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Is an egg-killer present in rice?

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Abstract. The genetic model for hybrid sterility that an allelic interaction at the S_5 locus induces the abortion of megaspores has been proposed as partial clarification of the wide compatibility in Asian rice cultivars; this model predicts the presence of an egg-killer. The present study was carried out in order to confirm that the proposed S_5^i allele acts as an egg-killer against its counterpart, the S_5^j allele, in the Indica-Japonica hybrid. A conspicuous feature of an egg-killer is the high rate of its transmission into the progeny through the egg. Backcrossing experiments were conducted using the Indica-Japonica hybrid in which the S_5^i and S_5^j alleles were assumed to be involved. Although an egg-killer was easily identified by these backcross experiments, it was not detected in the Indica-Japonica hybrid, which suggests that the proposed genetic mechanism for hybrid sterility in Asian rice should be viewed with caution.

Key words: *Oryza sativa* – Indica-Japonica hybrid – Hybrid sterility – Female sterility

Introduction

Hybrid sterility due to allelic interaction was first reported in *Nicotiana* (Cameron and Moav 1957); an alien gene (pollen killer) in *N. plumbaginifolia* caused the degeneration of pollen not carrying it when the chromosome on which it occurred was added to the *N. tabacum* complement. The genetic basis for hybrid sterility between species seems to be complex. Accumulated evidence, however, suggests that genes causing gametic abortion are wide-spread between and within plant species (Sano 1983; Scoles and Kibirge-Sebunya 1983). The majority of the ones detected so far are gametophytic type and have been classified into three types, gamete eliminator, pollen-killer, and egg-killer, depending on the gametes affected. The first two have been frequently detected in plants (Sano 1993), while only one example of an egg-killer has been reported – in a maize-*Tripsacum* hybrid (Maguire 1963). An extra chromosome derived from *Tripsacum* was transmitted to a high proportion of the progeny through the egg. This was explained by assuming the presence of an egg-killer on the extra chromosome that induces the abortion of megaspores not carrying it, although data were limited. Ikehashi and Araki (1986) proposed that an egg-killer is involved in the genetic mechanism of hybrid sterility observed in Asian rice cultivars (*Oryza sativa*). The experiment presented here was carried out to confirm its presence.

Since Kato et al. (1928) observed F_1 sterility barriers between the Indica and Japonica types of Asian rice, a number of studies have been reported on the mechanism of hybrid sterility in varietal crosses of rice (Oka 1988; Morishima et al. 1992). Hybrid sterility due to allelic interaction at a single locus has also been reported in some varietal crosses within *O. sativa* (Kitamura 1962; Oka 1964). A recent discovery was the detection of the wide compatibility gene, S_5^n, which has been recognized as a gene that greatly reduces seed infertility in the Japonica-Indica hybrid (Ikehashi and Araki 1986). These investigators also asserted that there are additional two alleles at the S_5 locus and that an S_5^i allele from the Indica parent induces the abortion of megaspores carrying the other allele (S_5^j from Japonica parent) in the heterozygote ($S_5^iS_5^j$), suggesting that

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the S_5^i allele may act as an egg-killer against S_5^j. This locus has attracted the attention of rice breeders since the hybrid sterility gene might prevail among commonly used breeding materials (Ikehashi and Araki 1984; 1988, Lin et al. 1992). Despite the fact that in the present investigation we found much circumstantial evidence for the existence of an egg-killer, we failed to detect any egg-killer in the Indica-Japonica hybrid.

