SHORT COMMUNICATION

GENE DOSAGE EFFECT OF WAXY ALLELES ON AMYLOSE
CONTENT IN ENDOSPERM STARCH OF RICE

KAZUTOSHI OKUNO

Division of Genetics, National Institute of Agricultural Sciences
Hiratsuka 254

Received February 27, 1978

The waxy (glutinous) endosperm character in rice, *Oryza sativa* L., is controlled by the *wx* gene belonging to linkage group I, chromosome 6 (Iwata and Omura 1971). The *wx* locus is known to control the constitution of endosperm starch in different cereals, and no other locus has so far been found in rice. The starch of non-waxy endosperm contains both amylopectin and amylose molecules, whereas the waxy endosperm starch contains only amylopectin and no amylose, or very little amylose (Taki 1959). In the present study, the dosage effect of *wx* alleles on the amount of amylose formed in rice endosperm was investigated by using hybrid seeds.

The waxy mutant used in the present experiment was selected from the progenies of M₁ plants of a rice cultivar Norin 8 treated with ³²P. The waxy mutant had a single recessive allele at the *wx* locus (Table 1). The mutant was reciprocally crossed

Table 1. Segregation in F₂ of crosses between *wx* mutant and Norin 8 and between *wx* mutant and genetic tester

Cross combination	F ₁ phenotype	F ₂		Total	χ^2 (3:1)
		Non-waxy*	Waxy		
Mutant × Norin 8	Non-waxy	166	43	209	2.183
Norin 8 × Mutant	Non-waxy	130	44	174	0.008
Mutant × Tester	Waxy	0	132	132	
Tester × Mutant	Waxy	0	183	183	

* Non-waxy includes translucent endosperm. The F₂ segregation ratio showed a good fit to the 2:1:1 ratio, if non-waxy was divided into transparent and translucent types ($\chi^2=0.655$).

with Norin 8 by the hot water emasculation technique. The endosperm is a triploid structure carrying two complements of genes from the female parent and one from the male parent. Hence, the hybrids of *wx* mutant × Norin 8 and Norin 8 × *wx* mutant have genotypes *+wxwx* and *++wx*, respectively, and the mutant and Norin 8 have *wxwxwx* and *+++*, respectively. The *+wxwx* endosperm was translucent and distinguishable not only from *wxwxwx* but also from *+++* endosperm. On the other

hand, the hybrid seeds with $++wx$ were transparent and visually indistinguishable from $+++$. The crude starch solution from $++wx$ endosperm, however, was more lightly blue black than that from $+++$ endosperm when stained with I_2 -KI solution.

The starch samples were prepared from milled endosperm of seeds with the above four genotypes by using the alkali method (Yamamoto *et al.* 1973). The grain powder, removing embryos, was stirred for 3 h in diluted aqueous alkaline (0.2% NaOH) solution under a low temperature (5°C) and washed with distilled water. Fine endosperm powder passing through 100-mesh screen was used to estimate the amount of amylose and other carbohydrates. The amount of amylose present in the prepared starch was estimated by the iodine amperometric titration method (Larson *et al.* 1953). Into a beaker were introduced 1 ml of 5N potassium hydroxide solution containing an amount of 4 mg of starch sample, 84 ml of distilled water, 10 ml of normal hydrochloric acid and 5 ml of 0.4N potassium iodide (KI) solution. The mixture was kept at 10°C and stirred at a constant rate. The mixture was titrated with increments of a standard 0.00157N potassium iodate (KIO_3) solution. The total amount of carbohydrates was determined by the phenol sulfuric acid method using maltose as a standard.

