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P. Wu · G. Zhang · N. Huang · J. K. Ladha

Non-allelic interaction conditioning spikelet sterility in an F_2 population of indica/japonica cross in rice

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Abstract Significant segregation of spikelet fertility occurred in an F_2 population derived from a spikelet fertility-normal F_1 hybrid produced by a cross between 'Palawan', a japonica variety, and 'IR42', an indica variety. To identify factors controlling the fertility segregation, we used 104 RFLP markers covering all 12 rice chromosomes to investigate the association of spikelet fertility and marker segregation. We found that the segregation of two sets of gene pairs was significantly ($P < 0.001$) associated with fertility segregation. The first pair of genes was linked to RFLP marker RG778 on chromosome 12 and RFLP markers RG690/RG369 on chromosome 1. A significant reduction in fertility was observed when the plants were homozygote at RG778 with the indica allele as well as homozygote at RG690/RG369 with the japonica allele. The second pair of genes was linked to RG218 on chromosome 12 and RG650 on chromosome 7, respectively. The recombinant homozygote at these two loci showed a significant reduction on spikelet fertility. The non-allelic interaction effect was further modified by a gene linked to RG778, resulting in even lower fertility. The results of this study provides the first evidence of chromosomal localization of sporophytic sterility genes whose interaction can result in a reduction of spikelet fertility in the F_2 derived from fertility-normal F_1.

Key words *Oryza sativa* L. · Spikelet fertility · Non-allelic interaction · Restriction fragment length polymorphism (RFLP)

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P. Wu[1] · G. Zhang · N. Huang (✉) · J. K. Ladha
The International Rice Research Institute, PO Box 933, Manila, The Phillippines

Present address:
[1] Department of Soil Science and Plant Nutrition, Zhejiang Agricultural University, Hangzhou, 310029, Zhejiang, China

Introduction

Since Kato et al. (1928) observed F_1 sterility barriers between indica/japonica types of Asian rice, many studies have shown that hybrid sterility in the F_1 populations of wide crosses in rice is a common phenomenon (see reviews by Oka 1988; Morishima et al. 1992). On the basis of the segregation distortion of morphological and isozyme markers, this hybrid sterility has been explained by allelic interaction at several loci in the rice genome (Nakagahra 1972; Ikehashi and Araki 1986, 1988; Yanagihara et al. 1992; Lin et al. 1992; Lin and Ikehashi 1991; Sano 1990, 1993; Zhang and Lu 1993; Zhang et al. 1994). The interaction between indica and japonica alleles such as $S\text{-}5^i$ and $S\text{-}5^j$ at the $S\text{-}5$ locus on rice chromosome 6 can lead to partial female (or male for other loci) gamete abortion.

It has also been observed that wide crosses can produce partially sterile F_2 plants derived from self-pollinated F_1 plants whose fertility is as perfectly normal as that of their parents (Oka and Doida 1962; Oka 1978). The degree of fertility in F_2 progenies varies from cross to cross and is determined by their genotypes. This phenomenon is explained by a model of non-allelic interaction. It is assumed that genes from both parents are recombined in F_2 progenies and that some recombinants carry incompatible loci from both parents, resulting in the segregation of fertility in the F_2 population (Oka and Doida 1962; Oka 1978). A true breeding partly sterile line can be obtained from these crosses (Oka and Doida 1962; Oka 1978). It is, however, quite complicated to test the non-allelic interaction hypothesis of hybrid sterility segregation in an F_2 population with morphological and isozyme markers because only limited numbers of these markers are present in each cross.

The advent of DNA markers such as RFLP allows hundreds of markers to be surveyed in a single F_2 population. Segments of DNA, and therefore the genes carried on them, from both parents can be compared

pair-wise to examine the effects of a non-allelic interactions. With RFLP markers, Cowen et al. (1992) determined that two genes linked to two RFLP markers located on two different chromosomes interact with each other to produce much higher embryo-like structure from anther culture. This work exemplifies the power of using RFLP markers to study the effects of gene interaction.

In our study, we exploited the advantage of the existence of a well-saturated RFLP map in rice (Causse et al. 1994) to study the effect of gene interaction in an F_2 population with fertility segregation. We were able to identify two sets of markers linked to genes that were derived from both parents and which interacted with each other, resulting in the formation of partly sterile F_2 plants.

