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Chromosomal location of genes conditioning low amylose content of endosperm starches in rice, *Oryza sativa* L.

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Summary. Eight dull mutants that lower the amylose content of rice endosperm as well as waxy mutant and a cultivar with common grains were crossed in a diallele manner. The amylose content of F_1 and F_2 seeds was determined on the basis of single grain analysis. It was concluded that the low amylose content of dull mutants is under monogenic recessive control. Alleles for low amylose content are located at five loci designated as *du-1, du-2, du-3, du-4* and *du-5*. These loci are independent of *wx* locus located on chromosome 6. The five *du* loci have an additive effect in lowering the amylose content. Two loci, *du-1* and *du-4*, were found to be located on chromosomes 7 and 4, respectively.

Key words: Gene analysis – Endosperm mutants – Low amylose content – Dosage effect

Introduction

In rice, the amylose content of endosperm starch is one of the important characteristics determining eating and cooking qualities (Juliano et al. 1965; Kurasawa et al. 1969). Varietal differences in amylose content have been investigated in rice breeding programs for improving grain quality (Williams et al. 1958; Juliano et al. 1964; Reyes et al. 1965; Webb et al. 1968; Inatsu et al. 1974; Juliano 1979). These reports indicated that a wide diversity in amylose content existed among nonwaxy rice cultivars. It was reported that the amylose content in nonwaxy cultivars is determined by a single dominant gene with major effect and several modifying genes with minor effect (Bollich and Webb 1973; IRRI 1976; McKenzie and Rutger 1983). Kumar et al. (1987) reported that the amylose content in nonwaxy varieties was determined by multiple allelic series at the *wx* locus.

Recently, several mutants affecting the amylose content of starch in rice endosperm were produced by mutagenesis (Amano 1981; Satoh and Omura 1981; Okuno et al. 1983; Yano et al. 1985). Among them, mutants with low amylose content were characterized by dull endosperm and were designated as "dull" mutants. Okuno et al. (1983) reported that low amylose content in one of the dull mutants is controlled by a single recessive gene (*du*) that is nonallelic to the *wx* gene. Waxy plants are devoid of any amylose in the endosperm and in the pollen grains. The present study was undertaken to determine the allelic relationships of various *du* loci and their chromosomal location.

Materials and methods

Mutant stocks

Eight dull mutants and one waxy mutant were used in this study. These mutants were induced by treating fertilized egg cells of nonwaxy rice cultivar, Kinmaze, with N-methyl-N-nitrosourea (MNU) solution (Satoh and Omura 1981). Endosperms of all low amylose mutants were dull and could be distinguished from normal and waxy lines.

Mode of inheritance and tests for allelism

F_1 seeds from reciprocal crosses among the dull mutants, waxy mutant and Kinmaze were produced in greenhouse and harvested at maturity. The F_1 plants were grown in the paddy field and were harvested at maturity. The F_1 and F_2 seeds were dehulled and thoroughly milled. The amylose content of milled rice grains was determined on single grain basis using the colorimetric method with an Autoanalyzer (Technicon).

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Table 1. Amylose contents of F_1 seeds from the crosses between low amylose mutant lines and the original cultivar, Kinmaze

Line	Amylose content (%)[a]		
	Selfed	Kinmaze × Mutant	Mutant × Kinmaze
EM-12	4.1	15.5	13.2
EM-15	4.4	17.4	15.3
EM-57	3.8	15.4	12.5
EM-69	3.3	15.3	–[b]
EM-79	2.0	15.8	14.1
EM-85	3.7	16.5	13.4
EM-98	1.5	11.7	6.0
EM-140	5.7	12.3	14.1
Kinmaze	15.7		

[a] Amylose contents were calculated on the dry weight basis, indicating the mean value of five seeds
[b] No data

Table 2. Segregation for amylose content in F_2 seeds of the crosses between low amylose mutant lines and the original cultivar, Kinmaze

