Distorted segregation of the esterase isozyme genotypes in barley (*Hordeum vulgare* L.)

Takeo KONISHI^{1,*}, Kyoko ABE¹, Seiji MATSUURA¹
and Yuko YANO²

¹Research Institute for Bioresources, Okayama University, Kurashiki 710

²Institute of Genetic Resources, Faculty of Agriculture,
Kyushu University, Fukuoka 812

(Received 6 August 1990)

ABSTRACT

Distorted segregation of the esterase isozyme genotypes was observed in F₂ population which was produced from a cross combination between 'Ko A' and 'Mokusekko 3' of barley varieties. After examining the segregation of esterase isozyme genotypes in B₁F₁ hybrids derived from the reciprocal backcrosses, it was made clear that the distorted segregation was caused by certation between pollens of different genotypes, independent of the female genotypes. Furthermore, the certation was controlled by a newly designated gene, *Ga*₂, at the locus which was linked with the multilocus, *Est*₁, *Est*₂ and *Est*₄, for esterase isozymes at the long arm of chromosome 3.

The distorted segregation ratios did not significantly vary among F₂ populations derived from F₁ hybrids of the same combination which were grown in different years. Mechanically mixed pollens of the parents, however, could not induce the certation.

1. INTRODUCTION

In barley, only a few genetic studies on distorted segregation have been conducted, although such phenomena were observed sometimes in hybrid populations. Tabata (1961) first reported the distorted segregation of waxy individuals in a hybrid population between 'Coast II' and 'Waxy Orderbrucker', and proposed a gametophytic factor, *Ga*, which was linked with the waxy locus *wx*, on chromosome 1. Takahashi and Hayashi (1966) also observed the distortion of *cu*₂ segregation in several hybrid populations of 'Choshiro-hen' carrying the *cu*₂ gene for curly growth, suggesting that the distorted segregation might be due to the certation between pollens of different genotypes.

In a study of linkage between two loci for resistance to Barley Yellow Mosaic Virus (BaYMV) and esterase isozymes, the present authors happened to find the distorted segregation for esterase isozyme genotypes in F₂ population between

* Present address: Institute of Genetic Resources, Faculty of Agriculture, Kyushu University, Fukuoka 812, Japan.

'Ko A' and 'Mokusekko 3', and genetically analyzed the phenomenon.

2. MATERIALS AND METHODS

F₁ hybrids which were derived from a cross combination between 'Ko A' and 'Mokusekko 3' were grown in the field every year from 1985 to 1988, and segregation of the esterase isozyme genotypes in their F₂ seedlings was examined. Furthermore, the reciprocal backcrosses were made, and their B₁F₁ individuals were also tested for the esterase isozyme genotypes. For linkage analysis between two loci for the distorted segregation and esterase isozymes, segregation of the esterase isozyme genotypes in each of the F₃ progenies heterozygous for esterase isozymes of the same single cross combination was examined, and the recombination value was estimated by the following formulae which were proposed by Nakagahra et al. (1972).

The recombination value (p) is

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

where a , b and c are the numbers of F₃ progenies exhibiting deficient, normal and excess segregation of the esterase isozyme genotype of 'Mokusekko 3', respectively.

The standard deviation of recombination value (s_p) is

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

The mechanically mixed pollens which were produced from the parents, 'Ko A' and 'Mokusekko 3', in different proportions were pollinated on each of the parents, and their hybrids were tested for the segregation of the esterase isozyme genotypes.

The esterase isozyme genotypes in a hybrid population were examined individually at the seedling stage by the means of a starch gel electrophoresis described by Konishi et al. (1989).

3. RESULTS

Segregation in F₂ populations derived from the hybrids which were grown in different years

Table 1 indicates segregation of the esterase isozyme genotypes in F₂ populations derived from F₁ hybrids between 'Ko A' and 'Mokusekko 3' which were grown in different four years, 1985–1988. When the certation did not occur, the segregation of P₁:F₁:P₂ genotypes in F₂ population should be in a ratio of 1:2:1 theoretically. However, in this cross combination, the segregation was distorted significantly in every F₂ population: The frequency of segregants carrying the P₁ genotype was much higher than the theoretical value (25%), and that of the P₂

Table 1. Segregation of the esterase isozyme genotypes in F_2 populations derived from F_1 hybrids between 'Ko A' (P_1) and 'Mokusekko 3' (P_2) which were grown in different years, 1985–1988

Year	Segregation [#]				χ^2 (1:2:1)
	P_1 type	F_1 type	P_2 type	Total	
1985	104 (41.6%)	131 (52.4%)	15 (6.0%)	250	63.94***
1986	126 (42.0)	159 (53.0)	15 (5.0)	300	83.22***
1987	97 (39.1)	132 (53.2)	19 (7.7)	248	50.10***
1988	120 (40.1)	162 (54.2)	17 (5.7)	299	73.05***
Total	447 (40.7)	584 (53.2)	66 (6.0)	1,097	269.25***

[#] P_1 type: *Af-null-Su/Af-null-Su* genotype at *Est*₁-*Est*₂-*Est*₄ loci for esterase isozymes.