Materials and methods

Plant material and sterility measurement

The parents used in this study were 'Palawan', an upland wide compatibility variety (Kumar and Virmani 1992), as female parent, and 'IR42', an indica variety, as male parent. The F_1 hybrids were grown in a greenhouse to produce an F_2 population. Normal spikelet fertility of the F_1 hybrid relative to that of the parents was observed. A pot experiment with 250 F_2 plants, 20 F_1 plants and the two parents, respectively, was conducted in a greenhouse at the International Rice Research Institute in 1993. The rice plants were maintained under flooded conditions during the growing period. The spikelet fertility of each F_2 plant was measured at maturity by percent filled grain of the main panicle. The F_2 plants with similar spikelet fertility as that of their parents were considered to be normally fertile.

RFLP map and linkage analysis

DNA was extracted from fresh leaves of the tiller plants of the population (Dellaporta et al. 1984), digested with six restriction enzymes *Eco*RI, *Eco*RV, *Sca*I, *Hin*dIII, *Xba*I and *Dra*I, separated on 0.9% agarose gel, transferred to Hybond N^+ membranes (Amersham, Chicago) and probed with a [^{32}P] dCTP-labelled probe (Feinberg and Vogelstein 1984). The parents were checked for polymorphism with RFLP markers provided by Steve Tanksley, Cornell University, USA. Polymorphic markers were then surveyed in the F_2 population. An RFLP marker map with 104 informative RFLP markers mapped on 12 chromosomes was constructed with the program MAPMAKER (Macintosh v1) (Lander et al. 1987) at a minimum LOD of 3. The recombination fraction expressed in centi-Morgans (cM) was calculated by the Kosambi mapping function (Kosambi 1944). The order of the markers was established using multiple point analysis at a LOD of 3.0.

Statistical analysis

The percentage of spikelet fertility was converted into arc-sin values before statistical analysis. To determine if any single locus was associated with spikelet fertility segregation in an F_2 population, one-way analysis of variance (ANOVA) was conducted using the SAS GLM procedure (SAS 1985). To identify epistatic interactions on spikelet fertility segregation, all possible pair-wise interactions of 104 RFLP marker loci were conducted with two-way ANOVA. The interaction was considered to be significant if the F value exceeded the criteria at a probability less than 0.1% taking into consideration that the spikelet fertility is strongly subjected to environmental effects. The Chi-square test was performed to examine fitness of the frequencies of the marker loci against expectation from Mendelian segregation. The effect of the heterozygosity of a single marker locus on spikelet fertility was tested by regression analysis between percent heterozygosity of the 104 markers and spikelet fertility of the 231 plants.

Results

Distribution of spikelet fertility in F_2 population

The distribution of spikelet fertility in the F_2 population is shown in Fig. 1. For comparison, the average spikelet fertility of the two parents, F_1 plants and F_2 plants is also shown in Fig. 1. The spikelet fertility of F_1 plants was 81% basically the same as that of the parents, indicating again that the spikelet fertility of the F_1 was normal. The spikelet fertility of the F_2 plants, on the other hand, segregated from as low as 5% to as high as 95%. Although the distribution of spikelet fertility in the F_2 population can be seen to be continuous, two peaks are obvious. The first peak is around 10% spikelet fertility. A total of 56 F_2 plants have a spikelet fertility of less than 40%. The second peak is around 80% of spikelet fertility. The segregation of spikelet fertility observed in this cross is similar to that observed by Oka and Doida (1962) and Oka (1978). The results suggest that some major factors may be involved, resulting in the spikelet fertility segregation, but that minor factors influencing spikelet fertility may also exist.

RFLP marker segregation

Most RFLP marker loci fit the expected 1:2:1 Mendelian segregation ratio. Only a small portion of the markers showed skewness towards either the indica or japonica parent. The genotypic frequency of 5 marker loci which were found to be associated with fertility segregation (next section) in F_2 are shown in Table 1. Except for RG778 which showed a significant segregation

Fig. 1 Distribution of spikelet fertility (%) in an F_2 population with 231 individuals derived from 'Palawan'/'IR42'