Cross	Segregation in F_2 seeds			χ^2 (3:1)
	High amylose	Low amylose	Total	
Kinmaze × EM-12	182	58	240	0.089
Kinmaze × EM-15	217	80	297	0.594
Kinmaze × EM-57	62	23	85	0.192
Kinmaze × EM-69	71	13	84	4.063*
Kinmaze × EM-79	76	20	96	0.889
Kinmaze × EM-85	82	27	109	0.003
Kinmaze × EM-98	74	23	97	0.086
Kinmaze × EM-140	81	28	109	0.028

* Significant at the 5% level

Chromosomal location of du genes

Two of the dull mutant lines, EM-12 and EM-98, were crossed to nine types of trisomics (Iwata and Omura 1975, 1976). F_1 plants of each cross were separated into disomic and trisomic plants based on plant morphology as described by Iwata et al. (1970). The F_2 seeds from disomic and trisomic F_1 plants were classified into dull and normal endosperm types. In the cross of trisomic A × EM-98, we inferred the genotypes of the F_2 individuals from the segregation pattern of endosperm character in the F_3 seeds.

Results

Amylose content of eight dull mutant lines ranged from 2.0–5.7%, compared with 15.7% of the original cultivar, Kinmaze (Table 1). The degree of translucency of endosperm of the mutants was correlated with the amylose content of endosperm starch. Amylose content of F_1 seeds from the reciprocal crosses between low amylose mutants and Kinmaze is shown in Table 1. The amylose content of F_1 seeds from the crosses of seven mutant lines (with the exception of EM-98) used as a pollen parent and Kinmaze as a maternal parent was similar to that of Kinmaze. A slight difference in amylose content existed between reciprocal crosses. In the cross of Kinmaze with EM-98, the amylose content of F_1 seeds was much lower than that of Kinmaze.

The frequency distributions for amylose content of F_2 seeds from the above-mentioned crosses are shown in Fig. 1. It seemed that the difference in amylose content of parents shown in Table 1 and Fig. 1 was caused by the temperature effect during the ripening period (Asaoka et al. 1984). In six crosses (excluding Kinmaze × EM-98 and Kinmaze × EM-140), the segregation for amylose content of F_2 seeds showed a bimodal distribution, corresponding to the amylose content of dull mutants and Kinmaze. The F_2 seeds from the crosses of Kinmaze × EM-98 and Kinmaze × EM-140 also segregated into

Table 3. Amylose content of F_1 seeds of the crosses among low amylose mutant lines and waxy mutant line. Figures in *parenthesis* are the amylose contents of self-pollinated seeds

Female parent	Male parent								
	EM-12	EM-15	EM-57	EM-69	EM-79	EM-85	EM-98	EM-140	EM-21
EM-12	(4.1)	13.4	3.1	–[b]	13.3	13.6	9.8	9.4	10.5
EM-15	–	(4.4)	–	13.3	–	1.9	–	–	12.0
EM-57	2.8	–	(3.8)	14.3	13.2	12.4	10.0	8.2	–
EM-69	13.8	15.0	13.5	(3.3)	3.1	15.0	9.6	10.5	–
EM-79	13.7	–	14.0	2.7	(2.0)	14.9	10.7	8.9	–
EM-85	12.5	4.0	13.3	13.4	11.1	(3.7)	11.4	11.7	–
EM-98	6.9	–	6.8	9.2	9.3	7.4	(1.5)	2.8	2.2
EM-140	15.1	–	14.6	16.6	16.1	(15.9)	11.4	(5.7)	12.1
EM-21[a]	7.5	–	7.8	8.3	8.4	8.4	1.0	3.2	(0)

