Mutants for rice storage proteins. III. Genetic analysis of mutants for storage proteins of protein bodies in the starchy endosperm

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ABSTRACT

Two mutants for rice storage protein in the starchy endosperm, 13b-L and 57-H, were crossed with their original variety, Kinmaze. From the results obtained in the F_1 and F_2 seeds, it was concluded that 13b-L and 57-H are controlled by single recessive genes, which were designated as *esp-1* and *esp-2*, respectively. The SDS-PAGE indicates that both mutant genes are regulatory genes for the protein bodies. The segregation ratio in F_2 of the cross between *esp-1* and trisomic type F and the cross between *esp-2* and trisomic type G fitted that of a trisomic segregation. These results suggest that *esp-1* and *esp-2* are located on chromosomes 10 and 9, respectively.

Key Words: rice, mutant, storage protein, SDS-PAGE, gene analysis.

1. INTRODUCTION

There are a large number of genes for seed storage proteins in cereals. *O2* (Opaque-2) is a regulatory gene (Soave *et al.* 1978) and some *Zp* genes are structural genes for zein subunits (Lee *et al.* 1976, Soave *et al.* 1982) which are alcohol soluble proteins in maize endosperm. Hordein, the storage protein of barley grain, consists of three groups of polypeptides called B, C and D hordein. Each group is coded by a complex locus designated as *Hor 2*, *Hor 1* and *Hor 3*, respectively (Blake *et al.* 1982, Faulks *et al.* 1981, Shewry *et al.* 1980, 1983). The studies on chromosomal localization of the genes for gliadin and glutenin which are alcohol soluble and acid and/or alkaline soluble proteins of wheat endosperm, respectively, have also been done (Galili and Feldman 1984, Jackson *et al.* 1983, Payne *et al.* 1980, 1982, 1984).

In rice, Kataoka (1978) and Kambayashi *et al.* (1984) reported that the grain protein content is controlled by polygenes, but there are few studies on the major gene for grain protein. Tanaka *et al.* (1980) isolated and characterized two types of protein bodies from rice endosperm. One type of protein body

(PB-I) was a spherical particle with lamellar structure 1–2 μm in diameter. The other type (PB-II) was stained homogeneously and had no lamellar structure. PB-I is the deposition site of prolamin and PB-II is that of glutelin. The polypeptide constitution of protein bodies can be distinguished by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) analysis of crude protein extracted from rice endosperm. In previous studies, four types of mutants for storage proteins were selected and some of them have been evaluated as suitable breeding material of rice endosperm protein (Kumamaru *et al.* 1987). These were mutants in which the polypeptide composition in the protein bodies in rice endosperm were remarkably changed (Kumamaru *et al.* 1986). The present study deals with the genetic behavior of the gene for each polypeptide in two mutant lines, and the identification of the chromosome on which these genes are located.

2. MATERIALS AND METHODS

Materials: Two mutant lines for seed storage protein, CM 21 and CM 1787, were used in this experiment. They were induced by N-methyl-N-nitrosourea from a rice cultivar, Kinmaze, and have been stocked at Plant Breeding Laboratory, Kyushu University and were screened by Kumamaru *et al.* (1987). CM 21 is a mutant which has a remarkably lower intensity of the 13b polypeptide band and a slightly higher intensity of 57 kDa polypeptide band than that of Kinmaze (Fig. 1). In CM 1787, the intensity of the 57 kDa and 10–39 kDa polypeptide bands is higher and lower, respectively, than that of Kin-

Fig. 1. SDS-PAGE of the storage protein in the F_1 and F_2 seeds of the cross between Kinmaze and CM 21.

1) Kinmaze, 2) F_1 seed of the cross between Kinmaze $\times$ CM 21, 3) F_1 seed of the cross between CM 21 $\times$ Kinmaze, 4) CM 21, 5–8) CM 21 type (13b-L) in F_2 seeds, 9–14) Kinmaze type (normal) in F_2 seeds.

