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Inheritance of two endosperm protein loci in rice (*Oryza sativa* L.)

L. F. O. Chen [1] and L. C. Chen [2]

[1] Institute of Botany, Academia Sinica, Nankang 11529, Taipei, Taiwan, Republic of China
[2] Department of Agronomy, Chia-Yi Agricultural Experiment Station, Chia-Yi 60014, Taiwan, Republic of China

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Summary. Previous studies indicated two types of phenotypic protein markers as two minor bands of SDS-PAGE for rice storage protein. A variant derived from a Pakistani variety, Dular, was found to show a mobility variant with Band 11, a relatively faster-moving band as compared to Band 10, while most of the other cultivated rices exhibited Band 10 at a molecular weight of around 100–110 K. Band 11 was also observed in several wild rice species. How this variant occurred is not known. Another marker is characterized by the presence of either Band 56 (slower-migrating band) or Band 57 (faster-migrating band) in most cultivars at a molecular weight of about 28–27 K. Most *indica* varieties developed in Taiwan have Band 57 and *japonica* varieties have Band 56. Genetic analysis of F_1, F_2 and F_3 seeds from interstrain crosses indicated that Band 10 versus Band 11 and Band 56 versus Band 57 are due to codominant alleles at two loci. Tests of independent inheritance between these two loci (Band 10/11 versus Band 56/57) indicated that there is no linkage between them. Both of these two protein loci encode for endosperm proteins and mostly belong to the minor polypeptide subunits of the glutelin fraction of rice seed proteins. Studies on reciprocal crosses indicate dosage effects as exhibited in band patterns. Variations in band intensity were frequently observed when the maternal genotype was different.

Key words: Rice (*Oryza sativa* L.) – Seed protein loci – Codominance – Inheritance – SDS-PAGE

Introduction

Rice (*Oryza sativa*) seed storage proteins have been characterized and intensively studied in the past decade (Juliano 1972; Juliano and Boulter 1976; Padhye and Salunkhe 1979; Yamagata et al. 1982; Yamagata and Tanaka 1986; Chen and Cheng 1986; Takaiwa et al. 1987). Most of these proteins were believed to accumulate in two types of protein bodies of starchy endosperm (Tanaka et al. 1980; Yamagata and Tanaka 1986). Rice endosperm is a triploid tissue derived from two dosages of maternal genes and one dosage of paternal genes.

Previously, most genetic studies on characters of rice endosperm were primarily on waxy gene and amylose content (Iwata and Omura 1971; Sano 1984; Okuno 1978; Kumar and Khush 1988). Okuno (1978) indicated the gene dosage effect of waxy alleles on the amylose content in endosperm starch of rice. Kumar and Khush (1988), from the study on inheritance of amylose content in rice, pointed out that different dosage effects tended to occur in different crosses. Protein content in rice grain was reported to be controlled by polygenes (Kataoka 1978; Kambayashi et al. 1984). Varietal differences in electrophoretic zymograms of rice seed proteins have been recently documented (Sarkar and Bose 1984; Damardjati et al. 1985; Endo 1987; Chen et al. 1987). Takaiwa et al. (1987) pointed out by Southern hybridization that there were four or five copies of glutelin genes per haploid rice genome. Recently, Kumamaru et al. (1987) assigned two genes for rice storage proteins in the starchy endosperm on chromosome 9 and 10, by using two mutants derived from the N-methyl-N-nitrosourea treatment. Chen et al. (1987) reported two potential phenotypic markers for rice seed protein profiles, after sceening 118 rice cultivars. A variant with an extra minor band in the high molecular weight zone was observed in Dular. This extra minor band was shown to be a mobility variant of normal type. Another phenotypically distinct marker in rice endosperm proteins was the presence of a minor band of either Band 56 or Band 57 in most rice

cultivars at a molecular weight of around 27–28 K (Chen et al. 1987). We studied the inheritance of these two previously reported markers in rice.

Materials and methods

Materials

Two accessions of rice, Dular'a' and Dular'b', were used in this investigation. Dular'a' has an extra band in the high molecular weight region (>66 K), which was identified to be a mobility variant with a relatively faster-migrating Band 11 instead of the normal Band 10 present in the second accession Dular'b'. The nomenclature for numbering bands follows Bushuk and Zillman (1978) and Chen et al. (1987). The Dular'a' accession was received from IRRI (International Rice Research Institute, Manila, the Philippines) and was maintained as a breeding line at the Chia-Yi Agricultural Experiment Station. The Dular'b' accession was collected from TARI (Taiwan Agricultural Research Institute, Wu-Feng, Taichung, Taiwan, ROC). We hereafter refer to Dular'a' as Dular(CY) with variant band and Dular'b' as Dular(TA) with normal band. At least 50 seeds of the Dular (CY) were assayed individually by SDS-PAGE and all showed the variant allele. Reciprocal crosses between Dular(CY) and Dular(TA) were made, and F_1 and F_2 seeds were assayed for this variant allele. Some F_3 seeds of various crosses were also analyzed for phenotypic segregation of this variant allele.

