in crosses between Indica and Japonica rice. However, a Javanica variety, Ketan Nangka (WCV), showed typical hybrid sterility when crossed to the Indian varieties N22 and Jaya. Further, Dular, another WCV from India, showed typical hybrid sterility when crossed to an IRRI line, IR2061-628-1-6-4-3(IR2061-628). By genetic analyses using isozyme markers, a new locus causing hybrid sterility in crosses between Ketan Nangka and the Indicas was located near isozyme loci *Est-1* and *Mal-1* on chromosome 4, and was designated as S-9. Another new locus for hybrid sterility in the crosses between Dular and the IR2061-628 was identified and was found linked to four isozyme loci, *Sdh-1*, *Pox-2*, *Acp-1* and *Acp-2*, on chromosome 12. It was designated as S-15. On the basis of allelic interac-
tions causing female-gamete abortion, two alleles were found at S-9, S-9^{kn} in Ketan Nangka and S-9^i in N22 and Jaya. In the heterozygote, S-$9^{kn}/S$-9^i, which was semi-sterile, female gametes carrying S-9^{kn} were aborted. The hybrid of Dular and IR2061-628, with a genetic constitution of S-$15^{Du}/S$-15^i, was semi-sterile and the female gametes carrying S-15^{Du} were aborted. A Japonica tester variety, Akihikari, and an Indica variety, IR36, were found to have neutral alleles, S-9^n and S-15^n, at these loci, in addition to S-7^n and at S-7. The accumulation of three neutral alleles into a breeding line should help solve the hybrid sterility problem in wide crosses of rice.

Key words *Oryza sativa* · Hybrid sterility · Gamete abortion · Wide compatibility · Wide cross

Introduction

Hybrid sterility limits the application of wide crosses in plant breeding. A number of genetic mechanisms leading to hybrid sterility have been reported. Of these, hybrid sterility due to gamete abortion in heterozygotes is not widely known in cultivated plants. Female-gamete abortion due to an allelic interaction was first reported in distant crosses of rice by Kitamura (1962). Gamete abortion by an allelic interaction was also found in tomato (Rick 1966). Since then gametocidal genes have been reported in several plant species (reviewed by Endo 1990).

The semi-sterility of F_1 hybrids between the Indica and Japonica varieties of rice has been attributed to an allelic interaction at the S-5 locus, where Indica and Japonica varieties have S-5^i and S-5^j, respectively, and some Javanica varieties have a neutral allele, S-5^n. The S-$5^i/S$-5^j genotype produces semi-sterile panicles due to the abortion of female gametes carrying S-5^j. Such an abortion does not occur in S-$5^n/S$-5^i and S-$5^n/S$-5^j genotypes. The parent homozygous for S-5^n is called a wide-compatible variety (WCV) (Ikehashi and Araki 1986). Recently, an RFLP marker RG213 on chromo-

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some 6 was found to be closely linked to the *S-5* locus and is being used to identify wide-compatible lines (Zheng 1992; Yanagihara et al. 1995). This *S-5ⁿ* has been incorporated into Indica and Japonica varieties to overcome the problem of sterility in wide crosses and hybrid rice breeding (Araki et al. 1988; Ikehashi 1991). The *S-5ⁿ* allele has been effective in a large number of Indica-Japonica crosses to produce fertile hybrids. In the varietal screening program, some hybrids showed semi-sterility despite the presence of *S-5ⁿ* in one of the parents (Ikehashi et al. 1991). Female gamete abortion in hybrids between Aus (summer rice in the Indian Subcontinent) and some Javanica varieties was found to be caused by allelic interaction at the *S-7* locus on chromosome 7 (Yanagihara et al. 1992). Another locus, *S-8*, for female gamete abortion in a hybrid between a Korean Indica variety and some Javanica varieties was identified, linked to two isozyme loci, *Cat-1* and *Pox-5* on chromosome 6 (Wan et al. 1993).

In the present paper we report two additional loci for hybrid sterility in cultivated rice. All of these loci are found to be independent of each other.

Materials and methods

Plant material

Varieties used for genetic analyses and their marker genotypes are shown in Table 1. Dular and N22 belong to the Aus group or summer rices grown in the Bengal region of the Indian Subcontinent. Jaya is an Indian variety developed from a cross of TN1/T141. IR36, IR50 and IR2061-628-1-6-4-3 (IR2061-628) are Indica varieties developed at the International Rice Research Institute (IRRI). Akihikari, Akamai I and FL 123 are Japonica tester lines. Ketan Nangka is a Javanica variety from Indonesia. Of these, N22 and Dular are known

to be wide-compatible varieties (WCV) like Ketan Nangka. They produce fertile hybrids when crossed with many of the IRRI varieties and breeding lines, as well as with the Japonicas (Table 2).