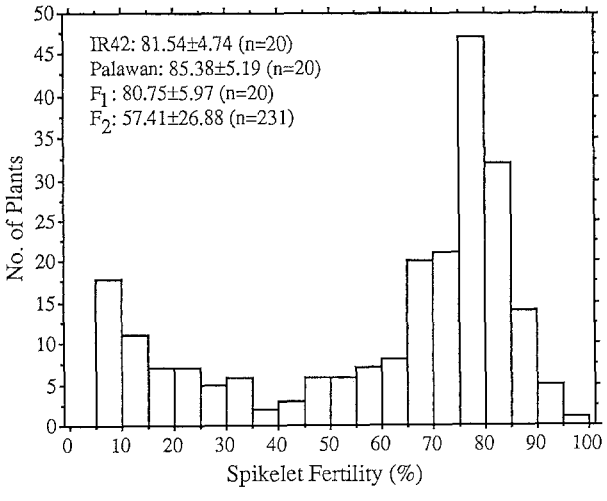

Table 1 Chi-square test of genotypic frequencies of RFLP markers with significant effects on spikelet fertility

Marker	Genotype[a]			χ^2
	II	IJ	JJ	
RG690	25.0	55.9	19.0	2.31[ns]
RG778	40.8	32.1	27.1	16.55**
RG369	28.0	55.5	16.1	4.47[ns]
RG218	23.7	52.1	24.2	0.25[ns]
RG650	21.8	50.0	28.8	0.84[ns]

** Significant at 1% level, ns, non-significant
[a] I, Indica; J, japonica

distortion with a skewness towards the indica genotype and lower heterozygous genotype, all of the other 4 markers had normal segregation.

RFLP marker loci associated with spikelet fertility segregation

Analysis of variance for the association between single marker loci and spikelet fertility variation was conducted for 104 RFLP markers using the SAS GLM procedure. All possible epistatic interaction effects were also tested. No significant association between single marker loci and spikelet fertility was detected ($P < 0.001$). A highly significant non-allelic interaction effect was observed between RG778 located on chromosome 12 and 2 closely linked marker loci, RG369 ($P < 0.0001$) and RG690 ($P < 0.001$) located on chromosome 1, and between RG218 located on chromosome 12 and RG650 ($P < 0.001$) located on chromosome 7 (Table 2 and Fig. 2).

To analyze the genetic mechanism underlying the detected non-allelic interaction effect, we compared the averages of spikelet fertility on nine possible combinations between interacting market loci. The homozygous combinations of an indica genotype of RG778 with japonica genotypes of RG369 and RG690 showed a considerable effect on the means of spikelet fertility (Table 3). The averages of spikelet fertility of the combination were only about 20%, much lower than that of other combinations (Table 3), indicating that the lower fertility in some F_2 plants was due to the effects of the genes linked to the 3 markers. Since RG369 and RG690 are closely linked together, and there is a great similarity in spikelet fertility distribution among the genotypes, we would expect that both RG369 and RG690 reflect the effect of the same gene on chromosome 1 (Fig. 2).

Table 2 Analysis of variance for the association of RFLP marker loci with spikelet fertility in an F_2 population derived from 'Palawan'/'IR42'

Source	*df*	*F* value	*P* value	Chromosome no.
RG778 × RG369	4	6.70	0.00005	12, 1
RG778 × RG690	4	5.22	0.00051	12, 1
RG218 × RG650	4	5.05	0.00067	12, 7

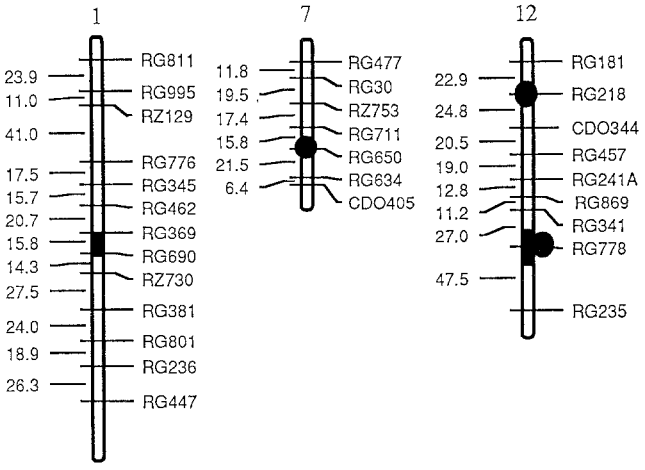

Fig. 2 Chromosomal location of sporophytic sterility genes. The *solid bars* on chromosomes 1 and 12 represent one pair of interacting genes linked to RG778 and flanked by RG369 and RG690. The *solid circles* on chromosome 12 and 7 represent another interacting gene pair linked to RG218 and to RG650. The effect of a gene linked to RG778 on the interaction between RG218 and RG650 is also indicated by the *solid circle*. RFLP markers are to the *right*, and to the *left* are map distances in cM based on the Kosambi function