[a] Waxy mutant line
[b] No data

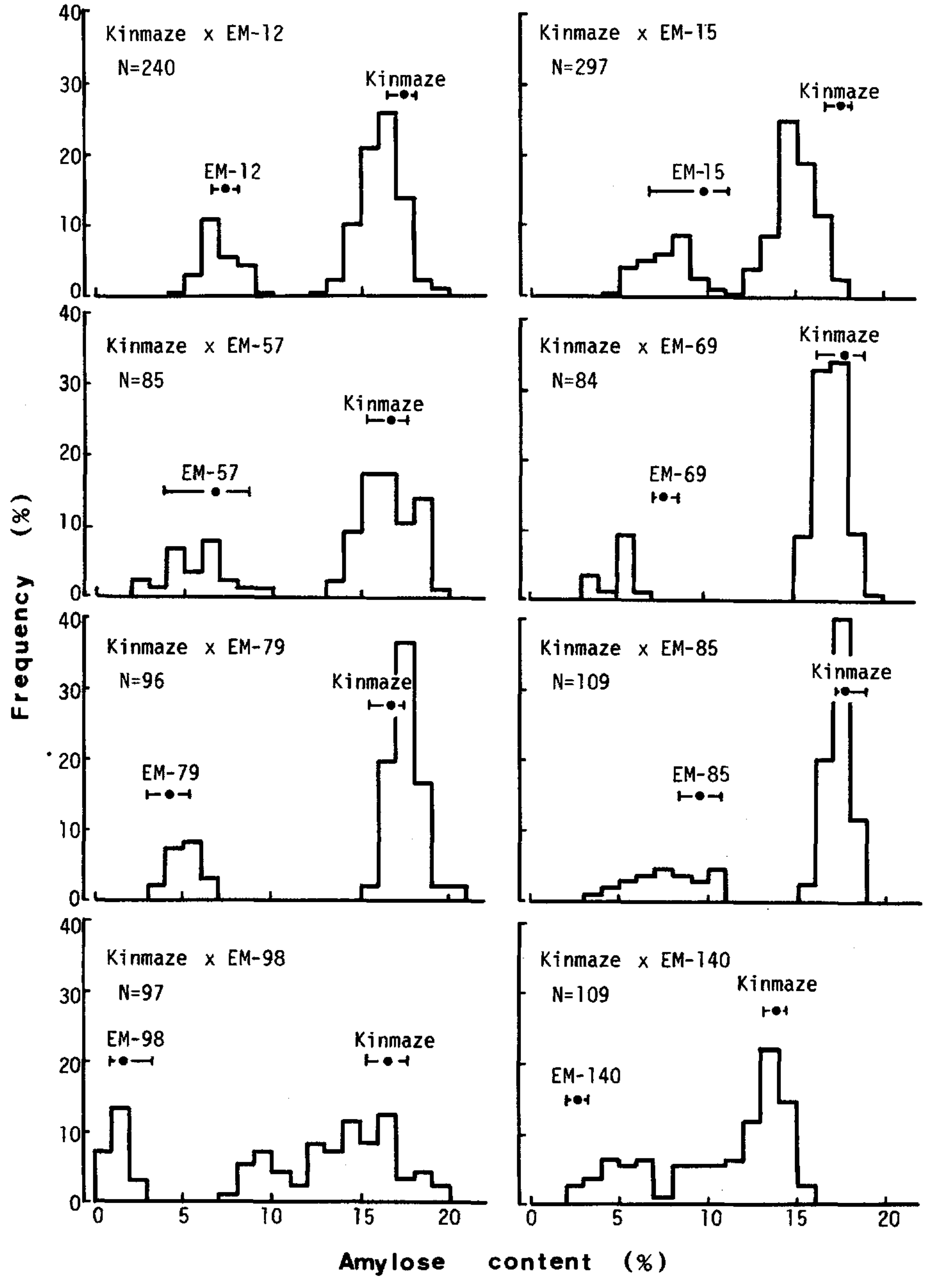

Fig. 1. Frequency distributions for amylose content of F_2 seeds from the crosses between the original cultivar, Kinmaze, and low amylose mutants lines; ⊢—●—⊣ indicates the mean values and the ranges of parents