Additional varieties examined to determine F_1 hybrid sterility are shown in Table 2. Of these, Ingra is an Aus variety; CPSL017 is a breeding line which is wide-compatible like Ketan Nangka; Banten is a Javanica variety and Yeong Pung is a Korean Indica variety developed from a cross of Milyang 23 × 2/IR2061-481. All of them are candidates for constructing a system to detect allelic differences at hybrid sterility loci (S-loci).

Genetic analysis using marker genes

According to the method for analyzing one-locus allelic interaction, which causes F_1 semi-sterility (Ikehashi and Araki 1986), a series of three-way crosses (A/B//C) were prepared after confirming that a hybrid A/C produced semi-sterile panicles and the hybrid B/C was fertile. The progeny of A/B//C would thus be composed of semi-sterile plants, expected from A/C and fertile ones, expected from B/C, in a ratio of 1:1. When a backcross A/C//C was studied, the progeny resulted in the semi-sterile plants expected from A/C and the fertile ones from C/C in a ratio of 1:1. If any genetic markers co-segregate with semi-sterility, a locus for the semi-sterility will be indicated by such markers.

Three-variety crosses or backcrosses were studied to confirm the 1:1 segregation of fertile versus semi-sterile plants: Akamai I/N22//Ketan Nangka and N22/Ketan Nangka//Ketan Nangka (Table 3), Jaya/Ketan Nangka//Ketan Nangka, and Akihikari/Jaya//Akihikari (Table 4), IR36/Dular//IR2061-628 and Akihikari/IR2061-628//Akihikari (Table 5).

The spikelet fertility was determined by counting fertile and sterile spikelets on the upper half of 3–4 panicles for each plant.

Test for distorted segregation

To detect distorted segregation of marker genes, which may indicate female gamete abortion, isozyme markers were studied in progenies of backcross populations. The backcrosses were made with F_1 plants as female so that any segregation distortion from a 1:1 ratio may indicate abortion of female gametes.

Table 1 Rice varieties used in the present study and their marker alleles at respective loci[a]

Parental varieties	Marker gene and chromosome											
	Chrom.6		Chrom.7		Chrom.4					Chrom.12			
	Est-2 *S-5*	*Cat-1* *S-8*	*Est-9* (*S-7*)	*Rc*	*Ph* [*S-9(t)*]	*lg*	*Est-1*	*Mal-1*	*Sdh-1* [*S-15(t)*][b]	*Pox-2*	*Acp-1*	*Acp-2*
N22	1	2	1	−	−	+	1	2	1	0	1	0
Jaya	2	1	2	−	+	+	1	2	1	1	1	1
Ketan Nangka	1	2	1	−	−	+	0	1	2	1	2	0
Banten	0	2	1	−	−	+	0	1	2	0	2	0
Akamai I	0	2	1	+	−	+	0	1	2	0	2	0
IR2061-628	2	1	2	−	+	+	1	2	1	1	1	1
Dular	1	2	1	+	−	+	0	1	2	0	2	0
IR36	2	1	2	−	+	+	1	2	1	1	1	1
Akihikari	0	2	1	−	−	+	0	1	2	0	2	1
IR50	2	1	2	−	+	+	1	2	1	1	1	1
FL123	0	2	1	−	−	−	0	1	2	0	2	0

[a] + and − denote dominant and recessive alleles, respectively, while the isozyme allele systems are quoted from Morishima and Glaszmann (1991)

[b] Three hybrid sterility loci and two new loci are shown under the marker loci

Table 2 Spikele fertility in various F_1 hybrids

Crosses	Mean %	Fertility[a]	Site and year of testing	
N22/Ketan Nangka	48.2	S	Tukuba	(1992)
N22/CPSL017	88.3	F	Tukuba	(1992)
N22/IR36	82.3	F	Tukuba	(1992)
N22/Akihikari	91.6	F	Tukuba	(1992)
N22/Ingra	91.5	F	Nanjing	(1993)
N22/Yeong Pung	87.6	F	Nanjing	(1993)
N22/Dular	87.8	F	Nanjing	(1993)
Akamai 1/N22	87.6	F	Takuba	(1993)
Banten/N22	92.3	F	Takuba	(1992)
Jaya/Ketan Nangka	38.6	S	Tukuba	(1992)
Jaya/Akihikari	46.3	S	Tukuba	(1992)
Jaya/IR36	92.6	F	Tukuba	(1992)
Jaya/Ingra	91.8	F	Nanjing	(1993)
Jaya/N22	90.8	F	Nanjing	(1993)
Jaya/Yeong pung	92.3	F	Nanjing	(1993)
Jaya/Dular	93.6	F	Nanjing	(1993)
IR2061-628/Dular	35.7	S	Tukuba	(1992)
IR2061-628/Akihikari	47.8	S	Tukuba	(1992)
IR2061-628/IR36	88.3	F	Tukuba	(1992)
IR2061-628/Ketan Nangka	91.2	F	Tukuba	(1992)
IR2061-628/Ingra	92.3	F	Nanjing	(1993)
IR2061-628/Yeong Pung	89.6	F	Nanjing	(1993)
IR2061-628/N22	87.8	F	Nanjing	(1993)
IR2061-628/Jaya	92.3	F	Nanjing	(1993)
Dular/IR36	86.7	F	Tukuba	(1992)
Dular/Akihikari	88.6	F	Tukuba	(1992)
Dular/Ingra	89.6	F	Nanjing	(1993)
Dular/Yeong Pung	93.2	F	Nanjing	(1993)
Ketan Nangka/Dular	90.3	F	Nanjing	(1993)
CPSL017/Ketan Nangka	60.3	S	Nanjing	(1993)
Akamai 1/Ketan Nangka	91.2	F	Tukuba	(1992)
Banten/Ketan Nangka	91.7	F	Tukuba	(1992)
Banten/Dular	87.6	F	Tukuba	(1992)
Banten/Akihikari	93.8	F	Tukuba	(1992)
Banten/Yeong Pung	42.5	S	Nanjing	(1993)
Banten/N22	86.7	F	Nanjing	(1993)
Banten/IR2061-628	43.6	S	Kyoto	(1994)