Fig. 2. SDS-PAGE of the storage protein in the F_1 and F_2 seeds of the cross between Kinmaze and CM 1787.

1) Kinmaze, 2) F_1 seed of the cross between Kinmaze $\times$ CM 1787, 3) F_1 seed of the cross between CM 1787 $\times$ Kinmaze, 4) CM 1787, 5-7) CM 1787 type (57-H) in F_2 seeds, 8-13) Kinmaze type (normal) in F_2 seeds.

maze (Fig. 2). Therefore, the mutant types of CM 21 and CM 1787 have been named 13b-L and 57-H, respectively (Kumamaru *et al.* 1987).

Cross: Reciprocal crosses were made between two mutant lines and Kinmaze. The proteins of F_1 seeds and F_2 seeds of each cross were analysed by SDS-PAGE.

Trisomic analysis was applied to determine the chromosome on which the genes for storage protein mutants are located. The trisomic series used in this study have been derived from a desynaptic mutant induced from Kinmaze (Sato unpublished). In the crosses of mutant lines with trisomic series, F_1 plants were classified into disomic and trisomic plants based on their phenotypic expression, as reported by Iwata *et al.* (1970). The storage proteins of F_2 seeds were analysed by SDS-PAGE.

Protein extraction: The individual seeds of F_1 and F_2 seeds were struck by hammer, and the tissue was then placed in a micro test tube (1.5 ml) with 600 μ l of 0.125 M Tris-HCl pH 6.8 containing 4% SDS, 8 M urea, 5% 2-mercaptoethanol and 20% glycerin and incubated for 4 hours at 30°C. After centrifugation (5 min., 15,000 r.p.m.), the supernatants were applied to SDS-PAGE.

SDS-PAGE: Extracted storage proteins of F_1 and F_2 seeds were electrophoresed using the discontinuous buffer system of Laemmli (1970) on a slab

gel and were stained by 0.05% Coomassie brilliant blue R-250 in 7% acetic acid and 50% methanol and destained by 7% acetic acid.

3. RESULTS AND DISCUSSION

Fig 1 shows the SDS-PAGE of the proteins of F_1 and F_2 seeds from the reciprocal crosses between Kinmaze and CM 21. SDS-PAGE patterns of both F_1 seed proteins were of the normal type, i.e., Kinmaze, and only two types, normal and 13b-L, were segregated in F_2 seeds, though the segregation of 57 kDa polypeptide in F_2 seeds is not clear. These results show that 13b-L is recessive to the normal and there is no gene dosage effect. The segregation mode in the F_2 seeds of the cross between CM 21 and an original cultivar are shown in Table 1. The segregation ratio showed a good fit to the expected 3:1 ratio. The result suggests that the 13b-L in CM 21 is controlled by a single recessive gene.

Table 1. *Segregation of normal and mutant types in F_2 seeds of the cross between Kinmaze and mutants*

Cross combination	Phenotype of F_1 seeds	Segregation in F_2 seeds			χ^2 (3:1)
		Normal	Mutant	Total	
Kinmaze $\times$ CM 21	Normal	163	48	211	0.57
$\times$ CM 1787	Normal	144	56	200	0.96

Fig. 2 shows the SDS-PAGE of the protein of F_1 and F_2 seeds from the reciprocal crosses between Kinmaze and CM 1787. Both F_1 seeds showed the normal type, and the normal and 57-H types were segregated in the F_2 seeds. Moreover, 57-H type always accompanied with lower intensity of 10–39 kDa polypeptides than that of Kinmaze. The concurrent change of these polypeptides may result from pleiotropic expression of gene for enriched 57 kDa polypeptide or close linkage between gene for 57 kDa and genes for 10–39 kDa polypeptides. The results show that, as with 13b-L, this trait is recessive to the normal and there is no gene dosage effect. The segregation in the F_2 seeds of the cross between CM 1787 and an original cultivar are shown in Table 1. The segregation ratio showed a good fit to the expected 3:1 ratio. The result suggests that 57-H in CM 1787 is controlled by a single recessive gene.