Materials and methods

Plant materials

The materials used were two rice accessions, IR36 (Indica type) and T65*wx* (a near-isogenic line of 'Taichung 65' with *wx*, Japonica type). 'Taichung 65' (designated T65) and IR36 were assumed to carry the S_5^j and S_5^i alleles, respectively, whose allelic interaction induces the abortion of megaspores (Ikehashi and Araki 1986). T65*wx* has *wx* (glutinous endosperm) from 'Kinoshita-mochi' (Japonica type) and is a derivative from BC_{14} plants (Oka 1974). The *wx* locus is located on chromosome 6 and loosely linked with S_5^j (about 25% in recombination value) according to Ikehashi and Araki (1988). As T65*wx* showed semi-sterility in the cross with IR36 as did T65, the genotype of T65*wx* was assumed to be the same as that of T65 except for the *wx* locus. Two additional marker genes, *C* (chromogen) and *alk* (alkali spreading), are also located on chromosome 6, and the genotypes of T65*wx* and IR36 were expected to be $wx\text{-}C\text{-}S_5^j\text{-}alk$ and $Wx\text{-}C^+\text{-}S_5^i\text{-}alk^+$, respectively (Ikehashi and Araki 1986, 1988). A problem in examining the genetic mechanism of hybrid sterility is that the responsible genes are rather complex and the manner of segregation often becomes obscure. Semi-sterility was also observed in microspores of the F_1 hybrid of T65*wx* × IR36 although the S_5^i/S_5^j genotype was expected to induce the abortion of megaspores only. This caused a low seed setting when the F_1 was backcrossed as the pollen parent. For reasons of simplicity, in this paper only data on seed fertility are presented.

The proposed genetic model

The proposed genetic model is that the S_5^i allele induces abortion of megaspores carrying the S_5^j allele in the heterozygote (S_5^i/S_5^j), whereas the S_5^n allele induces no abortion of megaspores with either of the other alleles (Ikehashi and Araki 1986). All of the homozygotes (S_5^i/S_5^i, S_5^j/S_5^j and S_5^n/S_5^n) are fertile so that semi-sterility occurs only in the hybrid between S_5^i/S_5^i and S_5^j/S_5^j. 'Ketan Nanka' (Javanica type, designated N), which is called a "wide compatibility variety", carries S_5^n and produces fertile hybrids when crossed with either of the Indica (I) and Japonica (J) types, although the J × I F_1s are generally semi-sterile. Hybrid derivatives with the *wx*, *alk*, and *C* genes from N had a significantly higher seed fertility than plants with the corresponding alleles from I in the cross of (J × N) × I, where J, N, and I were assumed to carry S_5^j, S_5^n, S_5^i, respectively. The result observed in the cross of (J × N) × I showed that a gene(s) responsible for fertility was present on chromosome 6 but not that S_5^i induces the abortion of megaspores with S_5^j in the heterozygote (S_5^i/S_5^j). Accordingly, further evidence is needed to ascertain the presence of an egg-killer in the hybrid.

A test for the presence of an egg-killer

A conspicuous feature of an egg-killer is its high rate of its transmission through the egg (Maguire 1963). Since S_5^i is expected to act as an egg-killer against S_5^j, only S_5^i can be transmitted to the progeny through the egg. As a result, the backcross of (T65*wx* × IR36) F_1 with pollen of T65*wx* (S_5^j/S_5^j) gives only semi-sterile plants in the progeny but the backcross to stigmas of T65*wx* gives fertile and semi-sterile plants in an 1:1 ratio (Fig. 1). Thus, S_5^i is easily maintained in the progeny as long as the heterozygote is maternally backcrossed with T65*wx*. On the other hand, backcrossing of the F_1 with pollen of IR36 gives only fertile (S_5^i/S_5^i) plants since S_5^j is aborted during megasporogenesis. Accordingly, T65*wx* was used as the recurrent parent in the present experiment to confirm a high transmission of S_5^i in the heterozygote (S_5^i/S_5^j) through the egg.

Results

T65*wx* × IR36 F_1 showed 51.3% seed fertility, which was not different from the 54.1% of the T65 × IR36 F_1. The T65*wx* × IR36 F_1 was reciprocally backcrossed with T65*wx* (Table 1). The mean fertilities in the BC_1F_1 were lower when the F_1 was used as the pollen parent (52.5%) rather than as the female parent (71.2%). The numbers of *Wx* and *wx* segregants were almost equal in the BC_1F_1 generation, although the number of *wx* segregants was expected to be less than that of *Wx* segregants when the F_1 was used as the female parent. Regardless of the direction of cross, *wx* segregants tended to show higher seed fertilities than *Wx* segregants (57.5% versus 48.3% and 76.8% versus 64.7%, Table 1). The higher seed fertilities observed in *wx* segregants could not be clarified by S_5^i and S_5^j only because, when backcrossed with pollen of T65*wx*, all of

Table 1. Segregations for seed fertility in the first backcross generation by using T65*wx* as the recurrent parent