[a] S and F denote semi-sterility and hybrid fertility, respectively

Table 3 Distribution of spikelet fertility classified by marker genotype in Akamai I/N22//Ketan Nangka and N22/Ketan Nangka//Ketan Nangka. Numbers underlined are assumed recombinants

Genotype	Number of plants in % spikelet fertility class										Total	Mean (%)
	10	20	30	40	50	60	70	80	90	100		
Akamai I/N22//Ketan Nangka												
Rc^+/Rc^-	0	0	0	0	_3_	_2_	11	10	7	5	38	72.5**
Rc^-/Rc^-	2	1	2	3	4	2	8	_7_	_5_	_3_	37	56.3
$Mal-1^1/Mal-1^1$	_1_	_1_	0	_1_	_2_	_1_	10	11	6	5	38	70.1**
$Mal-1^2/Mal-1^1$	1	0	2	2	5	3	9	_6_	_6_	_3_	37	58.8
$Est-1^0/Est-1^0$	0	0	0	0	_1_	_2_	11	10	9	7	40	75.1**
$Est-1^1/Est-1^0$	2	1	2	3	6	2	8	_7_	_3_	_1_	35	54.3
N22/Ketan Nangka//Ketan Nangka												
$Est-1^1/Est-1^0$	0	2	6	14	19	18	_4_	_3_	_5_	_1_	72**	48.3**
$Est-1^0/Est-1^0$	0	0	_1_	_2_	0	_3_	3	7	6	6	28	67.3
$Mal-1^2/Mal-1^1$	0	2	5	12	17	17	_4_	_3_	_6_	_2_	68**	51.2**
$Mal-1^1/Mal-1^1$	0	0	_2_	_4_	_2_	_4_	3	7	5	5	32	65.5

** Shows significant difference between two genotypes at 1% (t test)

Table 4 Distribution of spikelet fertility classified by marker genotype in Jaya/Ketan Nangka//Ketan Nangka and Akihikari/Jaya//Akihikari. Numbers underlined are assumed recombinants

Genotype	Number of plants in % spikelet fertility class										Total	Mean (%)
	10	20	30	40	50	60	70	80	90	100		
Jaya/Ketan Nangka//Ketan Nangka												
$Est\text{-}9^1/Est\text{-}9^1$	0	0	0	2	1	0	7	9	23	12	54**	77.6**
$Est\text{-}9^2/Est\text{-}9^1$	0	1	6	22	20	16	6	7	8	2	88	50.1
$Est\text{-}1^0/Est\text{-}1^0$	0	0	0	0	0	1	8	11	22	11	53**	78.9**
$Est\text{-}1^1/Est\text{-}1^0$	0	1	6	24	21	15	5	5	9	3	89	49.8
$Mal\text{-}1^1/Mal\text{-}1^1$	0	0	0	1	6	1	9	8	22	11	56*	76.3**
$Mal\text{-}1^2/Mal\text{-}1^1$	0	1	6	23	15	15	4	8	9	3	86	53.5
$Est\text{-}2^1/Est\text{-}2^1$	0	0	2	13	10	9	5	9	16	8	72	61.3
$Est\text{-}2^2/Est\text{-}2^1$	0	1	4	11	11	7	8	7	15	6	70	60.4
$Cat\text{-}1^2/Cat\text{-}1^2$	0	0	3	13	12	7	6	9	17	5	72	60.0
$Cat\text{-}1^1/Cat\text{-}1^2$	0	1	3	11	9	9	7	7	14	9	70	61.6
Akihikari/Jaya//Akihikari												
$Est\text{-}2^0/Est\text{-}2^0$	0	0	0	1	2	5	9	12	10	8	47*	76.2**
$Est\text{-}2^2/Est\text{-}2^0$	0	1	6	15	17	12	5	6	4	6	72	51.6
$Est\text{-}9^1/Est\text{-}9^1$	0	0	2	9	9	9	6	8	8	8	59	63.2
$Est\text{-}9^2/Est\text{-}9^1$	0	1	4	7	10	8	8	10	6	6	60	61.8
$Cat\text{-}1^2/Cat\text{-}1^2$	0	0	3	10	8	9	6	9	6	6	57	60.3
$Cat\text{-}1^1/Cat\text{-}1^2$	0	1	3	6	11	8	8	9	8	8	62	64.6
$Est\text{-}1^0/Est\text{-}1^0$	0	1	2	7	8	8	6	10	7	9	58	65.1
$Est\text{-}1^1/Est\text{-}1^0$	0	0	4	9	11	9	8	8	7	5	61	59.8
$Sdh\text{-}1^2/Sdh\text{-}1^2$	0	0	2	8	10	7	7	9	8	8	59	62.3
$Sdh\text{-}1^1/Sdh\text{-}1^2$	0	1	4	8	9	10	7	9	6	6	60	60.8

