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High amylose mutants of rice, *Oryza sativa* L.

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Summary. Five mutant lines of rice with increased amylose content in starch granules were identified among floury endosperm mutants. The amylose contents of the mutants ranged from 29.4% to 35.4% and were about twice as high as that of the normal counterpart. Starch properties of the high amylose mutants were analyzed by column chromatography, X-ray diffractometry, photopastegraphy and scanning electron microscopy. The high amylose mutants produced longer unit chains of amylopectin than those of the normal counterpart as well as an increased amount of amylose. A X-ray diffractogram of starch in the mutant was characterized by a type B pattern, while that in the normal counterpart showed a type A pattern which is typical for starches of common cereals. The temperatures at the initiation of gelatinization of the mutants were much higher than that for the normal counterpart. The endosperm cells of the mutant were loosely packed with irregular round-shaped starch granules, whereas those of the normal counterpart were densely packed with polyhedral starch granules. Judging from the results obtained, it was concluded that starch properties of the high amylose mutants of rice were similar to those of the amylose-extender (*ae*) mutant of maize.

Key words: *Oryza sativa* L. – Endosperm – High amylose mutant – Starch properties

Introduction

Starch, in general, consists of two kinds of polysaccharides, amylose and amylopectin molecules. There are four loci which control the synthesis of amylose and amylopectin molecules in starch granules of maize kernels (Kramer et al. 1958). Amylose-extender (*ae*), dull (*du*) and sugary-2 (*su-2*) genes produce an increased amount of amylose, while the waxy (*wx*) gene produces only amylopectin.

In contrast with maize, only two types of starches, waxy (glutinous) and nonwaxy (nonglutinous), have been hitherto identified in endosperm cells and pollen granules of rice. Nonwaxy starch granules contain about 20% amylose and 80% amylopectin in Japanese rice cultivars (Inatsu et al. 1974), whereas waxy starch granules contain only amylopectin and no amylose. The production of amylose and amylopectin in endosperm cells is controlled by *wx* alleles on Chromosome 6 (Iwata and Omura 1971). Recently, low amylose mutants which were designated as "dull" were induced by radiation (Okuno 1976) and chemical mutagens (Isono et al. 1979). However, no mutant with increased amylose content in starch granules has been found in rice. Satoh and Omura (1981) induced various kinds of endosperm mutants in rice. Attempts were thus made to select high amylose mutants out of these mutants so that high amylose mutants of rice could be successfully selected.

The present study deals with the grain characteristics and starch properties of high amylose mutants of rice.

Materials and methods

Thirty floury and 21 white-core mutant lines of the rice cultivar, Kinmaze, were cultivated at the Agronomy Farm of Kyushu University, Fukuoka Prefecture, in 1981. The description of floury and white-core mutants were reported by Satoh and Omura (1981). All of the materials were self-fertilized and harvested at maturity. Brown rice of the mutants and their original cultivar were ground to pass through a 60-mesh screen. The fine rice powder was gelatinized in a mixture of

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1N sodium hydroxide and 20% ethanol at room temperature. The amount of amylose was colorimetrically determined using the Technicon Autoanalyzer. The amylose content was calculated on a dry weight basis.

Starch granules of three floury mutant lines, EM-10, EM-16 and EM-129, and their normal counterpart were isolated from milled endosperm by a modification of the method reported by Yamamoto et al. (1973).

The methods for debranching of starches with *Pseudomonas* isoamylase (EC. 3.2.68) and gel filteration of debranched materials on a column of Sephadex G-75 have been reported by Ikawa et al. (1981). Crystalline *Pseudomonas* isoamylase was purchased from Hayashibara Biochemical Laboratories, Inc., Okayama, Japan. Fraction I, II and III, and the intermediate fraction were separated according to the wavelength of absorption maxima (λ_{max}) of iodine-carbohydrate complex as follows: Fraction I (Fr. I), $\lambda_{max} \geqq 620$ nm, Intermediate Fr., 620 nm $> \lambda_{max} \geqq 600$ nm, Fr. II, 600 nm $> \lambda_{max} \geqq 540$ nm, Fr. III, 540 nm $> \lambda_{max}$. The amount of carbohydrates was determined by the phenol-sulfuric acid method (Dubois et al. 1956). The number of reducing ends was determined using the method of Hizukuri et al. (1981). The average length of unit chains was calculated by dividing the amount of carbohydrates by the number of reducing ends.

The temperature at the initiation of gelatinization of starch granules was analyzed by photopastegraphy (Kainuma et al. 1968a, b). X-ray diffractometry of starch granules of EM-16 and Kinmaze was recorded with a spectrodiffractometer. The sections of endosperm tissues of the high amylose mutant and the normal counterpart were observed by scanning electron microscopy.