Cross		Genotype	Number of plants examined	Seed fertility (%)									Mean
Female	Male			15	25	35	45	55	65	75	85	95	
F_1	T65*wx*	*Wx/wx*	14		1	2	3	4	3		1		48.3
		wx/wx	12		2	1	1	2	2	2	2		57.4
		Total	26		3	3	4	6	5	2	3		52.5
T65*wx*	F_1	*Wx/wx*	6				1	1	1	3			64.7
		wx/wx	7					1		3	3		76.8
		Total	13				1	2	1	6	3		71.2

the progeny was expected to be S_5^i/S_5^j (Fig. 1A). This strongly suggests that a sterility gene(s) different from an egg-killer was present on chromosome 6. However, the manner of segregation for seed fertility seemed to be continuous. From Fig. 1A it was expected S_5^i should be easily maintained in the progeny when the F_1 was backcrossed with pollen of T65*wx*, giving only semi-sterile plants (S_5^i/S_5^j). Unexpectedly, fertile plants frequently segregated in the BC_2F_1, even if semi-sterile BC_1F_1 plants were backcrossed with T65*wx* (Table 2). In each backcross generation, plants were randomly selected for further crossings. The frequency of fertile plants increased as the backcross generation proceeded. This tendency was observed regardless of the direction of cross. In BC_4F_1, most of plants were fairly fertile as was the recurrent parent (Table 2). Thus, the present results indicate that it is hard to maintain S_5^i in the progeny, which is against the expectation from the proposed genetic explanation.

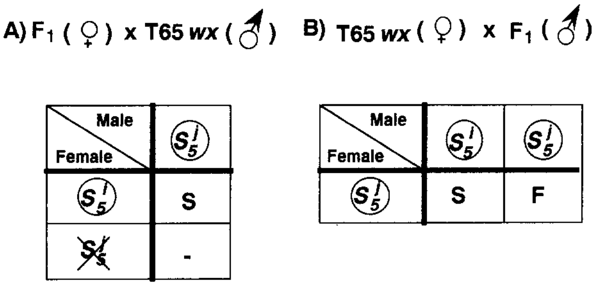

Fig. 1A, B. The mode of inheritance of an egg-killer (S_5^i) when the T65*wx* × IR36 F_1 was used as the female (**A**) and the male (**B**) parents in the backcross. On the basis of Ikehashi and Araki (1986), the genotypes of T65*wx* and IR36 are assumed to be S_5^j/S_5^j and S_5^i/S_5^i, respectively. S_5^i induces the abortion of megaspores carrying S_5^j in the heterozygote (S_5^i/S_5^j). Viable and inviable gametes are indicated by ○ and ×, respectively. Fertile (S_5^j/S_5^j) and semi-sterile (S_5^i/S_5^j) zygotes are indicated by *F* and *S*, respectively

Another expectation from the proposed genetic model is a deviation from a 3:1 ratio for marker genes linked with the S_5^j allele. Since the genotype of the F_1 was assumed to be *wx*-S_5^j/*Wx*-S_5^i, the segregation for *wx* was expected to show a deviation from a 3:1 ratio in the selfed seeds. Semi-sterile plants which were heterozygous for *wx* were examined for segregation distortion. However, the F_1 plants and semi-sterile plants in BC_1F_1 and BC_2F_1 showed a good fitness to a 3:1 ratio (Table 3), indicating that an egg-killer linked with *wx* was not involved in the cross.

Discussion

A conspicuous feature of an egg-killer is its high rate of transmission into the progeny through the egg. Despite the fact that there is much circumstantial evidence for

Table 2. Segregations for seed fertility in the backcross generations by using T65*wx* as the recurrent parent

Generation	Cross		Number of lines tested	Number of plants examined	Seed fertility (%)									Mean
	Female	Male			15	25	35	45	55	65	75	85	95	
BC_2F_1	BC_1F_1	T65*wx*	22	162	2	1	2	12	21	26	38	43	17	71.7
	T65*wx*	BC_1F_1	8	33			1	3	5	5	6	6	7	72.0
BC_3F_1	BC_2F_1	T65*wx*	7	30					3	1	7	12	7	78.4
	T65*wx*	BC_2F_1	4	17					1	2	5	4	5	77.9
BC_4F_1	BC_3F_1	T65*wx*	7	28						2	6	11	9	84.2
	T65*wx*	BC_3F_1	4	21						1	4	7	9	87.1
Control	(T65*wx*)			14								4	10	92.3