two groups for amylose content, but the distribution was broad. The difference in F_2 segregation patterns for amylose content in some crosses suggested the presence of dosage effect of *Wx* and/or *du* alleles responsible for amylose production in rice endosperm. Thus, the reduction of amylose content in F_1 seeds and the wide variation of amylose content in F_2 seeds from the cross between Kinmaze × EM-98 were probably due to the dosage effect of low amylose allele on amylose content in the endosperm. Segregation ratios for amylose content in F_2 seeds are shown in Table 2. The segregation of F_2 seeds in all the crosses, except Kinmaze × EM-69, agreed with the expected 3 high : 1 low ratio. The segregation of F_2 seeds from the cross of Kinmaze × EM-69 deviated slightly from the expected 3 : 1 ratio. These results suggest that the low amylose content of dull mutants is determined by single recessive genes.

Amylose contents of F_1 seeds from the crosses among low amylose mutants are given in Table 3. When the amylose content of F_1 seeds was similar to those of both parents, the two low amylose mutant lines were considered allelic to one another. Consequently, it was concluded that the mutant pairs EM-12 and EM-57, EM-15 and EM-85 and EM-69 and EM-79 have the allelic genes

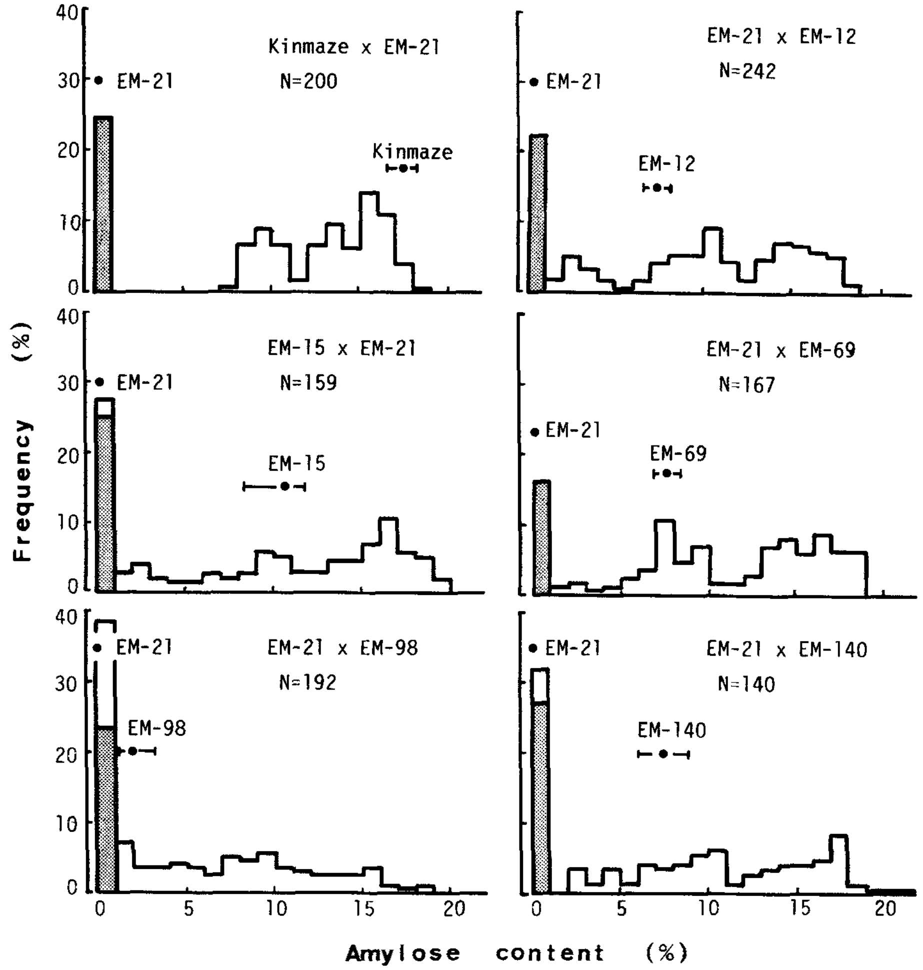

Fig. 2. Frequency distributions for amylose content of F_2 seeds from the crosses between low amylose mutant lines and waxy mutant line (EM-21); ⊢●⊣ indicates the mean values and the ranges of parents; ▨ indicates the F_2 seeds lacking amylose