Another locus in the molecular weight between 25 and 29 K shows at least two migration types of one minor band. Most *japonica* varieties developed in Taiwan have the slower-moving band (Band 56) and the *indica* varieties have the faster-moving band (Band 57) in this zone. Both Dular(CY) and Dular(TA) have the faster-moving band (Band 57). Koshihikari from Japan exhibits a slower-moving band (Band 56). Crosses were made between Koshihikari and Dular(TA). F_1, F_2 and F_3 seeds were checked for the genetic segregation of Bands 56 and 57.

Extraction and preparation of protein samples

Principal procedures for protein extraction and sample preparation followed Chen et al. (1987), except that samples were prepared on single grain basis. For each sample, 180 µl extraction buffer was applied.

SDS-PAGE (Sodium dodecyl sulfate – Polyacrylamide gel electrophoresis)

Gel preparations and electrophoretic procedures were the same as those described by Chen et al. (1987). For each gel, samples of two parental types were always included and Sigma SDS-7 molecular weight standards were applied. After electrophoresis, gels were stained by Coomassie Brillant Blue (0.12% (w/v) Coomassie blue, 50% methanol and 10% acetic acid) for 2 h and destained in 30% methanol with 10% acetic acid overnight with several changes. Band types of each individual was recorded.

Identification of tissue-specific and protein fraction of two loci

Polished rice grains from Taichung native no. 1, Koshihikari and Dular (CY) were analyzed for SDS-PAGE to clarify whether the examined loci were actually encoded for endosperm proteins, by comparing the profiles with the dehulled rice (including endosperm, embryo, pericarp and aleurone layer). Band types of the loci studied occur in both, revealing the endosperm protein characters. Sequential fractionation of seed storage proteins was also carried out, for further identification of protein fraction which the two loci encoded. The procedures of sequential fractionation were that of Chen and Cheng (1986).

Results

Analysis of Bands 10 and 11

SDS-PAGE analysis of crosses between Dular(CY) and Dular(TA) indicated that the reciprocal F_1 seeds exhibit both Band 10 and Band 11, however, variation in the band intensity of these two bands was frequently observed. In the F_2 seed populations, 1:2:1 segregation for Band 10:Band 10/11:Band 11, respectively, was observed (Fig. 1 and Table 1). At least 50 seeds of the parental types were analyzed and all were found to be homozygous. In addition, two other lines, IR1905 and Tetep (homozygous for Band 10), were crossed with Dular(CY). F_3 seeds of the two derived hybrid populations were also assayed for the segregation of Bands 10 and 11

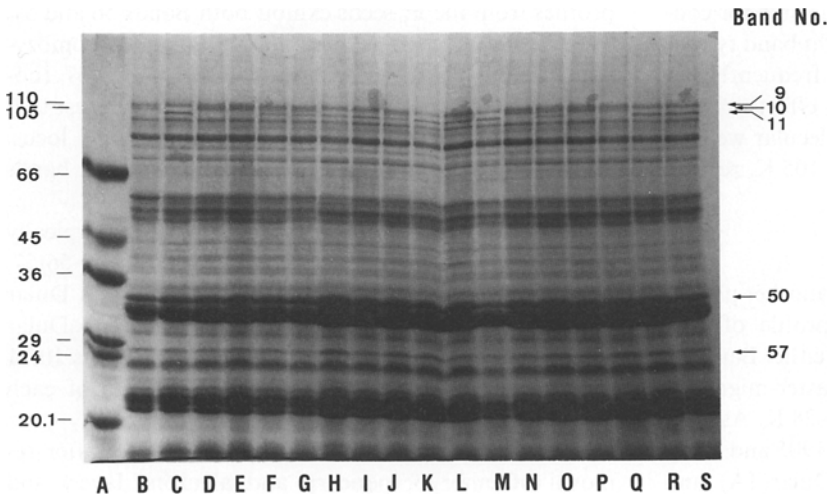

Fig. 1. Seed protein profiles of SDS-PAGE segregating for Band 10/11 from F_2 seeds of the cross between Dular (CY) and Dular(TA). *A*: protein molecular weight standard; *B* check variety–Taichung native no. 1; *C*: Dular(CY); *D*: Dular(TA); *E–S*: F_2 individuals. *B, D, E, N, P* and *Q* are homozygous for Band 10; *C, H, K, M, R* and *S* are homozygous for Band 11; *F, G, I, J, L* and *O* are heterozygous for Band 10/11

