New Endosperm Mutants Modifying Starch Characteristics of Rice, *Oryza sativa* L.

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Few genes which control the proportion of amylose and amylopectin components in starch granules have been found in rice, since waxy alleles were detected and located on Chromosome 6.[6,9] Recently, several kinds of endosperm mutants of rice were successfully induced by Okuno,[10] and Satoh and Omura.[13] The present report deals with newly induced mutants which are characteristic of the modification of starch properties in the endosperm of rice grains.

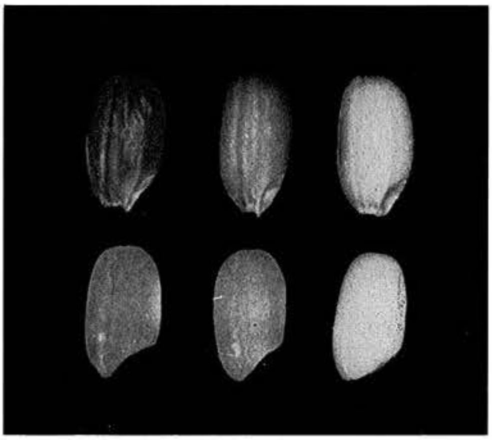

Plate 1. Endosperms of waxy mutant (right), dull mutant (middle) and nonwaxy counterpart (left)

Low amylose mutants[10,12]

1) Grain appearance

The endosperms of the mutants were compared with the nonwaxy counterpart (Plate 1). The endosperm of a new mutant became dull after drying and was visually distinguishable not only from waxy but also from nonwaxy endosperms. This mutant was designated as "dull" which is characteristic of dull endosperm.

2) Starch properties

The starch granules were isolated by using the alkali digestion method[14] from the endosperms of waxy and dull mutants which were induced by β-ray internal irradiation of the nonwaxy rice cultivar, Norin 8. The starch components were debranched with crystalline *Pseudomonas amyloderamosa* isoamylase and fractionated on a Sephadex G-75 column.[3,16] The amount of carbohydrate and the number of reducing ends in each fraction were determined by the phenol sulfuric acid method and a modification of Park-Johnson's method, respectively. The average length of unit chains was calculated from the division of the amount of carbohydrate by the number of reducing ends. Each fraction was divided according to the absorption maximum (λmax) of the iodine-carbohydrate complexes as follows: Fr. I, $\lambda max \geqq 620$ nm, intermediate Fr., $620 \text{ nm} > \lambda max \geqq 600$ nm, Fr. II, $600 \text{ nm} > \lambda max \geqq 540$ nm, and Fr. III, $540 \text{ nm} > \lambda max$.

Table 1 shows the distribution of starch components in the dull, waxy, and nonwaxy endosperms. The content of Fr. I (amylose) was 0% in the waxy mutant, 10.5% in the dull mutant and 19.4% in their nonwaxy

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Table 1. Effect of waxy and low amylose mutant genes on distribution of starch components after debranching with *Pseudomonas* isoamylase in rice

Cross Combination	Genotype of endosperm		Distribution of components (%)				Ⅲ/Ⅱ
	wx locus	*du* locus	Ⅰ	Int.	Ⅱ	Ⅲ	
+	+ + +	+ + +	19.4	2.4	17.0	61.2	3.6
du	+ + +	*du du du*	10.5	1.3	17.6	70.6	4.0
wx	*wx wx wx*	+ + +	0.0	1.5	19.8	78.8	4.0
+ × *du*	+ + +	+ + *du*	13.2	3.4	18.4	65.0	3.5
du × +	+ + +	+ *du du*	12.7	3.8	20.6	62.9	3.1
+ × *wx*	+ + *wx*	+ + +	10.1	3.6	19.2	67.1	3.5
wx × +	+ *wx wx*	+ + +	5.4	3.1	21.1	70.4	3.3
du × *wx*	+ + *wx*	+ *du du*	9.1	3.3	20.9	66.7	3.2
wx × *du*	+ *wx wx*	+ + *du*	4.9	3.2	20.2	71.7	3.5

counterpart, respectively. The amylose content of dull mutant was half as low as that of nonwaxy counterpart. The result indicated that the dull mutant was characterized by the reduction of amylose production in endosperm starch. The grain appearance of the dull mutant was preferentially related to the reduced amount of amylose. The contents of intermediate Fr. and Fr. II (longer unit chains of amylopectin) in the dull mutant were similar to those in the waxy mutant and the nonwaxy counterpart. The increased content of Fr. III (shorter unit chains of amylopectin) in the dull and the waxy mutants corresponded to the reduction in the content of amylose. No conspicuous difference in the relative ratio of Fr. III to Fr. II existed among these materials. This suggests the possibility that these materials possess a similar distribution of unit chain length of amylopectin.