** Shows significant difference between two genotypes at 1% (*t* test)

Table 5 Distribution of spikelet fertility classified by marker genotype in IR36/Dular//IR2061-628 and Akihikari/IR2061-628//Akihikari. Numbers underlined are assumed recombinants

Genotype	Number of plants in % spikelet fertility class										Total	Mean (%)
	10	20	30	40	50	60	70	80	90	100		
IR36/Dular//IR2061-628												
$Sdh\text{-}1^1/Sdh\text{-}1^1$	0	1	1	5	6	4	6	8	24	12	67	70.7**
$Sdh\text{-}1^2/Sdh\text{-}1^1$	0	0	6	12	13	5	8	8	5	1	58	53.6
$Pox\text{-}2^1/Pox\text{-}2^1$	0	0	0	2	1	0	13	13	23	12	64	79.5**
$Pox\text{-}2^0/Pox\text{-}2^1$	0	1	7	15	18	9	1	3	6	1	61	49.3
$Acp\text{-}1^1/Acp\text{-}1^1$	0	0	0	2	2	0	13	11	23	10	61	78.6**
$Acp\text{-}1^2/Acp\text{-}1^1$	0	1	7	15	17	9	1	5	6	3	64	50.3
$Acp\text{-}2^1/Acp\text{-}2^1$	0	0	0	2	2	1	13	10	24	8	60	78.1**
$Acp\text{-}2^0/Acp\text{-}2^1$	0	1	7	15	17	8	1	6	5	5	65	51.6
$Est\text{-}2^2/Est\text{-}2^2$	0	0	4	7	11	4	8	9	14	8	65	63.2
$Est\text{-}2^1/Est\text{-}2^2$	0	1	3	10	8	5	6	7	15	5	60	61.6
$Est\text{-}9^2/Est\text{-}9^2$	0	0	3	9	10	4	8	8	15	6	63	64.8
$Est\text{-}9^1/Est\text{-}9^2$	0	1	4	8	9	5	6	8	14	7	62	57.8
$Cat\text{-}1^1/Cat\text{-}1^1$	0	0	4	9	10	3	7	9	13	8	63	62.7
$Cat\text{-}1^2/Cat\text{-}1^1$	0	1	3	8	9	6	7	7	16	5	62	62.0
$Est\text{-}1^1/Est\text{-}1^1$	0	0	4	9	9	3	8	8	14	7	62	62.8
$Est\text{-}1^0/Est\text{-}1^1$	0	1	3	8	10	6	6	8	15	6	63	61.9
Akihikari/IR2061-628//Akihikari												
$Est\text{-}2^0/Est\text{-}2^0$	0	0	0	0	2	0	1	7	6	10	26**	76.8**
$Est\text{-}2^2/Est\text{-}2^0$	1	0	7	7	6	14	7	7	2	1	52	52.6
$Est\text{-}9^1/Est\text{-}9^1$	0	0	3	4	3	6	5	7	5	6	39	63.8
$Est\text{-}9^1/Est\text{-}9^1$	1	0	4	3	5	8	3	7	3	5	39	61.2
$Cat\text{-}1^2/Cat\text{-}1^2$	0	0	3	3	5	6	4	8	5	6	40	64.1
$Cat\text{-}1^1/Cat\text{-}1^2$	1	0	4	4	3	8	4	6	3	5	38	60.8
$Est\text{-}1^0/Est\text{-}1^0$	0	0	3	4	4	6	5	6	5	5	38	62.1
$Est\text{-}1^1/Est\text{-}1^0$	1	0	4	3	4	8	3	8	3	6	40	62.6
$Sdh\text{-}1^2/Sdh\text{-}1^2$	0	0	3	3	5	9	3	9	6	5	43	64.2
$Sdh\text{-}1^1/Sdh\text{-}1^2$	1	0	4	4	3	5	5	5	2	6	35	61.3