Table 1. Inheritance of mobility variants at the Band 10/11 locus in rice seed storage proteins

Crosses and genotypes	Generation	No. of progeny in each genotypic class			n	χ^2 1:2:1	p
		10/10	10/11	11/11			
Dular(TA) × Dular(CY) (10/10) (11/11)	F_2[a]	53	127	60	240	1.23	>0.25
Dular(CY) × Dular(TA) (11/11) (10/10)	F_2	73	146	70	289	0.10	>0.90
Dular(CY) × IR1905 (11/11) (10/10)	$F_{2:3}$	19	48	21	88	0.82	>0.50
	F_3/F_2	125	244	145	514	2.88	>0.10
Tetep × Dular(CY) (10/10) (11/11)	$F_{2:3}$	10	17	13	40	1.35	>0.50
	F_3/F_2	41	62	33	136	2.00	>0.25
Total		321	644	342	1307	0.95	>0.50

[a] F_2 = F_2 genotypes determined from zymotype of F_2 individuals
$F_{2:3}$ = F_2 genotypes determined by progeny test of eight F_3 individuals per F_2-derived family
F_3/F_2 = genotypes of F_3 individuals within segregating F_2 families

Table 2. Inheritance of mobility variants at the Band 56/57 locus in rice seed storage proteins

Crosses and genotypes	Generation	No. of progeny in each genotypic class			n	χ^2 1:2:1	p
		56/56	56/57	57/57			
Koshihikari × Dular(TA) (56/56) (57/57)	F_2[a]	78	165	82	325	0.34	≥0.90
Dular(CY) × IR1905 (57/57) (56/56)	$F_{2:3}$	22	33	13	68	2.44	>0.25
	F_3/F_2	121	223	126	470	1.33	>0.50
Tetep × Dular(CY) (56/56) × (57/57)	$F_{2:3}$	10	17	13	40	1.35	>0.50
	F_3/F_2	85	172	102	359	2.33	>0.25
Total		316	610	336	1262	2.03	>0.25

[a] F_2 = F_2 genotypes determined from zymotype of F_2 individuals
$F_{2:3}$ = F_2 genotypes determined by progeny test of eight F_3 individuals per F_2-derived family
F_3/F_2 = genotypes of F_3 individuals within segregating F_2 families

(Table 1). The results indicate that a structural gene controlled by two codominant alleles is responsible for coding Band 10/11. In the heterozygotes, a light band type at either Band 10 or Band 11 location was frequently observed. This might suggest the dosage effects of the triploid character of endosperm. The molecular weights of Bands 10 and 11 are about 110 K and 105 K, respectively.

Analysis of Bands 56 and 57

As described in 'Materials and methods', another phenotypic distinct type in the SDS-PAGE profile of seed proteins of rice cultivars is the presence of either Band 56 (slower-migrating band) or Band 57 (faster-migrating band) at a molecular weight of about 27–28 K. As indicated in Table 2, parents Koshihikari, IR 1905 and Tetep are homozygous for Band 56, while Dular(TA) and Dular(CY) are homozygous for Band 57. Seed protein profiles from the F_1 seeds exhibit both Bands 56 and 57. The F_2 showed 1:2:1 segregation for Band 56 (homozygous), Band 56/57 (heterozygous) and Band 57 (homozygous) (Fig. 2 and Table 2). These data suggest that there are two codominant alleles at Band 56/57 locus. Variations in band intensity in the heterozygous bands were frequently observed.

The relationship between these two phenotypically distinct protein loci, Band 10/11 and Band 56/57, were studied from F_3 populations of the crosses Dular (CY) × IR1905, Dular(CY) × IR4547 and Tetep × Dular (CY). As the data of Table 3 show, loci for Bands 10/11 and Bands 56/57 are inherited independently of each other.

The seed protein profiles of polished rice (after removal of embryo, pericarp and aleurone layer) and