for low amylose content. It was also concluded that the low amylose genes in EM-98 and EM-140 are nonallelic to each other and also nonallelic to three other loci for low amylose content. The amylose content of F_1 seeds from the crosses between dull mutants and waxy mutant was greatly reduced. These results do not necessarily indicate an allelic relationship between each *du* loci and the *wx* locus, since the amylose content of F_1 seeds between waxy and nonwaxy lines is known to be influenced by the dosage effect of waxy alleles.

We also examined the amylose content of F_2 seeds from the crosses between dull mutants and waxy mutant. The segregation patterns for amylose content in the F_2 seeds from all these crosses were more complicated, as compared to those from the crosses between waxy mutant and Kinmaze, and those between dull mutants and Kinmaze (Fig. 2). These complex segregations are presumably due to the nonallelic segregation of the *wx* and *du* alleles. The F_2 seeds could be divided into three types corresponding to the waxy mutant, the dull mutant and kinmaze, and types heterozygous for *wx* and *du* alleles. From the results, it was concluded that the *du* alleles are nonallelic to the *wx* gene. The proportion of normal seeds among the F_2 seeds from the cross EM-21 × EM-98 was lower than those from the other crosses, suggesting the dosage effect of *du* alleles of EM-98 on amylose content. These results lead us to conclude that all *du* genes are different from *wx* locus on which chromosome 6 is located.

The frequency distributions of F_2 seeds with different amylose levels from the crosses among five dull mutants are shown in Fig. 3. There was transgressive segregation for amylose content in all the crosses. The major proportion of F_2 seeds had amylose contents comparable to that of Kinmaze. In addition, the double recessive type seeds containing amylose content lower than both the parents segregated in the F_2 seeds of all the crosses. These results confirmed that the five mutant lines have nonallelic *du* genes. We propose that the genes for low amylose content be tentatively designated as *du-1* (EM-12 and EM-57), *du-2* (EM-15 and EM-85), *du-3* (EM-69 and EM-79), *du-4* (EM-98) and *du-5* (EM-140).

The segregation ratios for *du-1* in the F_2 seeds of the crosses between one of the dull mutants (EM-12) and