Another set of genes responsible for fertility segregation in F_2 plants were linked to RG650 and RG218 (Table 2 and 4). The mean comparison shows that a significant reduction in spikelet fertility occurred when the japonica allele near RG650 was recombined with the indica allele near RG218, or vice versa (Table 4). These two sets of non-allelic gene interactions provide clear evidence that the spikelet fertility in F_2 plants of a wide cross in rice can be controlled by sporophytic sterility gene interactions, as hypothesized by Oka and Doida (1962).

To investigate the relationship between the two sets of interacting genes, we analyzed three-way interactions by examining the change on the average spikelet fertility of two pairs of gene interactions due to the addition of a third marker. The homozygous genotypes of RG778 were found to have interaction effects on the RG218/RG650 combination (Table 5). The spikelet fertility changed from 38% with the II/JJ genotype for RG218/RG650 (Table 4) to only 21% when the II genotype for the RG778 marker was also considered (Table 5). A weaker but similar effect of the JJ genotype of RG778 on the JJ/II genotype of the RG218/RG650 marker loci was also observed (Tables 4 and 5).

Effect of DNA marker skewness on gene identification

The fertility gene linked to RG778 was found to play an important role in F_2 fertility segregation (Tables 2, 3 and 5). It was also found that the locus was skewed towards the indica parent (Table 1). It was therefore important to know that the identification of the fertility gene linked to

Table 3 Average spikelet fertility (%) of different genotypic combinations between RG778 and RG369, RG690 markers. The numbers in parentheses are frequencies of the genotypes (%)

RFLP marker		RG778			χ^2
		II[a]	IJ	JJ	
RG369	II	52.08 ± 27.89 (12.0)	48.13 ± 32.19 (8.0)	62.94 ± 22.08 (8.5)	
	IJ	59.00 ± 29.21 (25.0)	66.91 ± 16.97 (17.0)	74.58 ± 22.31 (13.0)	
	JJ	20.00 ± 26.17 (5.5)	74/58 ± 22.31 (6.0)	54.50 ± 26.40 (5.0)	54.9**
RG690	II	61.58 ± 22.30 (9.2)	57.94 ± 26.58 (8.2)	57.50 ± 25.22 (8.7)	
	IJ	58.58 ± 29.20 (23.2)	63.58 ± 23.87 (18.4)	50.38 ± 27.09 (12.6)	
	JJ	21.11 ± 26.21 (8.7)	62.50 ± 27.71 (4.8)	56.92 ± 25.88 (6.3)	44.63**

** Significant at 1% level
[a] II, Indica homozygotes; JJ, japonica homozygotes; IJ, heterozygotes

Table 4 Average spikelet fertility (%) of different allele combinations between RG218 and RG690. The number in parenthesis are genotypic frequency (%)

RFLP marker		RG650			χ^2
		II[a]	IJ	JJ	
RG218	II	72.31 ± 11.29 (6.3)	53.97 ± 28.67 (13.9)	37.78 ± 34.29 (4.3)	
	IJ	42.31 ± 28.92 (12.5)	55.53 ± 29.10 (22.6)	61.18 ± 25.67 (16.4)	
	JJ	35.83 ± 26.54 (2.9)	63.62 ± 26.05 (13.9)	65.67 ± 18.31 (7.2)	3.61[ns]

[ns] Non-significant
[a] II, Indica homozygotes; JJ, Japonica homozygotes; IJ, heterozygotes;

Table 5 Effect of marker locus RG778 on the interaction between RG218 and RG650 on spikelet fertility (*NA* no plant in the population with this genotype)

RFLP marker		RG778		
		II[a]	IJ	JJ
RG218 × RG650	II/II	71.67 ± 15.26	72.14 ± 11.13	73.33 ± 12.58
	II/JJ	21.00 ± 33.05	80.00[b]	51.67 ± 25.17
	JJ/II	NA	51.67 ± 24.66	30.00 ± 14.14
	JJ/JJ	65.00[b]	67.50 ± 11.95	74.00 ± 7.42