No change in starch properties of the dull mutant was observed during the ripening period from three weeks after heading through maturity (Table 2). Therefore, the reduced production of amylose and the structure of amylopectin in the dull mutant are possibly determined at the earlier developmental stage in which the starch accumulation in endosperm cells is active.

3) *Inheritance of dull mutant*

The reciprocal crosses were made among the waxy mutant, dull mutant and their nonwaxy counterpart. The F_1 plants of the cross between the mutant and the nonwaxy counterpart were backcrossed with the dull mutant as a pollen parent. The endosperms, excluding pericarps and embryos, of F_1, F_2, Bc_1F_1 and their parent seeds were individually weighed and gelatinized overnight in 1 N potassium hydroxide at room temperature. The amount of amylose was colorimetrically determined using Technicon Autoanalyzer. The amylose content was calculated on the dry weight basis.

Table 2. Change in starch properties of a low-amylose mutant during ripening period

Days after heading	Distribution of components (%)				Ⅲ/Ⅱ
	Ⅰ	Int.	Ⅱ	Ⅲ	
21	9.5	2.3	19.4	68.8	3.5
26	11.8	1.6	18.9	67.7	3.6
32	10.1	1.6	18.4	69.9	3.8
43	10.8	2.3	18.7	68.2	3.6
60	10.5	1.3	17.6	70.6	4.0

There was no difference in amylose content between the reciprocal F_1 seeds of the dull mutant and the nonwaxy counterpart. The frequency distribution for the amylose content of F_2 and Bc_1F_1 seeds was evidently bimodal (Fig. 1, Fig. 2). F_2 seeds segregated to high amylose 177 : low amylose 64, indicating a good fit to the expected 3 : 1 ratio ($\chi^2=0.311$). Moreover, the segregation ratio in Bc_1F_1 seeds also fitted to the expected 1 : 1 ratio ($\chi^2=1.571$). From these results, it was considered

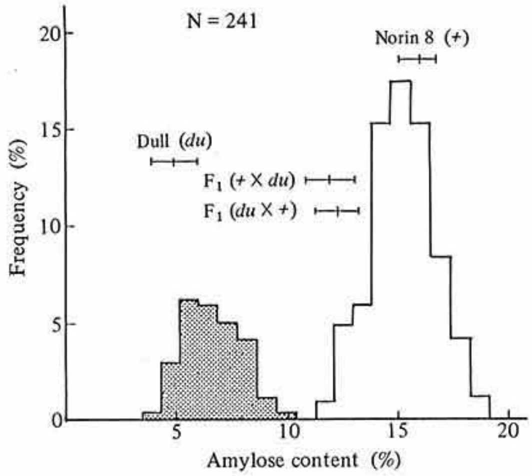

Fig. 1. Frequency distribution for amylose content in F_2 seeds of the cross between dull mutant and nonwaxy counterpart

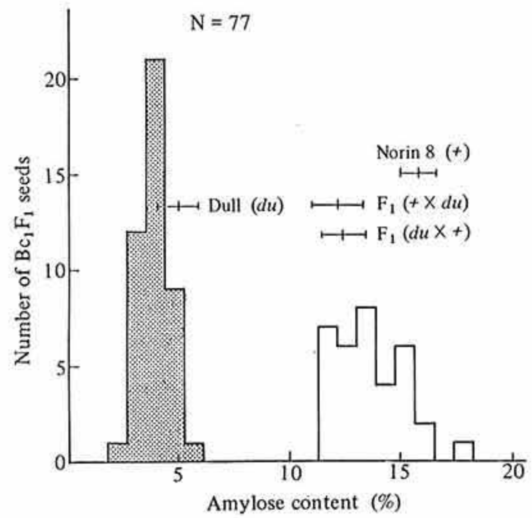

Fig. 2. Frequency distribution for amylose content in Bc_1F_1 seeds of (dull mutant × nonwaxy counterpart) × dull mutant

that the dull mutant is controlled by a single recessive gene which lowers the amylose production in starch granules.

The difference in amylose content existed between the reciprocal F_1 seeds of the waxy and the dull mutants. The segregation for amylose content in F_2 seeds of the cross between these two mutants was more complicated, presumably due to the nonallelic segregation of the waxy and the dull alleles, since the F_2 seeds could be divided into three types characteristic of the waxy mutant, the dull mutant and the nonwaxy counterpart. The results suggest that the dull allele (*du*) is nonallelic to the *wx* allele on Chromosome 6 and there is no linkage between *du* and *wx* loci.

4) Gene dosage effect

The starch granules were isolated from the endosperm of F_1 seeds of the reciprocal crosses among the waxy mutant, the dull mutant and their nonwaxy counterpart. The starch components debranched with isoamylase were analyzed using the above-mentioned method.

