

GENETIC ANALYSIS FOR DISTORTED SEGREGATION OF *Ef-1* GENE IN RICE

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Abstract

The present study was carried out to assess the genetic mechanism of the distorted segregation of earliness-1 gene (*Ef-1*) in cross between Indica and Japonica linkage tester. The linkage relationship was investigated in F₂ populations between the *Ef-1* gene on chromosome 10 and a new gametophyte gene. Distorted segregation of *Ef-1* appeared in F₂ populations and it was not related to the F₁ seed sterility and the F₂ seed fertility. The ratios of the distorted segregation did not vary significantly among F₂ populations derived from F₁ hybrids of the same cross combinations.

Ef-1 gene in B₁F₁ hybrids derived from the reciprocal backcrosses clearly showed that the distorted segregation was caused by a new gametophyte gene. The recombination value was calculated to be $24.2 \pm 5.4\%$ between *Ef-1* gene and gametophyte gene.

Introduction

Rice is a major food for more than one third of world's population in general and Asians in particular; nearly 90% of the world's rice is produced and consumed in this region. It is estimated that to feed the growing world population total food production will have to increase by 60% in the next 25 years (Khush, 1997). To cope with the increasing population and to achieve self sufficiency in rice production, there is need for new genes and improved genetic recombinants not found either in the cultivated varieties or their relatives (Akbar *et al.*, 2004).

Genetic analysis provides a guide line for the assessment of relative breeding potential of the parents or identifies best combiners in crops (Khattak *et al.*, 2004). Genetic studies on distorted segregation of morphological marker genes have been conducted, although such genetic phenomena were observed sometimes in rice of hybrid populations (Xu & Shen, 1992; Rha *et al.*, 1995, 1996). The characteristics of hybrid sterility, hybrid breakdown, hybrid weakness, and distorted segregation of genetic markers in rice are often observed in hybrid generations from the crosses between Japonica and Indica rice (Nakagahra *et al.*, 1972; Oka, 1974, 1989; Maekawa & Kita, 1985; Okuno, 1985; Sato *et al.*, 1987; Tsai, 1990; Xu & Shen, 1992; Rha *et al.*, 1995, 1996, 2000). It has already been reported that the gametophyte genes are responsible for the differential pollen fertilization in the F₁ populations from distantly related crosses (Sato *et al.*, 1987; Abe & Tsuda, 1988). Meanwhile for biochemical traits, the distorted segregation of isozyme gene in hybrid population between Japonica and Indica rice was reported (Second, 1982; Glaszmann, 1987; Guiderdoni *et al.*, 1989; Lin & Ikehashi, 1991; Rha, 2000).

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When wide crosses were performed in crops a large number of distorted segregations appeared in the selection of hybrid gametes due to the presence of gametophyte genes (Nakagahra, 1981; Abe & Tsuda, 1988; Sari-Gorla *et al.*, 1986; Xu & Shen, 1992; Rha *et al.*, 1995, 1996). Many loci related to gametophyte genes have been reported in rice viz., *ga-1* (Iwata *et al.*, 1964), *ga-4* and *ga-5* (Mori *et al.*, 1973), *ga-2* and *ga-3* (Nakagahra, 1972), *ga-6* (Maekawa & Kita, 1985) and *ga-10* (Kinoshita & Takamure, 1984), *ga-7* (Maekawa & Kita, 1985), *ga-8* (Nakagahra, 1981) and *ga-9* (Maekawa & Kita, 1985), *ga-11* (Lin & Ikehashi, 1991), *ga-14* and *ga-15* (Rha *et al.*, 1995), and *ga-16* and *ga-17* (Rha *et al.*, 1996).

Gene *Ef-1* for early heading was found in various Japanese rice varieties (Tsai, 1990; Sato & Hayashi, 1985). Using early heading trait for rice-barley cropping system is very valuable in Korea. Therefore in this study, the linkage relationship between the *Ef-1* gene on chromosome 10 and the gametophyte gene has been investigated in F₂ populations derived from the crosses between Indica and Japonica rice.

Materials and Methods

The materials used in this study included Indica type variety, Taichung Native 1 and Japonica type linkage tester. Crossing of the Indica and Japonica was performed. Randomized complete block design was followed in the experiment having three replications. Each treatment comprised of a single row of five-meter length in each replication. Inter row and inter plant distances were kept at 30 cm and 20 cm respectively.

The seeds, which were derived from the cross combination between Indica and Japonica was grown in experimental field of Sunchon National University, Korea. At the time of harvesting, crossed spikes were individually harvested/threshed to have seed for raising F₁ generation. After harvesting F₁ generation, seeds were planted in field, and then segregation of *Ef-1* gene in their F₂ plants was examined. Furthermore, the reciprocal backcross was made, and B₁F₁ individuals were also tested for the *Ef-1* gene and gametophyte gene. Segregation of the genotype in each of the F₃ progenies was also examined for heterozygous earliness. Recombination value (P) was estimated using the following formula, which was proposed by Nakagahra *et al.*, (1972).

