Gamete Abortion Locus Detected by Segregation Distortion of Isozyme Locus *Pgil* in *indica-japonica* Hybrids of Rice (*Oryza sativa* L.)

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In an attempt to stabilize the pollen fertility of *indica-japonica* hybrids of rice by the incorporation of segregation-distortion-neutral alleles, a locus causing distorted segregation of male gamete in *indica-japonica* hybrids was identified with an isozyme marker gene *Pgil* on chromosome 3. In seven F₂ populations of *indica-japonica* crosses and 25 F₃ lines from IR 36/Kamairazu, the *japonica*-type allele *Pgil*-2 was transmitted via male gamete with a ratio of 0.29 to 0.38 instead of the normal one of 0.5 due to the presence of a gametophyte gene *ga14*. The recombination value between the locus *ga14* and the marker gene *Pgil* was estimated to be 0.29 by using 57 F₃ lines of IR 36/Kamairazu. The male gamete transmission rate (*k*) of *ga14-j* was estimated to be 0.13~0.22 against the *ga14-i* instead of the normal one of 0.5 based on the data from the F₂ and F₃ lines. The locus *ga14* which reduced the frequency of male gametes harboring *ga14-j* in some *indica-japonica* hybrids was located in the order of *Pgil*~*ga14*~*bc1* on chromosome 3.

Key Words: *Oryza sativa* L, gamete abortion, gametophyte gene, pollen fertility, segregation distortion, wide cross.

Introduction

Utilization of pronounced heterosis in *indica-japonica* hybrids in rice had been difficult until the partial sterility in such F₁ hybrids was alleviated by using a female abortion-neutral gene *S5-n* (Ikehashi and Araki 1986, Ikehashi 1991, Zou 1989). In the past decade many *indica-japonica* hybrids were developed and tested for commercial cultivation. Such hybrids showed a normal spikelet fertility and a high level of heterosis in grain yield. However, the seed set hardly exceeded the level of 90% and was unstable under adverse conditions (Lu *et al.* 1999b). The unstable fertility of *indica-japonica* hybrids was partially attributed to pollen abortion caused by gametophyte genes (*ga*), which were detected by the distorted segregation of marker genes (Lu *et al.* 1999b). To increase and stabilize the seed set of such hybrids, it is

necessary to analyze *ga* genes and incorporate segregation-distortion-neutral alleles into the hybrids.

Up to now, 13 *ga* genes for segregation distortion, from *ga-1* to *ga-13*, have been identified on various chromosomes in rice (Iwata *et al.* 1964, Nakagahra 1972, 1981, 1986, Mori *et al.* 1973, Nakagahra *et al.* 1972, Maekawa *et al.* 1981, Maekawa 1982, Maekawa and Kita 1985, Kinoshita and Takamure 1984, Lin and Ikehashi 1993, Maekawa and Inukai 1992, Rha *et al.* 1994). Recently, in many instances, segregation distortion has been identified in studies of isozymes or RFLP markers (Ishikawa *et al.* 1987, Lin *et al.* 1992, Harushima *et al.* 1996). In our previous study, we identified a new *ga* locus which is responsible for the segregation distortion of an isozyme marker *Pgil* on chromosome 3. The locus was found to be related to pollen fertility (Lu *et al.* 1999c), and considered to lead to seed set instability due to the reduction of pollen fertility under adverse conditions (Lu *et al.* 1999b). The locus was independent from the *ga3* locus on the same chromosome (Lu *et al.* 1997), and has been registered as *ga14* (Nagato and Yoshimura 1998, Lu *et al.* 2000). In the current investigation, we report further studies on the loci *ga14*.

Mapping of a *ga* locus is difficult because no conventional method is available. However, a method to estimate a recombination value between a *ga* locus and a marker gene showing distorted segregation was proposed by testing F₃ lines to distinguish family lines in which the segregation distortion was similar or not to that found in the F₂ population. The recombination value between a marker gene and the *ga* locus can be estimated by the least square method using the ratios of different segregation types in F₃ lines (Iwata *et al.* 1964, Nakagahra 1972, 1981, Lin and Ikehashi 1993).

Materials and Methods

Plant materials and marker genes

The segregation distortion of *Pgil* was caused by male gametes as confirmed by our previous study (Lu *et al.* 1997). A total of 219 F₃ lines from an F₂ population of IR36/Kamairazu were analyzed to estimate the recombination value between *ga14* and *Pgil*. IR 36 is a typical *indica* cultivar released from the International Rice Research Institute (IRRI) which harbors an isozyme gene *Pgil*-1, while the *japonica* cultivar Kamairazu harbors *Pgil*-2.