The amperometric titration curves were obviously different between the four genotypes (Fig. 1). The amylose contents in the two homozygous genotypes, $+++$ and $wxwxwx$, were 12.23% and 0.00%, respectively (Table 2). The contents in heterozygous genotypes, $++wx$ and $+wxwx$, were in-between the values for the homozygous genotypes. Thus, the results of amylose determination demonstrated that the amount of amylose in endosperm starch increased with the number of non-waxy alleles. However, the amount of amylose was not linearly proportional to the dosage of wx alleles as

Fig. 1. Comparison of amperometric titration curves of endosperm starch between four genotypes.

Table 2. Amylose content in endosperm starch of four genotypes for *wx* alleles

Genotype	Amylose content (%)	Relative ratio (%)
<i>+</i> <i>+</i> <i>+</i>	12.23	100.0
<i>+</i> <i>+</i> <i>wx</i>	5.09	41.6
<i>+</i> <i>wx</i> <i>wx</i>	2.21	18.1
<i>wx</i> <i>wx</i> <i>wx</i>	0.00	0.0

Fig. 2. Increase of amylose content with the dose of non-waxy alleles.

A: maize (Tsai 1974).

B: rice (IRRI Annual Report 1975).

C: rice (the present study).

reported in an *Indica* cultivar of rice (IRRI Annual Reports 1975) and in maize (Tsai 1974). As shown in Fig. 2, the present experiment gave a different tendency of amylose content to increase with the number of non-waxy alleles from those reported previously. The intragenic recombination at the *wx* locus has been reported in maize (Nelson 1957, 1959, 1962; Briggs *et al.* 1965; Amano 1968) and in rice (Li *et al.* 1965) by pollen grain analysis of F_1 hybrids between waxy plants. Therefore, further studies will be carried out to manifest functional changes of the *wx* alleles.

ACKNOWLEDGMENT

The author is most thankful to Dr. K. Kainuma, head of Carbohydrates Laboratory, National Food Research Institute, for his kind support for conducting the present study.

LITERATURE CITED

- Amano, E., 1968 Comparison of ethyl methanesulfonate- and radiation-induced waxy mutants in maize. *Mutation Res.* **5**: 41-46.
- Briggs, R. W., E. Amano, and H. H. Smith, 1965 Genetic recombination with ethyl methanesulfonate-induced waxy mutants in maize. *Nature* **207**: 890-891.
- IRRI Annual Report, 1975 Factors that affect grain quality. p. 85.
- Iwata, N., and T. Omura, 1971 Linkage analysis by reciprocal translocation method in rice plant (*Oryza sativa* L.). II. Linkage groups corresponding to the chromosomes 5, 6, 8, 9, 10 and 11. *Sci. Bull. Fac. Agr., Kyushu Univ.* **25**: 138-153.
- Larson, B. L., K. A. Gilles, and R. Jenness, 1953 Amperometric method for determining the sorption of iodine by starch. *Anal. Chem.* **25**: 802-804.
- Li, H. W., S. Wang, and P. Z. Yeh, 1965 A preliminary note on the fine structure analysis of glutinous gene in rice. *Bot. Bull. Acad. Sinica* **6**: 101-105.
- Nelson, O. E., 1957 The feasibility of investigating "genetic fine structure" in higher plants. *Am. Nat.* **91**: 331-332.
- Nelson, O. E., 1959 Intracistron recombination in the Wx/wx region in maize. *Science* **130**: 794-795.
- Nelson, O. E., 1962 The waxy locus in maize. I. Intralocus recombination frequency estimates by pollen and by conventional analyses. *Genetics* **47**: 737-742.
- Taki, M., 1959 Studies on the chromatography of starches. III. On the separation and determination of amylose of starches by means of paper chromatography. *J. Agr. Chem. Soc. Japan* **33**: 245-249.
- Tsai, C. Y., 1974 The function of the waxy locus in starch synthesis in maize endosperm. *Biochem. Genet.* **11**: 83-96.
- Yamamoto, K., S. Sawada, and T. Onogaki, 1973 Properties of rice starch prepared by alkali method with various conditions. *J. Jap. Soc. Starch Sci.* **21**: 115-119.