Theor Appl Genet (1984) 68:467–473

Differential regulation of waxy gene expression in rice endosperm

Y. Sano
National Institute of Genetics, Misima 411, Japan

Received February 24, 1984; Accepted March 6, 1984
Communicated by G. S. Khush

Summary. In order to examine the effects of different alleles on the gene expression at the waxy locus, the *Wx* gene product which controls the synthesis of amylose was isolated from endosperm starch of rice plants and analysed by electrophoretic techniques. The major protein bound to starch granules was absent in most of waxy strains and increased with the number of *Wx* alleles in triploid endosperms, suggesting that the major protein is the *Wx* gene product. In addition to *wx* alleles which result in the absence or drastic reduction of the *Wx* gene product and amylose, differentiation of *Wx* alleles seemed to have occurred among nonwaxy rice strains. At least two *Wx* alleles with different efficiencies in the production of the major protein as well as amylose were detected. These alleles are discussed in relation to regulation of the gene expression.

Key words: *Oryza sativa* – *O. glaberrima* – *Wx* gene expression – Amylose content – Electrophoresis

Introduction

Recent investigations have revealed that the genomes of higher plants appear to contain substantial amounts of regulatory information associated with various facets of gene expression (Scandalios and Baum 1982). Although our knowledge of regulatory systems is quite limited, the accumulating evidence suggests that such regulatory systems might have played an important role leading to phenotypic diversity in crops.

In order to examine the expression of waxy (*wx*) gene in rice, the present study was undertaken by using electrophoretic techniques. The waxy (or glutinous) locus has been well documented by conventional genetic methods (Ikeno 1914; Nagao and Takahashi 1963; Iwata and Omura 1971; IRRI 1976) as well as by pollen analysis for a fine-structure map (Li et al. 1965, 1968). In addition, the waxy locus controls the production of amylose. The starch of nonwaxy endosperms contains both amylose and amylopectin, whereas waxy endosperms produce primarily amylopectin. In maize, the *Wx* gene product was assumed to be nucleoside diphosphate (NDP) sugar-starch glucosyl transferase, which controls the synthesis of amylose (Nelson and Rines 1962; Tasi 1974; Nelson et al. 1978; Echt and Schwartz 1981). In rice, amylose content is the major determinant of the eating, cooking and processing qualities (IRRI 1976). However, the inheritance of amylose content seems to be complicated among nonwaxy rice strains (IRRI 1976; McKenzie and Rutger 1983). I will report here that in addition to the *wx* alleles which result in the absence or trace of the gene product, there are at least two different *Wx* alleles which regulate the quantitative level of the gene product as well as amylose.

Materials and methods

Plant material

Seven *Oryza sativa* and three *O. glaberrima* strains were chosen from a collection assembled from various countries and preserved in the National Institute of Genetics, Misima, Japan. Their origins and endosperm characteristics are shown in Table 1. In addition, 20 waxy (or glutinous) cultivars were examined as to the presence or absence of the *Wx* gene product.

Two-dimensional electrophoresis

Seed polypeptides from nonwaxy and waxy strains were compared by means of a two-dimensional polyacrylamide gel

Table 1. The materials used

Species	Strain	Endosperm	Amylose content[a]	Origin
O. sativa	T65	Nonwaxy	14.2%	Taichung 65 from Taiwan
	N8	Nonwaxy	15.6%	Norin 8 from Japan
	108	Nonwaxy	25.0%	Peiku from Taiwan
	C8669	Nonwaxy	24.2%	A genetic tester through C.R.R.I., Cuttack, India
	C8005	Nonwaxy	24.1%	G. S. No 409 from India
	221	Waxy	0.0%	Malagkit Sinaguing from Philippines
	T65*wx*	Waxy	0.0%	An isogenic line of T65 with *wx* from Kinoshitamochi (derived from BC_{16})
O. glaberrima	W025	Nonwaxy	21.6%	From Sierra Leone (through S. Sampath)
	GM1	Nonwaxy	24.2%	A photoperiod non-sensitive strain with white grains selected by Oka (1977)
	GM2	Waxy	0.0%	A mutant from GM1 treated with EMS (Sano 1978)