F_1 type: *Af-null-Su/Ca-null-Nz*. P_2 type: *Ca-null-Nz/Ca-null-Nz*.

*** Significant at the 0.1% level.

χ^2 for heterogeneity (years)=1.06 ($P=0.80-0.70$).

genotype was clearly lower. Meanwhile, the frequency of heterozygotes showing the same genotype as that of the F_1 hybrid was fitted well to the theoretical value (50%). Their distorted segregation ratios did not vary significantly among the populations, indicating that the distortion was not influenced by natural conditions under which the F_1 hybrids were grown in the field.

Segregation in B_1F_1 hybrids of the reciprocal backcrosses between 'Ko A' and 'Mokusekko 3'

As shown in Table 2, two kinds of genotypes were detected in the B_1F_1 hybrids of each backcross, but their segregation ratios were much different among the combinations. When F_1 hybrids of the single cross combination between 'Ko A' and 'Mokusekko 3' were used as the female parent, the segregation fitted well to a ratio of 1:1. In the reverse combinations of which the F_1 hybrids were pollinated

Table 2. Segregation of the esterase isozyme genotypes in B_1F_1 hybrids derived from the reciprocal backcrosses between 'Ko A' (P_1) and 'Mokusekko 3' (P_2)

Cross	Segregation [#]				χ^2 (1:1)
	P_1 type	F_1 type	P_2 type	Total	
$F_1^+ \times P_1$	32 (44.4%)	40 (55.6%)	—	72	0.89
$P_1 \times F_1$	87 (79.1)	23 (20.9)	—	110	37.24***
$F_1 \times P_2$	—	47 (46.5)	54 (53.5)	101	0.49
$P_2 \times F_1$	—	66 (88.0)	9 (12.0)	75	43.32***

[#] See the footnote in Table 1.

*** Significant at the 0.1% level.

⁺ F_1 hybrids derived from the cross combination between 'Ko A' and 'Mokusekko 3'.

as the male parent, however, the distorted segregation appeared, suggesting that the distortion was due to certation or selective fertilization. From these results, it is concluded that the certation is controlled by a single gene, Ga_2 , and competition occurs for fertilization between pollens carrying the different alleles, Ga_2 and ga_2 , after pollination. As to the allelic constitution of the parents, it is thought that 'Ko A' possesses the Ga_2 allele for the certation, while 'Mokusekko 3' should possess ga_2 . Furthermore, the degree of certation is not influenced by either of the female genotypes, 'Ko A' or 'Mokusekko 3'.

Linkage relationship between two loci for certation and esterase isozymes

Segregation for the esterase isozyme genotypes in each of F_3 progenies heterozygous for the esterase isozymes was examined, using 100 seedlings per progeny. The F_3 progeny showing the distorted segregation for the esterase isozymes was easily distinguished from that indicating the normal or non-distorted segregation, as shown in Fig. 1.

Of the 30 F_3 progenies tested, 23 exhibited the distorted segregation, while only 7 indicated the normal segregation. From the numbers of progenies of the both types, the recombination value between the two loci for the certation (Ga_2) and esterase isozymes (Est_1) was estimated to be $11.7 \pm 4.14\%$, using the formulae proposed by Nakagahra et al. (1972).

Fig. 1. Frequencies of the P_1 (Ko A) and P_2 (Mokusekko 3) genotypes in each of F_3 progenies derived from the F_2 heterozygotes for esterase isozymes. Open circle: distorted segregation. Closed circle: normal segregation ($P_1=P_2=25\%$).

Segregation in derivatives from pollination of mixed pollens

Fresh pollens were collected from the parents, 'Ko A' (P_1) and 'Mokusekko 3' (P_2), and mechanically mixed in different proportions of P_1 and P_2 pollens in volume; 100:0 (P_1 only), 75:25, 50:50, 25:75 and 0:100% (P_2 only), respectively. These mixed pollens were pollinated to each of the parents. After examining the esterase isozyme genotypes of the derivatives from pollination of mixed pollens, it could be detected how many pollens from each of the parents contributed to produce hybrids or self-pollinated individuals.

