A New Mutant Gene Lowering Amylose Content in Endosperm Starch of Rice, *Oryza sativa* L.

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The present study was undertaken to investigate the starch properties and inheritance of a newly induced mutant of rice. The endosperm of the mutant is dull and can be readily distinguished from the waxy as well as nonwaxy endosperms. The amylose content of the dull mutant was half as low as that of the nonwaxy counterpart. No conspicuous differences regarding the distribution of unit chain length of amylopectin existed between dull mutant and the normal counterpart. The starch properties of the dull mutant were determined three weeks after heading. Judging from the genetical analysis for amylose content of the dull mutant, it was considered that amylose production in the dull mutant is controlled by a single recessive gene which is nonallelic to the *wx* alleles. The amylose content did not decreased proportionally with the number of *du* alleles, in contrast to the proportional reduction in amylose content with the number of *wx* alleles. The existence of a low amylose mutant gene makes it possible to improve the eating quality of rice grains.

KEY WORDS : rice, low amylose mutant, starch properties, inheritance.

Introduction

Starch is, in general, composed of two types of polysaccharide, amylose and amylopectin molecules. No mutant gene which controls the proportion of amylose and amylopectin molecules in starch granules has been found in rice, since IKENO (1914) indentified the waxy alleles. The *wx* locus on Chromosome 6 (IWATA and OMURA 1971) controls the starch synthesis in endosperm cells of rice grains. The starch of nonwaxy endosperm contains both amylose and amylopectin, whereas the waxy endosperm contains only amylopectin and no amylose, or very little amylose. In maize, the *wx* locus has been considered to reflect the presence of a structural gene coding for the starch granule-bound NDPG-starch glucosyltransferase which plays an important role for the normal synthesis of amylose (TSAI 1974).

OKUNO (1976) induced a new endosperm mutant of rice by ³²P treatment. The endosperm of this mutant was dull and could be distinguished from the waxy as well as nonwaxy endosperms. The "dull" mutant was found to be a low amylose mutant based on the determination of amylose content in endosperm starch using the iodine amperometric titration (OKUNO 1976). The finding of a low amylose mutant indicates the existence of a new endosperm character. Recently, SATOH and OMURA (1981) reported the existence of some "dull" mutants with a phenotype similar to that of the "dull" mutant described here. The present study deals with further analysis of the starch properties and the inheritance pattern for amylose concentration in this dull mutant.

Materials and Methods

The dull and waxy mutants detected in the progenies of M_1 plants of the rice cultivar, Norin 8, which had been treated with ^{32}P were analyzed in the present studies (OKUNO 1976, 1978).

Starch preparation: The starch granules were isolated from milled endosperms of waxy mutant, dull mutant and their normal counterpart by a modification of the method reported by YAMAMOTO *et al.* (1973). The endosperm was milled to pass a 60-mesh screen after removing the embryo and pericarp. The fine rice powder was stirred in a cold aqueous alkaline solution (0.2% sodium hydroxide) for three hours in a cold room and washed with distilled water to reach neutrality. The starch granules were then screened through a 200-mesh screen and subsequently washed twice with ethanol and once with acetone.

Debranching of starches with isoamylase and gel filtration of debranched starches: The methods for debranching of starches with *Pseudomonas* isoamylase (EC 3.2.1.68) and gel filtration of debranched starches on a column of Sephadex G-75 have been reported previously (IKAWA *et al.* 1981). Crystalline *Pseudomonas* isoamylase was purchased from Hayashibara Biochemical Laboratories, Inc., Okayama, Japan. Fractions I, II and III, and intermediate fraction were separated according to the wavelength of absorption maxima ($\lambda_{\max}$) of iodine-carbohydrate complexes as follows: fraction I (Fr. I), $\lambda_{\max} \geq 620$ nm, intermediate Fr., $620 \text{ nm} > \lambda_{\max} \geq 600$ nm, Fr. II, $600 \text{ nm} > \lambda_{\max} \geq 540$ nm, Fr. III, $540 \text{ nm} > \lambda_{\max}$.