[a] II, Indica homozygotes; JJ, japonica homozygotes; IJ, heterozygotes;
[b] Only a single plant in the population with these genotypes

RG778 was not due to the skewness of the RG778 locus. The frequencies of all nine genotypes (%) are shown in Table 3. The overall χ^2 test was significant, showing that the observation deviated from the expected Mendelian ratio. Close examination showed that the deviation was mainly due to the lower frequencies of heterozygotes and higher frequencies of homozygotes of the indica genotype at the RG778 locus (Tables 1 and 3). The frequencies of the critical genotype, II/JJ for RG778/RG369 or RG778/RG690, were 5.5% and 8.7% which were close to the expected (6.25%) and about the same as that of other double homozygous classes (Table 3). The average spikelet fertility in the critical class was 20% or 21%, which was less than half of all other double homozygous classes. Statistical tests among all homozygous classes showed that the difference was highly significant ($P < 0.0001$). We therefore concluded that the skewness of the RG778 marker did not have a significant effect on non-allelic interaction analysis for spikelet fertility.

Discussion

The sterility of hybrids derived from distantly related rice varieties can be classified into allelic interaction (gametophytic) (Oka 1957, 1974), non-allelic interaction (sporophytic) (Oka and Doida 1962) and nuclear-cytoplasm interaction (Shinjyo 1969, 1972). F_1 sterility was found to be controlled by sets of genes that affect the development of both the male and female gametes (Oka 1957). More recently, a multiple allele locus (*S*–5) on chromosome 6 was found to be responsible for hybrid sterility by allelic interaction (Ikehashi and Araki 1986,

1988). Female gametes were aborted in the genotype of $S\text{-}5^i/S\text{-}5^j$, whereas the neutral allele $S\text{-}5^n$ did not cause abortion in the heterozygotes of $S\text{-}5^i/S\text{-}5^n$ and $S\text{-}5^j/S\text{-}5^n$. Since then, the $S\text{-}5^n$ allele has been used to overcome hybrid sterility (Araki et al. 1990), and the donor of $S\text{-}5^n$ is called a wide-compatibility variety (WCV). In our experiment, 'Palawan' was used as it has been recognized as a WCV (Kumar and Virmani 1992) carrying $S\text{-}5^n$. We hoped to see normal fertility in the F_1 as well as F_2, but what we observed was a strong spikelet fertility segregation in the F_2 population, suggesting an additional genetic mechanism underlying the sterility problems in the progenies of wide crosses. The same phenomenon had been observed previously, and a two-loci model of sporophytic genes had been proposed (Oka and Doida 1962, Oka 1978) to explain spikelet fertility segregation in F_2 populations. If the genotypes of fertile parent plants are assumed to be $A_1A_1a_2a_2$ and $a_1a_1A_2A_2$ respectively, recombinants with the $a_1a_1a_2a_2$ genotype would produce in the sporophytic tissue some adverse effect on the development of the male and/or female gametes. With the aid of molecular markers, we are able to identify factors conditioning the spikelet fertility of F_2 plants. Spikelet fertility was significantly reduced when an indica allele linked to RG778 was recombined with a japonica allele linked to RG369/RG690 to form the $a_1a_1a_2a_2$ genotype (Table 3). Spikelet fertility was also significantly reduced when an allele linked to RG650 was recombined with an allele linked to RG218 to form the $a_1a_1a_2a_2$ or $A_1A_1A_2A_2$ genotypes (Table 4). We have therefore identified four genes whose interaction can lead to spikelet fertility segregation in an F_2 population derived from fertility-normal F_1 hybrids.

The identification of interacting loci conditioning spikelet fertility in an F_2 population has practical implications in both genetic as well as breeding studies. For example, anther culture is commonly used to generate double haploid lines from pollen of F_1 hybrids, and fertility problems are commonly observed in the regenerated lines. One plausible factor might be the formation of recombinants of the fertility genes identified in this study. If this is confirmed by further study, parents should then be selected for anther culture at least partially on the basis of the sporophytic genes conditioning the fertility in recombinants. It is recommended that the fertility of F_2 plants from a wide cross be examined before anther culture is initiated.

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