The isozyme locus *Pgil* is located at a map point 12 on

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chromosome 3 (Kinoshita 1995). The isozyme analysis was conducted according to the method of Glaszmann *et al.* (1988) with minor modifications by the authors.

Transmission rate of *Pgil-2* in *indica-japonica* hybrids

The transmission rate of *Pgil-2* is defined as the rate of gametes which actually transmit the allele of *Pgil-2* to progenies. Seven F_2 populations from *indica-japonica* hybrids which showed distorted segregation of the marker gene *Pgil* were used to estimate the transmission rate (Tr) of the *japonica*-type allele *Pgil-2*. The *indica* and *japonica* parents harbor the *Pgil-1* and *Pgil-2* alleles, respectively (Table 1). The transmission rate (Tr) of *Pgil-2* was calculated by the formula $Pgil-22/(Pgil-11+Pgil-22)$, where the doubled allele symbols indicate homozygotes. Out of the 219 F_2 plants from IR 36/Kamairazu, 99 were heterozygous for *Pgil* with a seed set range of 25.9-86.8% and gave sufficient population sizes for F_3 lines. Out of them, 57 were selected at random and tested for segregation types. Twenty five showed the same segregation type as that found in the F_2 population, and were used to calculate the Tr of *Pgil-2*.

Estimation of the recombination value between *gal4* and *Pgil*

The male transmission rates of *gal4-j* and *gal4-i* are denoted by k and $1-k$, respectively. The recombination value between *gal4* and *Pgil* is denoted by q . Therefore, the frequencies of male gametes harboring *gal4-j~Pgil-2*, *gal4-j~Pgil-1*, *gal4-i~Pgil-2* and *gal4-i~Pgil-1* can be calculated by the formula $k(1-q)$, kq , $(1-k)q$ and $(1-k)(1-q)$, respectively. The frequencies of the female gametes harboring *gal4-j~Pg-1-2*, *gal4-j~Pgil-1*, *gal4-i~Pgil-2* and *gal4-i~Pgil-1* are $(1-q)/2$, $q/2$, $q/2$ and $(1-q)/2$, respectively. The expected frequencies of the *Pgil* genotypes in the F_2 will be given by the combination of the frequencies for the male and female gametes. The expected frequencies of the heterozygotes of *Pgil* are given in Table 2, with further classification into four genotypes in terms of *Pgil* and *gal*, resulting in three segregation types for *Pgil* (Table 3). The frequency of each genotype was obtained by testing the segregation in F_3 lines, where the *gal4-j~Pg-1-2/gal4-i~*

Pgil-1 and *gal4-j~Pgil-1/gal4-i~Pgil-2* genotypes show the same segregation distortion of *Pgil* genes as that in the F_2 population. The genotypes *gal4-j~Pg-1-2/gal4-j~Pgil-1* and *gal4-i~Pgil-1/gal4-i~Pgil-2* do not display any segregation distortion at the *Pgi-1* locus. The *gal4-j~Pgil-1/gal4-i~Pgil-2* genotype would shows an increase in the transmission rate of *Pgil-2*, in contrast to the F_2 segregation type. The recombination value q between the marker *Pgil* and the *gal4* locus can be estimated by the least square method using the ratios of different segregation types between the theoretical and observed frequencies of the segregation types (Nakagahra 1972, Lin and Ikehashi 1993).

Estimation of transmission rate of *gal4-j*

The transmission rate of *gal4-j* is defined as the rate of gametes which transmitted the allele of *gal4-j* to the progenies. In the F_2 population, the expected frequencies for *Pgil* genotypes were determined by the recombination value q between *gal4* and *Pgil*, together with the male transmission rate (k) of *gal4-j* as shown in Table 4. If the recombination value is estimated, then the transmission rate can be determined by the observed frequencies of homozygous genotypes, *i.e.*, *Pgil-11* and *Pgil-22* in the F_2 population and in the F_3 lines showing the same segregation as in F_2 (Nakagahra 1972, Lin and Ikehashi 1993).