** Shows significant difference between two genotypes at 1% (*t*-test)

Marker genes and linkage analyses

Genetic markers tested for segregation are listed in Table 1. Of these markers, *Est-2* and *Cat-1* test segregation distortion at the *S-5* and *S-8* loci, respectively (Yanagihara et al. 1995; Wan et al. 1993). *Est-9* and *Rc* test segregation distortion at the *S-7* locus (Yanagihara et al. 1992). *Ph* and *lg*, which are linked with a recombination frequency of 22%, are located on chromosome 4 (Kinoshita 1993). Three pairs of markers, i.e. *Sdh-1* and *Acp-1*, *Sdh-1* and *Pox-2*, and *Acp-1* and *Pox-2*, are linked with recombination frequencies of 35.6%, 16.6% and 23.9%, respectively, on chromosome 12 (Ishikawa et al. 1992). The isozyme analyses were carried out following the methods of Ishikawa et al. (1989) and Glaszmann et al. (1988).

To determine associations between isozyme loci and spikelet fertility, B_1F_1 populations of Jaya/Ketan Nangka//Ketan Nangka, N22/Ketan Nangka//Ketan Nangka, Akihikari/Jaya//Akihikari and Akihikari/IR 2061-628//Akihikari were grown at Nanjing Agriculture University in 1993, and F_1 populations from the three-way crosses, i.e. Akamai I/N22//Ketan Nangka, IR36/Dular//IR2061-628, and an F_2 of IR50/FL123, were grown at The National Agriculture Research Center, Tsukuba, in 1992 and 1993.

Estimation of allelic differences at new loci

Allelic differences at a new locus were estimated following the model of allelic interaction at *S-5* where, in *S-5^i/S-5^j* hybrids, female gametes carrying *S-5^j* abort, but no abortion occurs in *S-5^n/S-5^i* and *S-5^n/S-5^j* hybrids. Following this model we can determine the allelic constitution at any S locus. For three given varieties, A, B and N, if a hybrid A/B shows gamete abortion, but N/A and N/B do not show any sterility, the variety N possesses a neutral allele at the new locus. A modified rule can be given as follows: when N/A or N/B show a degree of sterility but give normal segregation of markers linked to the locus, then N may possess a neutral allele. This test was applied to the crosses in this experiment to estimate the allelic constitution at new loci.

Results

A new locus for semi-sterility in hybrids between N22 and Ketan Nangka and between Jaya and Ketan Nangka

In the progeny of Akamai 1/N22//Ketan Nangka and N22/Ketan Nangka//Ketan Nangka (Table 3), the level of spikelet fertility was significantly differentiated by marker genotypes at the *Est-1* and *Mal-1* loci. The lowerd fertility of the heterozygote for *Est-1* and *Mal-1* can be attributed to an allelic interaction at a new locus for sterility located near these markers, which is hereafter designated as *S-9(t)*. The lowered fertility of the homozygotes at the *Rc* locus in the cross Akamai I/N22//Ketan Nangka can be explained by allelic interaction at the *S-7* locus.

Similarly, in the progeny of Jaya/Ketan Nangka//Ketan Nangka (Table 4), the level of spikelet fertility was significantly correlated with distortion at marker loci *Est-1* and *Mal-1*, indicating allelic interaction at the new locus *S-9(t)*. The lowered fertility of the heterozygote for *Est-9* can be explained by the allelic interaction at *S-7*. The fact that the fertility level was not correlated with the markers *Est-2* and *Cat-1* indicated that there was no

allelic interaction at *S-5* and *S-8*. In the progeny of Akihikari/Jaya//Akihikari (Table 4) the level of spikelet fertility was correlated with the marker genotypes at the *Est-2* locus. This indicates that the semi-sterility in the F_1 of Akihikari/Jaya (Table 2) is caused by allelic interaction only at *S-5* among the known loci.

A new locus for semi-sterility of the hybrid between IR2061-628 and Dular

In the progeny of IR36/Dular//IR2061-628, the level of spikelet fertility was significantly correlated with marker genotypes at *Sdh-1*, *Pox-2*, *Acp1* and *Acp-2* (Table 5). The lowered fertility of the heterozygotes for these four loci was assumed to be caused by an allelic interaction at a new locus linked with these markers, hereafter designated as *S-15(t)*. Intervening gene symbols *S-10* to *S-14* were recently assigned to loci for sterility in hybrids between a cultivated rice and wild *Oryza longistaminata* (Sano et al. 1992; Kinoshita 1993; Sano 1994). The fact that the fertility level was not correlated with the genotypes at *Est-2*, *Est-9*, *Cat-1* and *Est-1* indicated that allelic interaction at *S-5*, *S-7*, *S-8* and *S-9(t)* was not responsible for semi-sterility in the hybrid between Dular/IR2061-628. In the progeny of Akihikari/IR2061-628//Akihikari (Table 5) the level of spikelet fertility was correlated with the marker genotypes at *Est-2*, indicating that the semi-sterility in the F_1 of IR2061-628/Akihikari was caused by the allelic interaction at *S-5*.