The amylose content in the heterozygous genotypes for *wx* alleles, ++ *wx* and +*wx wx*, was in-between the values in the homozygous genotypes (Table 1). The amount of amylose in endosperm starch increased proportionally with the dose of nonwaxy allele as reported elsewhere.[4,5,8,11] The content in the heterozygous genotypes, ++*du* and +*du du*, was also in-between those in the homozygous genotypes for *du* alleles (Table 1). These homozygous genotypes had similar content of amylose to one another, indicating no proportional change in amylose production with the dose of *du* alleles. The heterozygous genotypes for *wx* and *du* alleles showed similar amylose content to the heterozygous genotypes for *wx* alleles only. This suggests that *wx* alleles are epistatic to *du* alleles on the amylose production in endosperm cells.

High amylose mutants[15]

1) Screening of high amylose mutants

As there is no mutant that increases the amylose content of starch granules such as amylose-extender (*ae*) in maize, attempts were made to select the high amylose mutant out of the endosperm mutants reported by Satoh and Omura.[13] The endosperm of the mutants was milled to pass a 60-mesh screen. The fine powder was gelatinized in 1 N sodium hydrox-

Plate 2. Endosperms of high amylose mutants and nonwaxy counterpart (left)

Table 3. Amylose contents and grain characteristics of high amylose mutants and their original cultivar, Kinmaze

Line	Phenotype	Amylose content (%)	Grain			
			Length (mm)	Width (mm)	Thickness (mm)	Weight (mg)
Kinmaze	Normal	17.4	5.5	2.8	2.1	23.9
EM-10	Floury	29.4	5.4	2.7	1.8	17.6
EM-16	Floury	30.8	5.2	2.6	1.8	16.0
EM-72	Floury	34.1	5.2	2.6	1.9	17.2
EM-129	Floury	35.4	5.4	2.8	2.0	20.8
EM-145	Floury	32.4	5.3	2.7	1.9	17.6

ide and 20% ethanol solution. The amylose content was colorimetrically determined using Technicon Autoanalyzer. Five high amylose mutants could be successfully selected out of the mutants which are characteristic of floury endosperm (Plate 2).

2) Grain characteristics and starch properties

Grain characteristics and amylose content of the high amylose mutants of the nonwaxy rice cultivar, Kinmaze, are shown in Table 3. Amylose content of the mutants ranged from 29.4% to 35.4%. These values were about twice as high as that of Kinmaze. No difference in grain length and width could be found between the mutants and Kinmaze, but the mutants had thinner grains than Kinmaze. Therefore, the grain weight of the mutants was lower than that of Kinmaze.

Gelatinization temperature of starch granules was examined by photopastegraphy. The temperature of high amylose mutants was significantly higher than that of Kinmaze. X-ray diffraction pattern was of type B in the mutants, whereas type A is typical of cereal starch in Kinmaze (Table 4).

The cross sections of endosperm of the mutants and Kinmaze were observed with a scanning electron microscope. The endosperm of Kinmaze was densely packed with the polyhedral starch granules, while that of the mutants was loosely packed with irregular round starch granules.

Table 4. Properties of starch granules prepared from endosperm of high amylose mutants

Line	Phenotype	Gelatinization temperature (°C)	X-ray diffraction pattern
Kinmaze	Normal	52	A
EM-10	High amylose	68	—
EM-16	High amylose	69	B
EM-129	High amylose	63	—

From the results obtained in the present studies, it was concluded that the starch properties of the high amylose mutants of rice were similar to those of the amylose-extender (*ae*) of maize.

Conclusion

Starch is the polysaccharide composed of amylose and amylopectin molecules. The amylose is a long unbranched chain with α-1,4 glucosidic linkage, while the amylopectin has a branched structure with α-1,6 glucosidic linkage at the branching points. Waxy starch is, in general, composed of only amylopectin and nonwaxy starch is composed of about 20% amylose and 80% amylopectin in the Japanese rice cultivars.[7] In maize, the mutant genes such as amylose-extender (*ae*), dull (*du*) and sugary-2 (su_2) which increase the amylose content have been selected in addition to waxy (*wx*). However, few genes which modify the proportion of amylose and amylopectin components in starch granules have been detected in rice, since *wx* alleles were found by Ikeno.[6] Recently, the mutants for endosperm characters were induced in rice and some of them were demonstrated to be low amylose mutants which were designated as "dull".[10,13]

Although the amylose content of rice cultivars in Japan and other countries has been so far examined, there are only few reports that dealt with genetic analysis of the amylose content.[1,2,8] In addition, no other genes concerning amylose content, except for *wx* alleles, have been detected in rice. The present studies substantiated the existence of a new mutant gene controlling the reduction in amylose production and differing from *wx* alleles, and of high amylose mutants in rice. These endosperm mutants which control the amylose production in starch granules in endosperm cells make it possible to improve the textural quality and to develop a new scope for utilization of rice grains.

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