Analytical methods for distribution of starch components: The amount of carbohydrate 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 carbohydrate by the number of reducing ends.

Determination of amylose content in F_1 , F_2 and Bc_1F_1 seeds: Reciprocal crosses were made among the waxy mutant, dull mutant and their normal counterpart, respectively. The F_1 plants were self-fertilized. The F_1 plants of the cross between the dull mutant and the normal counterpart were backcrossed to the dull mutant. Each endosperm of F_1 , F_2 , Bc_1F_1 and their parent seeds was gelatinized overnight in 1 N potassium hydroxide at room temperature after removing the embryo and pericarp. The amount of amylose was colorimetrically determined using Technicon Autoanalyzer. The amylose content was calculated on a dry weight basis. The starch granules were also isolated from the endosperm of F_1 seeds of the reciprocal crosses among the waxy mutant, the dull mutant and their normal counterpart. The starch components debranched with isoamylase were analyzed using the above-mentioned method.

Results

The endosperm appearances of the mutants were compared to that of the normal counterpart (Fig. 1). The endosperm of the newly induced mutant became dull after drying and could be readily distinguished from the waxy as well as nonwaxy (normal)

endosperms. Therefore, the new endosperm mutant was designated as "dull". The size and shape of the brown rice grains did not vary among the three types of materials.

Figure 2 shows the elution patterns of isoamylase-debranched starches of the waxy mutant, the dull mutant and their normal counterpart. Three peaks appeared for the dull and normal starches and two peaks for the waxy starch. Fraction I appeared after the first elution in the normal counterpart which had typical amylose and amylopectin. This fraction corresponded to amylose according to the absorption maximum of iodine-carbohydrate complexes (620 nm-635 nm). Fractions II and III consisted of linear unit chains of amylopectin since the degree of polymerization in their peaks amounted to 33-40 and 10-12 glucose units, respectively. The dull mutant showed a smaller amount of Fr. I in the endosperm starch than the normal counterpart.

Table 1 shows the distribution of isoamylase-debranched starch components in these three materials. The content of Fr. I (amylose) was 0% in the waxy mutant, 10.5% in the dull mutant and 19.4% in their normal counterpart, respectively. The amylose content of the dull mutant was half as low as that of the normal counterpart. This result indicates that the dull mutant is characterized by a reduction of amylose production in the starch granules. The endosperm appearance of this mutant was preferentially related to the reduced amount of amylose. The contents of the intermediate Fr. and Fr. II in the dull mutant were similar to respective values in the waxy mutant and the normal counterpart. The content of Fr. III in the dull and waxy mutants increased in proportion with the decrease in the content of Fr. I, compared with the situation in the normal counterpart.

Fig. 1. Endosperms of waxy mutant (right), dull mutant (middle) and normal counterpart (left)

Fig. 2. Elution patterns of endosperm starches debranched with isoamylase in waxy mutant, dull mutant and normal counterpart.

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

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

Table 2. Changes in starch properties of the 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

The relative ratio of Fr. III to Fr. III was similar to one another, suggesting that these materials may possess an identical distribution of unit chains of amylopectin.

No change in the starch properties of the dull mutant was observed during the ripening period from three weeks after heading to maturity (Table 2). Therefore, the starch properties of the dull mutant which are characterized by a reduced amount of amylose are presumably determined up to the early stages of development when starch accumulation in the endosperm cells is more active.