Mapping of *S-9(t)*

As mentioned above, the progeny of Akamai 1/N22//Ketan Nangka (Table 3) indicated a new locus *S-9(t)* for semi-sterility, situated near the markers *Est-1* and *Mal-1*. This gene also caused semi-sterility in the cross of Jaya/Ketan Nangka//Ketan Nangka (Table 4). If the alleles of marker genes co-segregate completely with the alleles at *S-9(t)*, each of the marker alleles would only co-segregate with fertile or semi-sterile plants. However, there were a few plants which showed semi-sterility, but had a marker allele of high fertility, or normal fertility, with a marker allele of low fertility. Such plants were the result of recombination between the marker genes and the gene for semi-sterility, although there might have been interference by the *S-7* locus. These assumed recombinants between the *S-9(t)* and the markers are underlined in Tables 3 and 4; such recombinants could not be clearly identified due to the continuous variation in spikelet fertility.

In addition, 86 F_2 plants of IR50/FL123 cross were tested to obtain segregation ratios for *Ph*, *lg*, *Est-1* and *Mal-1*. The estimated recombination frequencies are shown in Fig. 1. Thus the recombination frequency between *S-9(t)* and *Est-1* was estimated to be 16.2–18.6%, and that between *S-9(t)* and *Mal-1* was
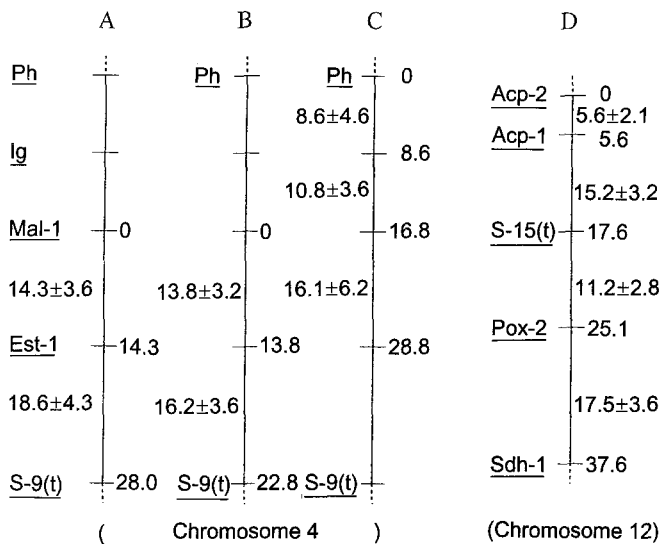
Fig. 1 Linkage map of isozyme markers on chromosomes 4 and 12, including the estimated position of S-9(t) and S-15(t), based on the data from Akamai 1/N22//Ketan Nangka (A), Jaya/Ketan Nangka//Ketan-Nangka (B), IR50/FL123 F$_2$ (C) and IR36/Dular//IR2061-628(D)

Table 6 Segregation of marker genotypes in IR2061-628/Dular//Dular

Locus	II	H	JJ[a]
Est-2		48	52
Est-9		49	51
Cat-1		47	53
Est-1		52	48
Sdh-1		31	69**
Acp-1		20	80**
Acp-2		23	77**

** Significant deviation from 1:1 ratio at 1%
[a] J: allele from K.N. or Dular
I: allele from IR line, N22 or Jaya
H: heterozygote

22.8–28.0%, while the recombination frequency between *Est-1* and *Mal-1* was calculated to be 13.8%–16.1%. The loci for *Est-1* and *Mal-1* were assigned to chromosome 4 by Guiderdoni et al. (1989), but they did not report on recombination values.

Mapping of S-15(t)

The recombination values between *S-15(t)* and the marker loci *Sdh-1, Pox-2, Acp-1* and *Acp-2* were calculated from the progeny of IR36/Dular//IR2061-628. The assumed recombinants are underlined in Table 5 and the recombination frequencies are given in Fig. 1. As shown in this figure *S-15(t)* is located between *Acp-1* and *Pox-2*. Previously reported recombination frequencies were: 16.6% between *Sdh-1* and *Pox-2*, 35.6% between *Sdh-1* and *Acp-1*, and 23.9% between *Acp-1* and *Pox-2* (Ishikawa et al. 1992). Thus, the present estimates are in good agreement with those reported earlier.