Results and Discussion

Transmission rate of *Pgil-2* in *indica-japonica* hybrids

The transmission rate (Tr) of *Pgil-2* ranged from 0.29 ± 0.11 to 0.38 ± 0.09 in the seven F_2 populations from F_1 hybrids of *indica-japonica* crosses (Table 1). The Tr in IR 36/Kamairazu was estimated to be 0.34 ± 0.02 with the 25 F_3 lines from the F_2 plants which showed the same mode of distorted segregation as that of *Pgil*.

Segregation types of F_3 lines in relation to *Pgil-2*

The segregation types of F_3 lines are shown in Table 2. All the lines which exhibited a distorted segregation in the same mode as that found in the F_2 gave a frequency of *Pgil-2* within the range of 0.20 to 0.40, while the lines with a normal segregation gave values ranging from 0.40 to 0.60. One line showed a significant increase of the frequency of *Pgil-2*, namely 0.67 which was different from the F_2 population, and was assumed to correspond to the recombinant genotype *gal4-j~Pgil-1/gal4-i~Pgil-2*. Besides, four lines exhibited ambiguous segregation patterns showing a non-significant deviation from normal with a probability between 0.1 and 0.05 (Table 2).

Recombination value between *gal4* and *Pgil*

The recombination value (q) between *gal4* and *Pgil* was estimated to be 0.29 by the least square method (Table 3). The four lines which showed an ambiguous segregation with a probability of 0.1~0.05 (Table 2) were excluded

Table 1. Transmission rate (Tr) of *Pgil-2* via male gametes in the F_2 of *indica-japonica* hybrids (Kyoto, 1995, 1998)

Crosses	<i>ii:ij:jj</i> ^{a)}	Tr
Taichung 65/IR 50	73: 94 :35	0.32 ± 0.09
IR 36/Kamairazu	74: 99 :46	0.38 ± 0.09
IR 36/Akamai 1	31: 41 :15	0.33 ± 0.14
IR 36/Calotoc	39: 78 :21	0.35 ± 0.12
3037/02428	114: 150 :55	0.33 ± 0.07
G 43/G 19	32: 52 :16	0.33 ± 0.13
G 18/Nekken 1	47: 57 :19	0.29 ± 0.11
IR 36/Kamairazu F_3 lines ^{b)}	923:1228:481	0.34 ± 0.02

^{a)} *ii:ij:jj* refers to *Pgil-ii:Pgil-ij:Pgil-jj*.

^{b)} The sum of 25 lines which showed the same distorted segregation as the F_2 population of IR 36/Kamairazu.

Table 2. Identification of segregation types and frequency of *Pgil* in 57 F₃ which were selected from heterozygous F₂ plants in IR 36/Kamairazu

Probability ^{a)}	Segregation type	Theoretical frequency ^{b)}	No. of F ₃ lines	Sum of segregation ratio		
				<i>Pgil</i> -11	: <i>Pgil</i> -12	: <i>Pgil</i> -22
P<0.05	F ₂	(1-q)2	25	923	:	1228 : 481
P>0.1	Normal	2q(1-q)	27	730	:	1366 : 695
P<0.05	Non F ₂	q ²	1	19	:	88 : 38
0.1>P>0.05	Not determined		4	142	:	217 : 121
Total			57	1814	:	2899 : 1335

^{a)} χ^2 test for both *Pgil*-11 : *Pgil*-22 = 1 : 1 and *Pgil*-11 : *Pgil*-12 : *Pgil*-22 = 1 : 2 : 1. A total of 90-150 seedlings for each F₃ line were tested except for three lines with less than 90 seedlings.

^{b)} q: Recombination value between *gal4* and *Pgil*.

Table 3. Estimation of recombination frequency according to segregation types of 53 F₃ lines selected from heterozygous F₂ plants for *Pgil* in IR 36/Kamairazu

F ₂ genotype	Segregation type	Theoretical frequency	No. of plants	
			Observed	Estimated by q=0.29
<i>gal4-i. Pgil-1/gal4-j. Pgil-2</i>	F ₂	(1-q) ²	25	26.7
<i>gal4-i. Pgil-1/gal4-i. Pgil-2</i>	Normal	2q(1-q)	27	21.8
<i>gal4-j. Pgil-1/gal4-j. Pgil-2</i>	Normal	2q(1-q)	27	21.8
<i>gal4-j. Pgil-1/gal4-i. Pgil-2</i>	Non F ₂	q ²	1	4.5
χ^2 probability				0.1-0.25

q: Recombination value between *gal4* and *Pgil*.