Table 3 shows frequencies of individuals exhibiting the allelic combination of 'Ko A' for esterase isozymes which were produced by pollens of 'Ko A' in the derivatives from mixed pollinations, together with numbers of derivatives examined. The frequencies fitted well to the proportions of 'Ko A' pollens among mixed pollens, indicating that no certation occurred by pollination with mechanically mixed pollens of the parents.

Table 3. Frequencies of individuals exhibiting the allelic combination of 'Ko A' for esterase isozymes (*Af-null-Su*) in the derivatives from pollination of mechanically mixed pollens between 'Ko A' and 'Mokusekko 3' in different proportions to each of the parents

Female	Male		Proportion of mixed pollens (%)			
	Ko A	100	75	50	25	0
	Mokusekko 3	0	25	50	75	100
Ko A		100.0 (32)*	77.0 (74)	56.4 (94)	15.6 (90)	0.0 (38)
Mokusekko 3		100.0 (9)	81.7 (60)	43.9 (66)	25.8 (66)	— —
Total		100.0 (41)	79.1 (134)	51.3 (160)	19.9 (156)	0.0 (38)

* Number of derivatives examined.

$\chi^2=4.018$ for the female parent of 'Ko A' ($P=0.2-0.3$).

$\chi^2=0.856$ for the female parent of 'Mokusekko 3' ($P=0.8-0.9$).

4. DISCUSSION

Distorted segregation of the esterase isozyme genotypes was observed in F_2 populations between 'Ko A' and 'Mokusekko 3'. Pollen fertility and seed set of the F_1 hybrids were normal or more than 95%, respectively. Furthermore, using B_1F_1 hybrids of their reciprocal backcrosses, it was made clear that segregation of the esterase isozyme genotypes in female gametes was regular ($P_1:P_2=1:1$), indicating that the distortion was independent of the female genotypes. From these results, it may be concluded that the distortion is caused by certation or

difference in fertilization between the pollen genotypes.

In the present study, the esterase isozyme genotype is a useful marker for examining the distorted segregation. Because, the isozyme alleles at the same locus are codominant, and the Ga_2 locus is linked with the esterase isozyme loci (Est_1 , Est_2 and Est_4) which are tightly linked at the terminal of the long arm of chromosome 3 (Konishi and Matsuura, 1987). The Ga_2 gene acts in the gametophytic phase of pollen, but expression of the gene can not be detected directly. Therefore, the distorted segregation of the esterase isozyme genotypes in a hybrid shows indirectly the difference in alleles at the Ga_2 locus between the parents. And the magnitude of distorted segregation was not influenced by different environmental conditions under which the F_1 hybrids were grown, suggesting that the action of the Ga_2 gene is stable.

The distorted segregation did not occur by pollination of mechanical mixture of Ga_2 and ga_2 pollens. In maize, however, the pollination of mixed pollens generally produced the distorted segregation (Ottaviano et al., 1982, 1988). The reason for the difference between barley and maize is uncertain. If the pollens carrying the ga_2 gene in the F_1 hybrids are already killed before fertilization, a half of the pollens should be sterile, but all the pollens are so well stained that the pollens seem to be fertile.

REFERENCES

- KONISHI, T., KAWADA, N., YOSHIDA, H. and SOHTOME, K. (1989) Linkage relationship between two loci for the barley yellow mosaic resistance of Mokusekko 3 and esterase isozymes. *Japan. J. Breed.* **39**, 423-430.
- KONISHI, T. and MATSUURA, S. (1987) Linkage analysis of $Est-4$ locus for esterase isozyme-4 in barley. *Barley Genet. Newsl.* **17**, 68-70.
- NAKAGAHARA, M., OMURA, T. and IWATA, N. (1972) Gametophyte genes and their loci on the eleventh linkage group of cultivated rice. *Japan. J. Breed.* **22**, 305-312.
- OTTAVIANO, E., PETRONI, D. and PE, M. E. (1988) Gametophytic expression of genes controlling endosperm development in maize. *Theor. Appl. Genet.* **75**, 252-258.
- OTTAVIANO, E., SARI GORLA, M. and PE, E. (1982) Male gametophytic selection in maize. *Theor. Appl. Genet.* **63**, 249-254.
- TABATA, M. (1961) Studies of gametophytic factor in barley. *Jpn. J. Genet.* **36**, 157-167.
- TAKAHASHI, R. and HAYASHI, J. (1966) Inheritance and linkage studies in barley. II. Assignment of several new mutant genes to their respective linkage groups by the trisomic method of analysis. *Ber. Ohara Inst. landw. Biol., Okayama Univ.* **13**, 185-198.