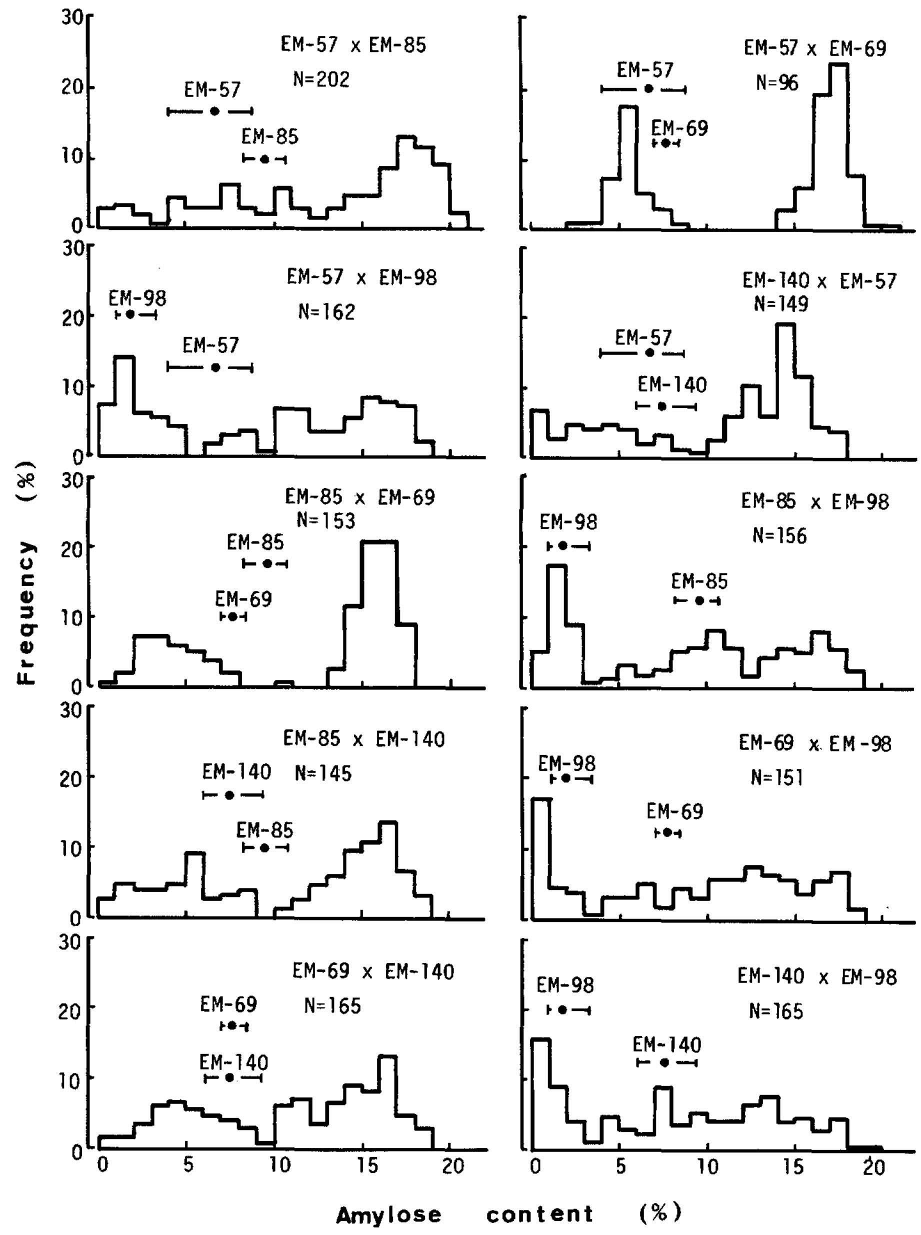

Fig. 3. Frequency distributions for amylose content of F_2 seeds from the crosses among low amylose mutant lines; ├─●─┤ indicates the mean values and the ranges of parents

nine types of trisomics are shown in Table 4. In the crosses with trisomic lines A, B, C and H, the segregation for dull endosperm in the F_2 seeds deviated from the expected 3 : 1 disomic ratio. When the recessive gene is located on the chromosome that is triplicate in the trisomic, the segregation ratios 8 : 1 and 44 : 1 are expected in disomic and trisomic portions of the F_2 population, respectively (Hermsen 1970). It was difficult to divide the F_2 seeds into disomic and trisomic fractions and the expected ratios could not be tested for fit to 8 : 1 and 44 : 1. However, the F_2 seeds of the cross with trisomic C segregated into 12 normal : 1 dull. These results showed that *du-1* is located on chromosome 7, which is the extra chromosome of trisomic C. We also examined the segregation ratio for *du-4* in the F_2 seeds of the crosses between EM-98 and nine types of trisomics. In the cross with trisomic A, the segregation for dull endosperm deviated from the expected 3 : 1 disomic ratio, although it was not typical for trisomic segregation (data not shown). We also examined the segregation pattern of F_2 individual genotypes in the cross of trisomic A × EM-98 (Table 5). In the disomic and trisomic portions of the F_2 population, the segregation agreed with the expected trisomic ratio. These results indicate that the gene, *du-4*, is located on chromosome 4, which is the extra chromosome of trisomic A.