Segregation distortion of markers due to S-5, S-7, S-9(t) and S-15(t)

In the cross of Akihikari/Jaya//Akihikari (Table 4) and Akihikari/IR2061-628//Akihikari (Table 5), segregation distortion at the *Est-2* locus was significant, indicating the known allelic interactions at *S-5*. In the cross of N22/Ketan Nangka//Ketan Nangka (Table 3), Jaya/Ketan Nangka//Ketan Nangka (Table 4), and IR2061-628/Dular//Dular (Table 6) there was normal segregation of *Est-2*, suggesting that the semi-sterility in these crosses was not due to *S-5*. The distortion of alleles

at *Est-9* found in Jaya/Ketan Nangka//Ketan Nangka (Table 4) indicated that the allelic interaction causing the abortion of female gametes possessing the Japonica-type allele from Ketan Nangka occurred at *S-7*, as *Est-9* is a marker for *S-7* (Yanagihara et al. 1992).

In the progeny of N22/Ketan Nangka//Ketan Nangka (Table 3) and Jaya/Ketan Nangka//Ketan Nangka (Table 4), a significant reduction of Japonica-type alleles from Ketan Nangka at the *Est-1* and *Mal-1* loci confirmed that female gametes possessing the Japonica-type allele were partially aborted by an allelic interaction at the new locus, *S-9(t)*.

In the backcross of IR2061-628/Dular//Dular (Table 6), the significant reduction in the frequency of heterozygotes at the *Sdh-1, Acp-1* and *Acp-2* loci confirmed that female gametes possessing the indica-type allele were partially aborted by an allelic interaction at the new locus, *S-15(t)*, which is located near *Sdh-1, Acp-1* and *Acp-2*.

Determination of allelic differences at S-9(t) and S-15(t)

As stated earlier, for three given varieties, A, B and N, if the hybrid A/B shows gamete abortion by allelic interaction between *S-X^a* and *S-X^b* at the locus *S-X*, while in the hybrids N/A and N/B there is no such allelic interaction, the variety N may possess a neutral allele *S-X^n*.

The reduction of the Japonica-type allele at *S-9* in Jaya/Ketan Nangka//Ketan Nangka and N22/Ketan Nangka//Ketan Nangka suggested that female gametes possessing the Japonica-type allele were aborted by an allelic interaction at this locus. Therefore, the two interacting alleles are designated as *S-9^{kn}* (Ketan Nangka) and *S-9^i* (Jaya and N22). At *S-9*, a portion of female gametes carrying *S-9^{kn}* were aborted in the heterozygote of *S-9^{kn}/S-9^i*.

In the B$_1$F$_1$ of Akihikari/Jaya//Akihikari, there was no allelic interaction at *S-9* as indicated by the normal segregation of alleles at *Est-1* and *Mal-1*. The F$_1$ hybrid Akihikari/Ketan Nangka was fertile (Ikahashi and Araki 1986). Therefore, Akihikari is considered to possess a neutral allele, *S-9^n*. Similarly, IR2061-628, IR36

and Dular showed normal fertility in their crosses with Ketan Nangka, and may also possess $S-9^n$ (Table 7). For the remaining varieties the allelic constitution at this locus is not known.

Reduction of the Indica-type allele at $S-15$ in the cross IR2061-628/Dular//Dular suggested that female gametes possessing the Indica-type allele were aborted by an allelic interaction at $S-15$ (Table 6). Therefore, two interacting alleles, $S-15^{Du}$ in Dular and $S-15^i$ in IR2061-628, can be designated. A portion of female gametes carrying $S-15^{Du}$ were aborted in the heterozygote $S-15^{Du}/S-15^i$. The F_1 hybrid of Akihikari/Dular was fertile (Table 2) and the B_1F_1 Akihikari/IR2061-628//Akihikari showed normal segregation of alleles at $Sdh-1$, $Acp-1$ and $Pox-2$ (Table 4). Therefore, Akihikari is considered to possess a neutral allele, $S-15^n$. Similarly, those varieties which showed normal fertility in their crosses with both IR2061-628 and Dular may possess $S-9^n$. Thus, Jaya, N22, Ketan Nangka and IR36 are donors of $S-15^n$, as summarized in Table 7 where allelic differentiations at other loci are also listed.

Discussion

An allelic interaction leading to hybrid sterility in rice was first reported at the $S-5$ locus on chromosome 6 (Ikehashi and Araki 1986), where the $S-5^i/S-5^j$ genotype produces semi-sterile panicles due to partial abortion of female gametes carrying $S-5^j$, whereas $S-5^n/S-5^i$ and $S-5^n/S-5^j$ do not. Since then, the $S-5^n$ allele has been incorporated into breeding lines to utilize the heterosis of Indica-Japonica hybrids in hybrid rice breeding (Ikehashi 1991; Yuan 1992; Zou et al. 1992).