Results and discussion

Five out of 30 floury mutants were selected as high amylose mutants. The amylose contents of these mutants ranged from 29.4% to 35.4%, while that of the normal counterpart was 17.9% (Table 1). The amylose contents of the mutants were about twice as high as that of the normal counterpart. The endosperm of the high amylose mutants was opaque and could be readily distinguished from that of the normal counterpart (Fig. 1). The length and width of the grains slightly decreased in EM-16 and EM-72 compared with those of the normal counterpart (Fig. 1). In the other mutants, no difference in the length and width of the grains was found between the mutant lines and the normal counterpart. All of the mutants had a thinner grains than the normal counterpart. The grain weights also significantly decreased in all of the mutants (Table 1). It was considered that the lower grain weights of the mutants were due to decreasing in grain size, particularly in grain thickness.

Table 2 shows the distribution of isoamylase-debranched starch components in the mutant (EM-16) and the normal counterpart. The contents of Fr. I which corresponds to amylose were 27.7% and 20.4% in the mutant and the normal counterpart, respectively. The content of the intermediate fraction in the mutant

Fig. 1. Endosperms of the high amylose mutant and the normal counterpart. From the *left*, Kinmaze, EM-10, EM-16 and EM-129, respectively

Table 1. Amylose contents and grain characteristics of high amylose mutants and 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
-16	floury	30.8	5.2	2.6	1.8	16.0
-72	floury	34.1	5.2	2.6	1.9	17.2
-129	floury	35.4	5.4	2.8	2.0	20.8
-145	floury	32.4	5.3	2.7	1.9	17.6

Table 2. Properties of isoamylase-debranched materials of endosperm starches of a high amylose mutant (EM-16) and normal (Kinmaze)

Line	Distribution of starch components (%)[a]				Fr. III/ Fr. II	Chain length at peak of	
	Fr. I	Int. Fr.	Fr. II	Fr. III		Fr. II	Fr. III
Kinmaze	20.4	3.7	16.7	59.2	3.5	38	14
EM-16	27.7	10.1	26.7	36.0	1.4	42	16

[a] The range of each fraction (Fr.) was divieded according to iodine-carbohydrate complexes as follows: Fr. I, $\lambda_{max} \geqq 620$ nm, Intermediate Fr., 620 nm $> \lambda_{max} \geqq 600$ nm, Fr. II, 600 nm $> \lambda_{max} \geqq$ 540 nm, Fr. III, 540 nm $> \lambda_{max}$

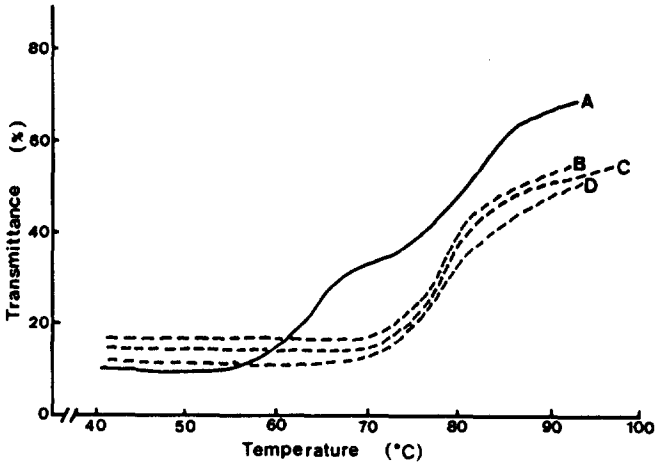

Fig. 2. Photopastegrams of endosperm starches of the high amylose mutants (EM-10, EM-16 and EM-129) and normal (Kinmaze). *A* Kinmaze, *B*, *C* and *D* EM-10, EM-16 and EM-129, respectively

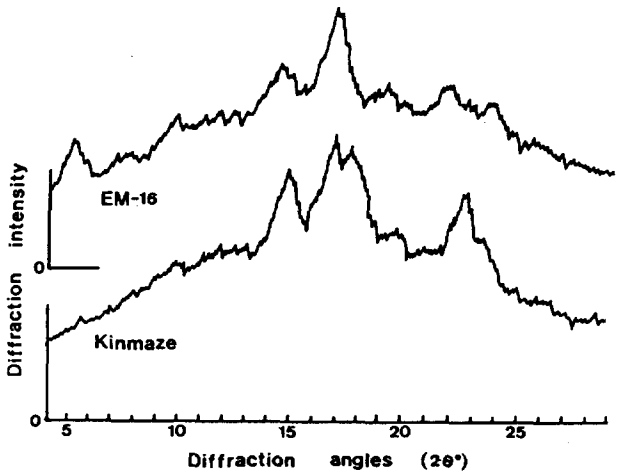

Fig. 3. X-ray diffractograms of endosperm starches of a high amylose mutant (EM-16) and normal (Kinmaze)

was 10.1% and was about three times as high as that of the normal. The content of Fr. II (longer unit chains of amylopectin) increased in the mutant, while the content of Fr. III (shorter unit chains of amylopectin) decreased in the mutant. The ratio of Fr. III to Fr. II was 3.5 in the normal and 1.4 in the mutant, suggesting the existence of a different distribution of unit chains of amylopectin in these two lines. From the results mentioned above, it was considered that the high amylose mutants could produce amylopectin with longer unit chains as well as increased amounts of amylose.