Discussion

Endosperm starch in cereal grains is composed of two kinds of molecules; amylose, which is primarily a linear chain of D-glucose; and amylopectin, which is a branched structure. Several mutant genes are known to affect the amylose content of endosperm starches in maize (Collins 1909; Eryster 1934; Mangelsdorf 1947; Brimhall et al. 1945, Vineyard and Bear 1952). A few genes that affect the amylose content in rice endosperm have also been reported (Satoh and Omura 1981; Okuno et al. 1983; Yano et al. 1985). The genetic analysis of a dull mutant with low amylose content indicated that this trait is controlled by a single recessive gene (Okuno et al. 1983). Mutants that lower the amylose content in the endosperm, excepting *wx* gene, have not been reported in other cereal crops.

Table 4. Segregation for *du-1* in F_2 seeds of the crosses between nine types of trisomics and EM-12. Figures in *parenthesis* are the numerical number of extra chromosome of trisomics. Designation of chromosome are based on the reciprocal translocation analysis (Nishimura 1961)

Type of trisomics	F_1 plant type	Segregation in F_2 seeds			χ^2 (3:1)
		+	*du*	Total	
C (7)	2n[a]	185	53	238	0.95
	2n+1[b]	219	19	238	36.76***
A (4)	2n	239	52	291	7.89**
	2n+1	217	46	263	7.91**
B (6)	2n	189	45	234	4.15*
	2n+1	188	43	231	5.02*
D (12)	2n	–[c]	–	–	–
	2n+1	82	17	99	3.24
E (11)	2n	278	73	351	3.31
	2n+1	472	132	604	3.19
F (10)	2n	299	60	359	13.15***
	2n+1	142	39	181	1.15
G (9)	2n	290	80	370	2.25
	2n+1	207	59	266	1.13
H (1)	2n	270	70	340	3.53
	2n+1	302	57	359	15.93***
L (2)	2n	260	60	320	6.67**
	2n+1	64	19	83	0.20

[a] Disomic plant
[b] Trisomic plant
[c] No data
*, ** and *** significant at the 5%, 1% and 0.1% levels, respectively

The results of this study show that there are at least five different loci with alleles that lower the amylose content in rice. Two of these loci, *du-1* and *du-4*, are located on chromosome 7 and 4, respectively. Different *du* genes have different effects on lowering the amylose content. The occurrence of double recessive types homozygous for different *du* loci suggested that each of the five *du* loci had an additive effect in reducing the amylose content.

The amylose content is one of the characters expressed in the endosperm, which is the triploid tissue consisting of one nucleus from a male gamate and two polar nuclei form a female gamate. The amylose content of endosperm starches has been known to increase in proportion to the number of *Wx* alleles in endosperm cells of maize (Sprague et al. 1943; Tsai 1974) and rice (Heu and Park 1976a, b; Okuno 1978; Kumar and Khush 1986). In this study, the dosage effect of *Wx* alleles on amylose content was reconfirmed. However, varying doses of *du-1, du-2, du-3* and *du-5* did not affect the amylose content. The gene dosage effect of *du-4* on amylose content was, however, observed. These results suggest the existence of differences in the regulatory mechanism of amylose production in rice endosperm. These mutants may prove to be useful tools for studying the genetic control of starch biosynthesis in rice endosperm.

Different countries have preferences for rices with different amylose content as the amylose content is one of the main factors determining the eating quality of rice grains. In general, Japanese consumers prefer rices with lower amylose content because of the viscous texture of low amylose cooked rice. Therefore, rice breeding programs in Japan have aimed at the development of cultivars with lower amylose content. The availability of *du*

Table 5. Trisomic segregation of *du-4* in the cross of trisomic A × EM-98. The genotypes of F_2 individuals were inferred from the segregation pattern of endosperm character in the F_3 seeds

Portion of F_2	Segregation in F_2					χ^2 value
Disomic plant	+ +	+*du*	*dudu*		Total	(4:4:1)
	32	33	8		73	0.02
Trisomic plant	+ + +	+ +*du*	+*dudu*	*dududu*	Total	(12:22:10:1)
	9	24	14	0	47	3.30

mutants opens up the possibility of developing rice cultivars with very low amylose content.

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