Table 3. Segregations for *wx* in the selfed seeds of semi-sterile plants during the backcross generations, assuming that their genotypes are *wx*-S_5^j/*Wx*-S_5^i

Generation	Number of plants	Mean seed fertility (%)	Segregation for *wx*			χ^2	
			Wx	*wx*	Total	$p = 0.5$	$p = 0.25$
F_1	4	51.3	531	148	679	3.72^{ns}	53.66^{**}
BC_1F_1	10	49.2	575	186	761	0.13^{ns}	99.22^{**}
BC_2F_1	4	58.3	702	230	932	0.05^{ns}	126.37^{**}

[a] An estimate of the recombination value (p) between S_5^i and *wx* was taken as 0.25 based on Ikehashi and Araki (1988). The frequency of *wx* segregants was estimated by $p/2$

** Significance at 1%; ns, non-significant the existence of an egg-killer (Ikehashi and Araki 1986, 1988), the present study failed to detect an egg-killer in the hybrid of T65*wx* × IR36. Instead of an egg-killer, the present results suggest the presence of a different type of sterility gene(s) on chromosome 6, although the genetic mechanism remains to be studied.

The interaction between the S_5^i and S_5^j alleles was proposed by Ikehashi and Araki (1986) as being a major genetic mechanism of hybrid sterility between the Indica and Japonica types. They also reported BC_1F_1 data as supporting evidence for the elimination of female gametes. They backcrossed the T65 × IR36 F_1 cross with pollen of IR36 and found that the frequencies of the *C* and *alk* genes derived from T65 were significantly more reduced in the progeny than expected, which is not in accordance with the present backcross data in which T65*wx* was used as the recurrent parent. When IR36 was used as the recurrent pollen parent, the S_5^j allele would be completely eliminated according to their model. The resultant plants should be homozygous for S_5^j, showing a rapid elimination of semi-sterile plants if the S_5^i and S_5^j alleles were mainly responsible for the hybrid sterility detected in the cross. However, they observed that semi-sterile plants frequently occurred after backcrossing with IR36 and that plants having *C* and *alk* from T65 tended to have lower fertilities than those having C^+ and alk^+ from IR36. This implies that there must be a gene(s) causing hybrid sterility that is different from the S_5^i or S_5^j allele on chromosome 6, which is in agreement with the present results as well as with those obtained by Nakagahra (1986).

Rice is considered to provide a unique opportunity for examining the genetic bases of hybrid sterility since hybrid sterility is frequently observed between and within species. Information on female sterility is, however, quite limited at present incomparison to what is available on male-sterility (Sano 1990). Both the gamete eliminator and egg-killer induce female sterility in the hybrids, but their distribution is unknown in rice. Such sterility genes are transmitted to a high proportion of the progeny through the egg. An extensive survey of cytoplasmic sterility in rice showed that except for those cultivars with male-sterile cytoplasms, most produce fertile plants when repeatedly backcrossed with T65 (Shinijyo 1984). Oka (1974) also showed a gradual recovery in seed fertility when T65 was used as the recurrent pollen parent in backcross experiments with the Indica type. These observations suggest that a gamete eliminator and an egg-killer, both of which induce the abortion of megaspores, may be rare in rice, if present at all. Oka (1964) reported an exception – an accession of the Indica type that maintained seed infertility after repeated backcrosses with T65. Recently, it was proven that this cross involves a gamete eliminator that induced the abortion of both micro- and megaspores (Y. Sano et al. unpublished). These observations have led us to consider that the mechanism of hybrid sterility is apparently more complex in rice than Ikehashi and Araki (1986) proposed. The present study indicates that the proposed genetic models for hybrid sterility in Asian rice cultivars should be viewed with caution, but does not deny the possibility of the usefulness of a fertility element on chromosome 6 in hybrid breeding. Further studies are needed to explain why the part of chromosome 6 including the S_5^n allele greatly suppresses the occurrence of semi-sterility in the Indica-Japonica hybrid.

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