The segregation pattern for amylose content in the F_2 seeds of the cross between the dull mutant and the normal counterpart and in the Bc_1F_1 seeds of the cross between F_1 plants (*dull* × normal) and the dull mutant is shown in Fig. 3 and Fig. 4. The F_1 seeds of the reciprocal cross between the dull mutant and the normal counterpart had intermediate value of amylose content between that in waxy and normal seeds. No difference in amylose content was detected between the reciprocal F_1 seeds of the dull mutant and the normal counterpart. The segregation pattern for amylose content of the F_2 seeds and Bc_1F_1 seeds was evidently bimodal. F_2 seeds segregated to high amylose 177: low amylose 64, indicating a good fit to the expected 3:1 ratio ($\chi^2=0.311$). The segregation

Fig. 3. Frequency distribution for amylose content in F_2 seeds of the cross between dull mutant and normal counterpart.

ratio in the Bc_1F_1 seeds also fitted to the expected 1:1 ratio ($\chi^2=1.571$). These results indicate that amylose production in the dull mutant is controlled by a single recessive gene lowering the amylose content in the starch granules.

On the other hand, a difference in amylose content was observed between the reciprocal F_1 seeds of the waxy and the dull mutants. When the waxy mutant was used as a maternal parent, the F_1 seeds showed a lower content of amylose than when it was used as a pollen parent, presumably due to the gene dosage effect of the *wx* allele on amylose production. As shown in Fig. 5, the segregation pattern for amylose content in the F_2 seeds of the cross between the waxy and the dull mutants was more complicated, presumably due to the nonallelic segregation of the waxy and the dull alleles. The F_2 seeds could be divided into three types characterizing the waxy mutant, the dull mutant and the normal counterpart. From the results, it was considered that amylose production in the dull mutant is controlled by the *du* alleles which differ from the *wx* alleles located on Chromosome 6 and that there is no linkage between *wx* and *du* loci.

The amylose content in the genotypes heterozygous for the *wx* and *du* alleles was intermediate between the values in the genotypes homozygous for these two alleles (Table 1). The amylose content decreased proportionally with the number of *wx* alleles, reflecting the gene dosage effect of *wx* alleles on amylose production. However, the genotypes heterozygous for *du* alleles, $++du$ and $+du du$, had an identical content of amylose, suggesting the absence of gene dosage effect of the *du* alleles on amylose production. The F_1 seeds of the reciprocal crosses between the waxy and the dull mutants showed a similar content of amylose to that of the F_1 seeds of the crosses between the waxy mutant and the normal counterpart. This result led us to consider that the *wx* allele is epistatic to the *du* allele with respect to amylose production in the starch granules of the endosperm cells.

Fig. 4. Frequency distribution for amylose content in Bc_1F_1 seeds of the cross between F_1 plants (dull mutant $\times$ normal counterpart) and dull mutant.

Fig. 5. Frequency distribution for amylose content in F_2 seeds of the cross between dull mutant and waxy mutant.

Discussion

Starch comprises two kinds of polysaccharide, amylose and amylopectin molecules. Amylose consists of a long linear chain with a α -1,4 glucosidic linkage, while amylopectin has a branched structure with both α -1,4 and α -1,6 glucosidic linkages by K. H. Meyer's definition.

In maize, there are several kinds of mutant genes which modify the proportion of amylose and amylopectin molecules in the starch granules of endosperm cells. Amylose extender (*ae*), dull (*du*) and sugary-2 (*su₂*) genes produce an increased amount of amylose, whereas the waxy (*wx*) gene produces endosperm and pollen starches containing only amylopectin.

However, mutant genes controlling the production of amylose and amylopectin have not been detected in rice, since the *wx* alleles were identified on Chromosome 6 (IKENO 1914, IWATA and OMURA 1971). Although the amylose content of the starch granules were genetically analyzed (BOLLIICH and WEBB 1973, IRRI ANNUAL REPORT 1975, CHOE 1977, MCKENZIE and RUTGER 1983), no other loci than the *wx* locus have been identified. Recently, a new type of endosperm mutant has been successfully induced and designated as "dull" (OKUNO 1976, SATOH and OMURA 1981). These mutants have the characteristic appearance of dull endosperm and contain a reduced amount of amylose.