Table 4. Expected and observed numbers of *Pgil* genotypes in the F₂ and F₃ of IR 36/Kamairazu

<i>Pgil</i> Genotype	Expected frequency ^{a)}	Number of plants			
		Observed		Expected with q=0.29	
		F ₂	F ₃ ^{b)}	F ₂ with k=0.22±0.07	F ₃ with k=0.13±0.02
<i>Pgil</i> -11	(1-q-k+2qk)/2	74	923	67.6	863
<i>Pgil</i> -12	1/2	99	1228	109.5	1316
<i>Pgil</i> -22	(q+k-2qk)/2	46	481	41.9	453

^{a)} q: Recombination value between *gal4* and *Pgil*.

k: Male transmission rate of *gal4-j*.

^{b)} Total number from F₃ lines which showed the same segregation type as the F₂.

from the calculation. However, some of the four lines could possibly be included in the 'Non F₂' type based on theoretical expectation.

Estimation of the transmission rate of *gal4-j*

With the recombination value of 0.29 between *gal4* and *Pgil*, the transmission rate (k) of *gal4-j* was estimated to be 0.22±0.07 and 0.13±0.02 for the F₂ population and for the sum of 25 F₃ lines which showed the same segregation types as those found in the F₂ in IR 36/Kamairazu, respectively (Table 4). Thus, *gal4-j* was transmitted at the rate of 13-22% against the *gal4-i* pollen grains instead of the normal rate of 0.5. The transmission rate of 0.13 is likely to be closer to the actual value as it was calculated based on a large population size.

The new locus *gal4* showed a recombination value of 0.29 with an isozyme marker gene *Pgil* located at the map point 12 on chromosome 3. It is assumed that the locus *gal4*

may be located between the *Pgil* and *bcl*. Because *Pgil* is near the end of chromosome 3 and the recombination value between *Pgil* and *bcl* was estimated to be 0.40 and 0.42, respectively using the F₂ from Ketan Nangka/IR36//Kamairazu and Minghui 63/G 43 (Fig.1). Recently, a gene locus *S-c* for hybrid sterility of pollen has been reported in an *indica-japonica* hybrid on chromosome 3 by using RFLP markers (Zhang et al. 1996). The locus *gal4* is likely to be *S-c* based on the map position. At the locus *gal4* some *japonica* varieties such as Kamairazu have an allele *gal4-j*, while *indica* cultivars such as IR36 have *gal4-i*. The *gal4-i/gal4-j* genotype showed a segregation distortion because of the reduction of the frequency of male gametes carrying *gal4-j*. The transmission rate of the allele *gal4-j* was estimated to be 0.13-0.22 against the allele *gal4-i*. The lack of pollen fertility may be due to the reduced growth speed of the pollen grain tube or low activity related to the *ga* allele.

Female gamete abortion genes can cause directly a

Fig. 1. Putative arrangement of *gal4* locus and the related markers on chromosome 3.

a) Map point on chromosome 3, after Kinoshita, 1995.

reduction of seed fertility, unlike male gamete abortion, because the amount of pollen grains is usually large and sufficient for fertilization. However, if many *ga* loci reduce the pollen fertility, spikelet fertility is likely to decrease due to a lower pollen activity, or to be unstable due to the insufficient number of viable pollen grains under adverse environmental conditions.

To utilize the pronounced heterosis of *indica-japonica* hybrid in rice, it is important to overcome the hybrid sterility associated with male or female gamete abortion. It was observed that a neutral allele *S5-n* at locus *S5* on chromosome 6 mitigated the female gamete abortion in common *indica-japonica* hybrids. The *S5-n* gene has been incorporated into *indica* or *japonica* cultivars to overcome the sterility problem in breeding through the use of *indica-japonica* crosses (Ikehashi and Araki 1986, Ikehashi 1991, Yuan *et al.* 1997). In our previous study, we confirmed the presence of neutral genes designated as *gal1-n* at *gal1* (Lu *et al.* 2000). We have attempted to incorporate the *gal1-n* gene from a donor cultivar Dular into other lines by selecting several lines up to F_8 using a linked isozyme marker *Est9*. The new lines can actually alleviate the segregation distortion of *Est9* that is caused by the *gal1* locus (Lu *et al.* 1999a). It is noteworthy that some cultivars like Ketan Nangka have a neutral allele *gal4-n* which did not cause segregation distortion in the genotypes *gal4-i/gal4-n* and *gal4-j/gal4-n* (Lu *et al.* 2000). The incorporation of *gal4-n* into breeding lines is being attempted in our laboratory.

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