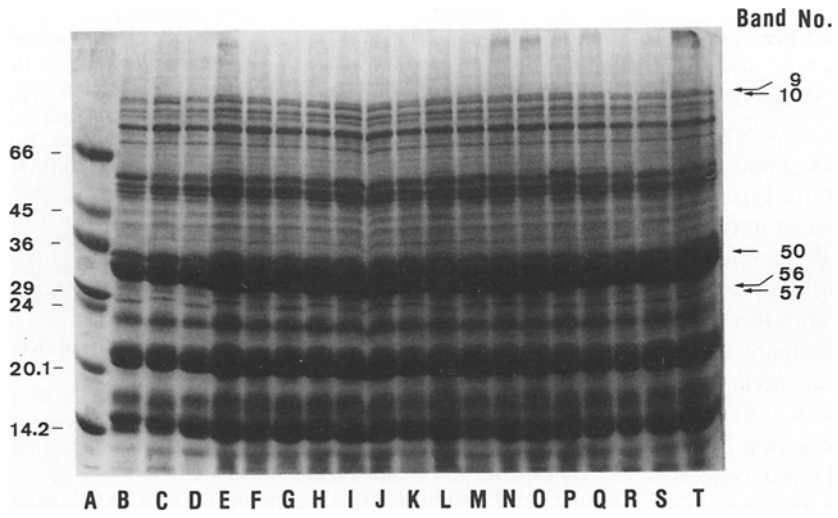

Fig. 2. Seed protein profiles of SDS-PAGE segregating for Band 56/57 from F_2 seeds of the cross between Dular(TA) and Koshihikari. *A*: protein molecular weight standards; *B*: check variety–Taichung native no. 1; *C*: Koshihikari; *D*: Dular(TA); *E–T*: F_2 individuals. *C, E, H, J, K* and *N* are homozygous for Band 56; *B, D, G, L, P* and *R* are homozygous for Band 57; *F, I, M, O, Q, S* and *T* are heterozygous for Band 56/57

Table 3. Test of independent inheritance between Band 10/11 and Band 56/57 loci by $F_{2:3}$ identification in rice seed storage proteins

Crosses[b]	Total	$F_{2:3}$ segregation for both loci[a]									χ^2	p
		1 : 2 : 1 : 2 : 4 : 2 : 1 : 2 : 1										
		Band										
		10 56	10/11 56	11 56	10 56/57	10/11 56/57	11 56/57	10 57	10/11 57	11 57		
Dular(CY) × IR1905	92	4	15	7	12	23	9	3	14	5	4.58	>0.50
Dular(CY) × IR4547	40	0	6	2	3	11	7	3	6	2	4.80	>0.50
Tetep × Dular(CY)	40	6	4	3	3	10	7	1	2	4	10.40	>0.10
Grand total	172	10	25	12	18	44	23	7	22	11	2.79	>0.90

[a] $F_{2:3} = F_2$ genotypes determined from progeny test of eight F_3 individuals per F_2-derived family
[b] Genotypes of Dular(CY) are Band 11/11 and Band 57/57, and Band 10/10 and Band 56/56 in IR1905, IR4547 and Tetep

brown rice (dehulled only) revealed that both Band 10 or 11 types and Band 56 or 57 types are present in both polished rice and brown rice. Therefore, protein bands encoded by loci Band 10/11 and Band 56/57 are endosperm characters. Bands 56 and 57 were more stable in assay in contrast to Bands 10 and 11, when the old stored seeds were used.

From sequential extractions, it appears likely that protein bands for both loci belong to minor subunits of glutelin fractions. Sequential extractions tend to lower the band intensity of Bands 10 and 11 in comparison with those of Bands 56 and 57. High molecular weight bands tend to degrade more easily when extracted samples were prolonged in the room temperature.

Discussion

Information on qualitative control of rice protein loci is limited. In this study, two loci for phenotypic protein markers were identified through SDS-PAGE in rice cultivars. Kumamaru et al. (1988) reported differences in storage protein on the basis of band intensity between normal and MNU induced mutant. The two endosperm protein loci, designated as *esp-1* and *esp-2*, were located on chromosomes 10 and 9, respectively (Kumamaru et al. 1987). The distinct variant types reported here are of a spontaneous nature and represent minor bands of rice storage proteins. Band 10 versus Band 11 and Band 56 versus Band 57 are characterized by mobility differences. These minor band show codominance, and single allele differences were detected in both Band 10/11 and Band 56/57.

Inheritance and genetic studies on rice protein loci are relatively rare (Kumamaru et al. 1987). Protein content in rice like other crops is controlled by polygenes (Kambayashi et al. 1984). Qualitative protein markers for major protein in rice are rare. Electrophoretic variations are mostly in minor bands (Chen et al. 1987; Damardjati

et al. 1985). The two protein loci described here can be easily analyzed as long as healthy seeds are properly stored. The loci, Band 10/11 and Band 56/57, are inherited independently (Table 3). We found most of *indica*-type rice has Band 57 and *japonica*-type rice has Band 56 in Taiwan's cultivated varieties. A possible third type with mobility in between Band 56 and 57 was also noted by Chen et al. (1987), however, it is difficult to separate from Band 57. Variations in band intensity were frequently observed in the heterozygotes of both loci. This might be due to the dosage effects of codominant alleles in the endosperm. In rice, inheritance of endosperm characters such as amylose content (Kumar and Khush 1988; Okuno 1978) and regulation of waxy gene action (Sano 1984; Okuno 1978) has shown differential dosage effects in different crosses. We are now studying more crosses to see if any cross combinations might exhibit differential dosage effects at these two loci.

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