$$P = (2c+b)/2(a+b+c)$$

where a, b and c in the formula represent the numbers of deficient segregation, normal segregation, and excess segregation of *Ef-1* gene in F₃ progenies, respectively. The standard deviation of recombination value (Sp) is given as follows:

$$Sp = \sqrt{p(1-p)/2(a+b+c)}$$

Results and Discussion

Distorted segregation of Ef-1 gene in F₂ populations: Distorted segregation of the *Ef-1* gene in F₂ population was obtained from F₁ hybrids, which were grown in different locations (Table 1). The segregation of dominant/recessive types in F₂ population theoretically would be expected in a ratio of 3:1, when the certation did not occur. The plants containing dominant/recessive types were distorted significantly in every F₂

populations in these cross combination. The frequency of the dominant type was much higher than the theoretical value (75%), while that of the recessive type was lower. The frequency of recessive type was 4.7% to 13.2%, significantly lower than expected 25% in the reciprocal cross (Table 2). Since distorted segregation ratios did not differ significantly among cross combinations and two locations, the distortion was obviously not influenced by cross combinations nor cultural conditions (Lin, 1991; Rha *et al.*, 1995, 1996; Rha, 2000). A large number of distorted segregations appeared in the selection of hybrid gametes due to the presence of gametophyte genes, when distantly related varieties crosses were performed in crops (Nakagahra, 1972, 1981; Sari-Gorla *et al.*, 1986; Abe & Tsuda, 1988; Xu & Shen, 1992; Rha *et al.*, 1995, 1996). From these results, it is suggested that the distorted segregation of *Ef-1* gene is caused by a gametophyte gene which is linked with *Ef-1* gene. It might be a new gametophyte gene in chromosome 10.

Distorted segregation of *Ef-1* gene in B₁F₁ hybrids: Reciprocal crossings between F₁ X Taichung Native 1 and Taichung Native 1 X F₁ were used to assess the segregation of *Ef-1* gene. Segregation ratios were quite different among the combination (Table 3). The segregation ratios were 32 and 29, fitting the expected 1:1 ratio, when F₁ hybrids of the Taichung Native 1 X linkage gene combination were used as the male parents and Taichung Native 1 was used as female parents. When the F₁ hybrids were used as the female parents, the ratio was 19:7 in the reverse combinations, being distorted from the 1:1 ratio significantly. The genotype in F₁ population obtained from Taichung Native 1 X linkage gene had good seed fertility ranging from 78.9% to 73.1% in different location (Table 4). Rha (2000) found that using B₁F₁ hybrids of backcrosses, the transmission rate of phosphoglucose isomerase isozyme gene was significantly not fitted with the expected value of 25%. Therefore the distorted segregation is not related with F₁ sterility but was controlled by a gametophyte gene, and competition occurred for differential pollen fertilization. It is suggested that the mean of seed fertility could be explained by pollen competition on the basis of these results.

Linkage relationship between *Ef-1* gene and gametophyte gene: Recombination value can be estimated between a gametophyte gene and *Ef-1* gene in F₃ data which was obtained from the heterozygous F₂ plants. The *Ef-1* gene segregation in each of F₃ progenies heterozygous for the earliness was investigated. The distorted segregation for the *Ef-1* showing the F₃ progeny was easily distinguished from the normal or distorted segregation. Recombination value can be estimated between a gametophyte gene and *Ef-1* gene from F₃ data when natural crossing the heterozygous F₂ plants. Only 7 lines indicated the normal segregation while 24 lines showed the distorted segregation out of 31F₃ tested progenies. The recombination value between *Ef-1* gene and gametophyte gene was calculated to be 24.2±5.4 % (Table 5). It was reported that distorted segregation was often found within *Oryza sativa* is most likely associated with gametophyte gene in microspores (Sano, 1990; Lin & Ikehashi, 1991). A linkage tester has two gametophyte genes (*ga-7*, *ga-9*) (Meakawa & Kita, 1985), which belonged to the third linkage group. Actually, their genes for distorted segregation were reported from the crosses between two varieties (Rha *et al.*, 1995, 1996). In most cases, altered segregation occurred in F₂ and in the backcrosses only when the F₁ was used as pollen parent.

On the basis of these results, it is suggested that the gametophyte genes might be related to the reproductive barrier, which exists between Indica and Japonica. Therefore, to understand the evolutionary processes of the genetic analysis determined in this experiment may be beneficial not only for breeding work, but also for investigating the functions and genetic bases of reproductive barriers in rice.

Table 1. Distorted segregation pattern for *Ef-1* in F_2 populations derived from F_1 hybrids. F_1 hybrids of the cross combinations between cultivar and linkage tester which were grown in different locations.

Cross combination	Growing location (F_1)	Number of F_2 plants		Frequency of recessive type (%)	Goodness of fit (3:1)
		Dominant	Recessive		
Taichung native 1 X linkage tester	Jeonju	476	96	18.9	23.77**
	Sunchoen	108	16	14.8	9.8*

** : $p < 0.01$

Table 2. Distorted segregation of *Ef-1* in F_2 population of reciprocal cross.

Cross combination	Number of F_2 plants		Frequency of recessive type (%)	Goodness of fit (3:1)
	Dominant	Recessive		
Taichung native 1 X linkage tester	238	41	4.7	15.8**
Linkage tester X taichung native 1	105	16	13.2	10.2**

** : $p < 0.01$

Table 3. Segregation of *Ef-1* in B_1F_1 hybrids derived from the backcross between cultivar and linkage tester.

Cross combination	Number of F_2 plants		Goodness of fit (3:1)	
	Dominant	Recessive		Total
Taichung native 1 X F_1	32	29	61	0.02**
F_1 X taichung native 1	19	7	26	8.14**

** : $p < 0.01$

Table 4. Percentage of seed fertility in F₁ hybrid of the cross between cultivars and linkage tester which were grown in different locations.

Cross combination	Growing location (F₁)	Seed fertility of F₁ hybrid (%)
Taichung native 1 X Linkage tester	Jeonju	78.9
	Sunchoen	73.1

** : p<0.01

Table 5. Recombination value between linkage tester and gametophyte gene.

Cross value* combination	Type of F₃ segregation			Recombination
	Low frequency type	Normal frequency type	High frequency type	
Taichung Native 1 X linkage tester	20	7	4	31
				24.2 ± 5.4

* : Recombination value (%) calculated by maximum likelihood method.

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