Photopastegrams of starches of three high amylose mutants and the normal counterpart are shown in Fig. 2. The normal counterpart showed a two-step gelatinization pattern, while the mutants showed a one-step gelatinization pattern. The temperature at the initiation of gelatinization of EM-10, EM-16 and EM-129 were 68 °C, 69 °C and 63 °C, respectively. These values were significantly higher than that of the normal counterpart.

Figure 3 shows X-ray diffractograms of the mutant (EM-16) and the normal counterpart. The diffractogram of the normal showed a type A pattern which is typical for endosperm starches in common cereals, while that of the mutant showed a type B pattern similar to that of starches of potato and amylose-extender mutant of maize (Brown et al. 1971).

The observations made with a scanning electron microscopy are shown in Fig. 4 A, B. The endosperm cells of the normal counterpart were densely packed with polyhedral starch granules, while those of the mutant were loosely packed with irregular round-shaped starch granules.

High amylose mutants have been reported to occur in maize (Vineyard and Bear 1952), barley (Walker and Merritt 1969) and pea (Hilbert and MacMasters 1946). In particular, three loci, *ae, du* and *su-2,* which increase the amylose content in starch granules of maize, were described by Kramer et al. (1958). The amylose-extender (*ae*) mutant of maize has been reported to produce starch granules characterized by a high birefringence end-point temperature (BEPT) and type B pat-

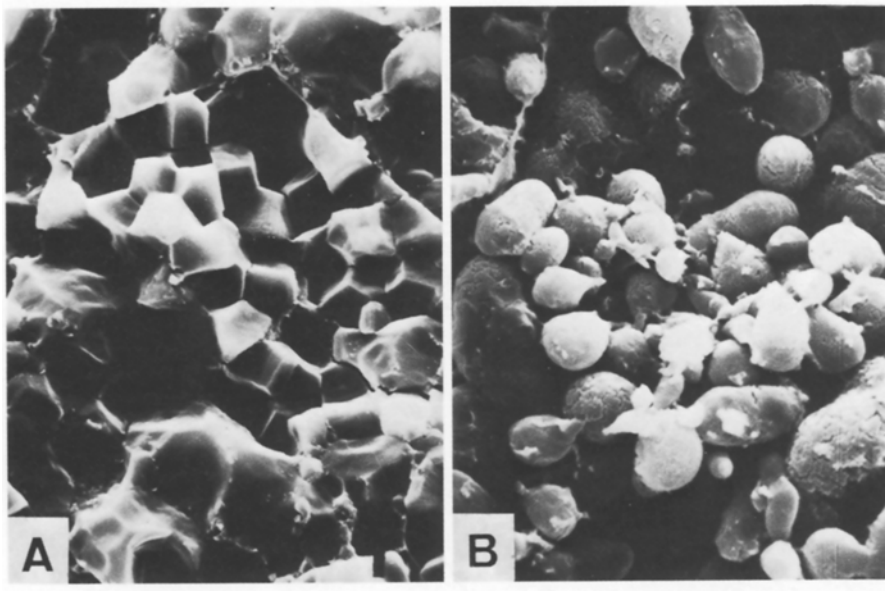

Fig. 4 A, B. Starch granules of a high amylose mutant (EM-16) and normal (Kinmaze) observed with a scanning electron microscopy. **A** Normal, **B** High amylose mutant

tern of X-ray diffractogram (Brown et al. 1971). As pointed out by Kainuma et al. (1968 b), BEPT is closely correlated with the temperature at the initiation of gelatinization determined in this study. These results suggest that the high amylose mutants of rice have starch properties similar to those of the *ae* mutant of maize. In addition, the endosperm cells of the *ae* mutant contain a large number of irregularly-shaped starch granules (Boyer et al. 1976). The observation with scanning electron microscopy indicates that the shape of the starch granules of the high amylose mutant in rice is also similar to that of the *ae* mutant in maize. Besides, it has been reported that the *ae* mutant of maize shows the characteristic distribution of longer unit chains of amylopectin in starch granules, compared with the normal counterpart and other mutants (Banks et al. 1974; Yamada and Taki 1976; Ikawa et al. 1978, 1981). The changes in the structure of endosperm starch were observed in the high amylose mutant of rice as well as *ae* mutant of maize. Judging from the results obtained, it was concluded that the high amylose mutants of rice have starch properties similar to those of the *ae* mutant of maize.

The *ae* mutant of maize is useful for elucidating the biosynthetic pathway of starch in endosperm cells (Boyer and Preiss 1981; Baba et al. 1982). Boyer and Preiss (1981) reported that kernels homozygous for the *ae* allele lacked one of the branching enzyme fraction (II b) and that the changes in starch-synthesizing enzyme of kernels of the *ae* mutant were closely correlated with the structural changes in endosperm starch of this mutant. Therefore, the high amylose mutants of rice make it possible to promote studies on biosynthesis of endosperm starch in rice.

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