The present investigation aimed at further analyzing the starch properties and the inheritance pattern of the dull mutant. From the results obtained, it was concluded that amylose production in the dull mutant is controlled by a single recessive allele at a locus different from the *wx* locus on Chromosome 6. It was also demonstrated that the *du* allele did not affect significantly the modification of amylopectin. These findings substantiated the existence of genes which control the proportion of amylose and amylopectin molecules in starch granules of rice in addition to the *wx* alleles.

Nonwaxy starch in rice grains of the Japanese cultivars is composed of about 20% amylose and 80% amylopectin (INATSU 1979). As reported by INATSU (1979), rice cultivars grown in Hokkaido which is the northernmost island of Japan had higher contents of amylose than those grown in the other regions. High amylose characteristics have been considered to adversely affect the eating quality of the Japanese rice cultivars. Assuming that the poor eating quality of rice grains is due to higher amylose content, emphasis should be placed on breeding low amylose cultivars. Therefore, it is considered that the presence of the *du* allele as one of the superior gene sources should contribute to the improvement of the eating quality of rice grains.

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イネ胚乳澱粉のアミロース含量を減少させる突然変異遺伝子

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澱粉は α -1,4 グルコシド結合より成る直鎖状のアミロースと α -1,4 および α -1,6 グルコシド結合より成る分岐状のアミロペクチンとから構成される多糖である。胚乳澱粉のアミロースとアミロペクチンの構成比を変化させる遺伝子として、トウモロコシでは、amylose extender (*ae*), dull (*du*), sugary-2 (*su*₂), waxy (*wx*) が見い出されているが、イネでは、第6染色体(第I連鎖群)に座乗するモチ性遺伝子(*wx*)が報告されているにすぎなかった。筆者らは、胚乳の外観がモチ・ウルチ性の中間を示す半透明の突然変異体(dull)を作出した。本報告では、新たに作出した dull 突然変異体の胚乳澱粉の性質および遺伝様式を調べた結果を述べる。

水稻品種農林8号の³²P 処理(β 線内部照射)の後代で選抜したモチ変異体, dull 変異体ならびに原品種の胚乳より, 200 メッシュ以下の澱粉粒を調製した。1 規定の水酸化ナトリウム溶液中で糊化した澱粉を *Pseudomonas amyloclavata* 由来のイソアミラーゼで処理し, α -1,6 グルコシド結合を切断した。枝切りした澱粉をセファデックス G75 で分画し, 各画分の全糖量をフェノール硫酸法で, 還元末端基数を Park-Johnson 法の改変法により比色定量した。dull 変異体と原品種の F₁, F₂, Bc₁F₁ 種子およびモチ変異体と dull 変異体の F₂ 種子のアミロース含量をオートアナライザーを用いて一粒づつ比色定量した。また, 上記3種の材料の間で正逆交雑し, F₁ 種子の澱粉の性質をカラムクロマトグラフィーにより調べた。

dull 変異体のアミロース含量は 10.5% であり, 原品種のアミロース含量の約 50% に減少した。アミロペクチンの鎖長分布については両者の間で顕著な差はなかった。dull 変異体の胚乳澱粉の性質は出穂後3週目までに決定された。

アミロース含量に関する遺伝分析の結果から, dull 変異体の胚乳澱粉における低アミロース化は, 第6染色体上の *wx* 座とは異なる単一の劣性主働遺伝子(*du*)によって支配されると考察した。*wx* 対立遺伝子の数量に

比例してアミロース含量は変化したが、*du* 対立遺伝子ではアミロース含量に対する遺伝子の数量効果は認められなかった。モチ変異体と *dull* 変異体の正逆交雑による F_1 種子について得られた結果から、アミロース合成に関して *wx* は *du* よりも上位であると推定した。

わが国の粳品種の食味改良への低アミロース遺伝子 (*du*) の有効性について論議した。