Trisomic analysis was applied to determine the chromosome on which these genes are located. The segregation pattern for 13b-L in the F_2 seeds of the crosses between CM 21 and five trisomic types is shown in Table 2. In the cross with trisomic type F, the segregation ratio of about 12:1 was observed

in the F_2 seed progenies from the trisomic F_1 plant and fitted to the trisomic ratio between 8:1 and 44:1. The result suggests that the gene for 13b-L is located on an extra chromosome of trisomic type F. As the extra chromosome of type F corresponded to chromosome 10 (Iwata and Omura 1971, 1975), it was concluded that the gene for 13b-L is located on chromosome 10.

Table 2. Segregation of normal and 13b-L in F_2 seeds of trisomic F_1 plants derived from the crosses between trisomics and CM 21

Type of trisomics (Chromosome)	Segregation in F_2 seeds			χ^2 (3:1)
	Normal	13bL	Total	
F (10)	190	16	206	32.63***
A (4)	41	9	50	1.31
E (11)	39	11	50	0.24
H (1)	41	9	50	1.31
L (2)	36	11	47	0.06

***: Significant at 0.1% level.

CM 1787 (57-H) was crossed with trisomic types B, C, D, E, F and G. The results are shown in Table 3. In the cross with type G, the segregation ratio was about 13:1 and significantly deviated from the 3:1 ratio. As the extra chromosome of type G corresponded to chromosome 9 (Iwata and Omura 1971, 1975), it was concluded that the gene for 57-H is located on chromosome 9.

Table 3. Segregation of normal and 57-H in F_2 seeds of trisomic F_1 plants derived from the crosses between trisomics and CM 1787

Type of trisomics (Chromosome)	Segregation in F_2 seeds			χ^2 (3:1)
	Normal	57-H	Total	
G (9)	186	14	200	34.56***
B (6)	38	12	50	0.03
C (7)	40	10	50	0.67
D (12)	42	8	50	2.16
E (11)	38	12	50	0.03
F (10)	44	6	50	4.51*

* and ***: Significant at 5% and 0.1% levels, respectively.

From the results obtained, it is suggested that the genes for 13b-L in CM 21 and 57-H in CM 1787 are designated as *esp-1* and *esp-2*, respectively, in which *esp* stands for endosperm storage protein.

The endosperm is triploid, with two of the genes from the maternal parent. Gene dosage effect has been reported to occur in F_1 and F_2 seed storage protein

in barley (Doll 1980), wheat (Burnouf *et al.* 1983, Dhaliwal 1977) and maize (Soave *et al.* 1981, Valentini *et al.* 1979). In the case of the mutant for the structural gene coding the polypeptide, the gene dosage effect, if any, should appear in the polypeptides of F_1 and F_2 seeds. In this study, the two polypeptides, the 13b and 57 kDa, showed no gene dosage effect (Fig. 1 and Fig. 2). On the other hand, in IEF and bidimensional electrophoresis (IEF/SDS) system, the intensity of some polypeptides in the protein composition of PB-I in CM 21 were reduced compared to that in Kinmaze (Kumamaru unpublished). These facts suggest that *esp-1* is a mutation of the regulatory gene for the polypeptides, being analogous to the case of the *O2* gene in maize (Soave *et al.* 1978). It has been reported that glutelin is deposited in PB-II (Tanaka *et al.* 1980), and that subunit groups of glutelin, the 22 to 23 kDa and the 37 to 39 kDa polypeptides, are formed through post-translational modification of the 57 kDa polypeptide (Yamagata *et al.* 1982, Yamagata and Tanaka 1986). Therefore, the enriched 57 kDa and the reduced glutelin polypeptides in CM 1787 is supposed to be due to the abnormal processing of glutelin. For no gene dosage effect in F_1 and F_2 seeds, it is thought that *esp-2* is not a structural gene but one of the genes for the post-translational processing of glutelin.

In conclusion, it is inferred that *esp-1* is a mutant of regulatory gene for the polypeptide of PB-I, and *esp-2* is that for PB-II.

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