In the utilization of the $S-5^n$ allele in breeding, however, varieties possessing this allele showed sterility in their crosses to a number of varieties, especially those from India. These results indicated that there may be additional alleles at $S-5$, or additional loci, for hybrid sterility. Through genetic analyses of such exceptional cases, two additional loci for hybrid sterility were identified. Yanagihara et al. (1992) reported that the sterility of the hybrid between a cultivar Ingra of the Aus group from India and a wide compatible cultivar CPSL017 was controlled by the $S-7$ locus located near $Est-9$ and Rc on chromosome 7. Wan et al. (1993) showed that the sterility of the hybrid between a Korean Indica variety, Yeong Pung, and Ketan Nangka was

independently controlled by two segregating genes $S-7$ and $S-8$. The latter was located near $Cat-1$ and $Pox-5$ on chromosome 6.

In this paper two more loci for hybrid sterility are identified. The sterility of hybrids between Jaya and N22, on one hand, and Ketan Nangka, on the other, was found to be controlled by $S-9$, located near $Mal-1$ and $Est-1$ on chromosome 4. The alleles of $S-9$ were shown to be differentiated into $S-9^{kn}$ in Ketan Nangka, $S-9^i$ in N22 and Jaya, and $S-9^n$ in the Japonica cultivar Akihikari. A hybrid between a wide-compatible variety, Dular, and IR2061-628-1 was semi-sterile, and the sterility was caused by allelic interaction at $S-15$, which is located near $Sdh-1$, $Pox-2$, $Acp-1$ and $Acp-2$ on chromosome 12. The alleles of $S-15$ were found to be differentiated into $S-15^i$ in IR2061-628-1, $S-15^{Du}$ in Dular, and $S-15^n$ in Japonica cultivars.

It is interesting to note that the sterility causing alleles at two loci, $S-8$ and $S-15$, were contributed by two sister lines IR2061-481-2 and IR2061-628-1, respectively. But most of the other sister lines of IR2061 showed normal fertility in their crosses with Dular, Ketan Nangka, Nekken 2, CPSL017 and IR36. To develop a tester system for hybrid sterility it is necessary to determine which donor variety among the parents of IR2061 contributed such sterility causing alleles at two loci.

So far, the semi-sterility of hybrids between distantly related cultivars was predominantly due to the interaction of $S-5^i$ and $S-5^j$ at $S-5$. But additional cases of hybrid sterility are found in the hybrids between Aus varieties and Javanicas like Ketan Nangka, as shown in Table 7. Thus hybrid sterility in different varietal combinations is explained by allelic interactions at different loci. In some hybrids, allelic interaction at two independent loci cause hybrid sterility.

A number of varieties, including Ketan Nangka and N22, were initially known to possess $S-5^n$ and were utilized as donors of wide compatibility genes. But, Ketan Nangka was found to possess sterility causing alleles at $S-7$, $S-8$ and $S-9$. N22 has sterility causing alleles at $S-7$ and $S-9$. However, an Aus variety, Dular, was found to have neutral alleles at all four loci, $S-5^n$, $S-7^n$, $S-8^n$ and $S-9^n$. A Japonica tester variety Akihikari was found to have the neutral alleles $S-7^n$, $S-8^n$ and $S-9^n$ and $S-15^n$ (Table 7). Therefore, the initial concept for wide compatibility needs to be modified. Ketan Nangka produces fertile hybrids in its crosses with a great num-

Table 7 Estimated allelic constitution at five S loci in tester varieties

Locus	IR36	Akihikari	Jaya	N22	K.Nangka	IR2061 -628	Dular	Ingra	Yeong pung	CPSLO 17	Banten
$S-5$	i	j	i	n	n	i	n	i	i	n	j
$S-7$	n	n	i	i	kn	n	n	ai	ai	cp	n
$S-8$	n	n	n	n	kn	n	n	n	yp	kn	n
$S-9$	n	n	i	i	kn	n	n	–	–	–	–
$S-15$	n	n	n	n	n	i	du	n	n	n	–

ber of Indica, as well as Japonica, varieties. In this context, this is a wide-compatible variety. But in terms of the number of neutral alleles at different loci this variety may not be the most wide-compatible.

Thus far, a number of loci for hybrid sterility have been identified. But the number of interacting alleles at each S-locus seems to be limited to three, of which one is neutral. Only at S-7 does there seem to be four alleles. It is interesting to note that some varieties, such as Akihikari and IR 36, have neutral alleles at many loci, as shown in Table 7. Therefore, it should be possible to accumulate the neutral alleles at all the loci to breed a line which can be crossed to any variety without resulting in hybrid sterility. To accomplish this a set of testers are needed. The results summarized in Table 7 should prove useful in this respect.

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