[a] 10 seeds per strain were examined (% dry basis)

electrophoresis according to O'Farrell (1975). All steps in the preparation were performed at 4 °C. Ten seeds of each strain were homogenized in 1 ml of 10 mM tris-HCl, pH 8.5. The extracts were then centrifuged at 12,000 rpm for 30 min. The protein concentrations were determined by the method of Lowry et al. (1951), using bovine serum albumin as the standard. The supernatant was diluted with sample buffer containing the following at the final concentrations indicated: 8.5 M urea; 2% (w/v) Triton X-100; 12.5 mM L-lysine; 2.5% (w/v) ampholine, pH 3–10; and 5% β-mercapto-ethanol (BME). Samples containing 150 µg protein were loaded onto 0.3 × 8-cm isoelectric focusing gels. Gels were run for 16 h at 320 V at room temperature, followed by 800 V for 1 h. The non-equilibrated gels were subjected to electrophoresis in the second dimension in 8.75% acrylamide slabs (13 × 0.1 × 15 cm). Gels were run at constant current (25 mA) and completed after the dye front (Bromphenol blue, 0.005%) had moved 7 cm into the separation gel. Gels were fixed in 12.5% trichloroacetic acid for 1 h at 60 °C and then were incubated in destaining solution (acetic acid : methanol : H_2O, 1 : 3 : 9) for 1 h. Proteins were stained in 0.2% Coomassie Blue R-250 in destaining solution for 2 h at 60 °C. Gels were subsequently destained by diffusion in destaining solution.

SDS gel electrophoresis

Starch granules were isolated from dried grains according to Schwartz and Echt (1982). After seed soaking for 12 h, the embryo was removed and 10 grains (or individual grain) were homogenized in 1 ml of buffer A (0.055 M tris-HCl, pH 6.8, 2.3% SDS, 5% BME, 10% glycerol). The suspension was filtered through a layer of Miracloth and centrifuged at 12,000 rpm for 1 min. The pellet was washed by three cycles of suspension and centrifugation in 1 ml of buffer A and two cycles in 1 ml of acetone. The starch granules were then dried in vacuo and stored at −80 °C. Five mg of the granules were mixed with 50 µl of buffer A and heated in a boiling water bath for 5 min. The gelled solution was cooled and an additional 100 µl of buffer A was added with gently stirring. The slurry was centrifuged at 12,000 rpm for 5 min, and the resulting supernatants were used for electrophoresis. SDS polyacrylamide gel electrophoresis was performed according to Laemmli (1970) using either 10% or 8.75% acrylamide gels. Molecular weights were determined from migration comparisons to Bio-Rad Low Molecular Weight Protein Standards in 10% acrylamide SDS gels.

Analysis of amylose content

Each endosperm was gelatinized overnight in 1 N potassium hydroxide at room temperature after removing the embryo and pericarp. The amylose content based on dry weight was colorimetrically determined by Technicon Autoanalyzer.

Results

Two-dimensional polyacrylamide gel electrophoresis

Examination of seed polypeptides in T65 (*Oryza sativa*, Japonica type) showed that it is possible to distinguish 22 major and more than 60 minor polypeptide components as revealed by isoelectric point and molecular weight. Comparisons of two-dimensional gels performed in T65 (*Wx*) and an isogenic line of T65 carrying *wx* (T65*wx*) revealed that the only difference detected was the presence of a subunit in T65 which was absent in T65*wx* (Fig. 1). This suggests that the *Wx* gene controls the production of the subunit. The molecular weight and isoelectric point were approximately 60,000 and pH 7.0, respectively.

Seed polypeptides from two nonwaxy (*Wx*) strains, 108 (*O. sativa*, Indica type) and W025 (*O. glaberrima*) were examined. The spot detected in T65 and not in T65*wx* was clearly recognized in both strains although they contained relatively greater amounts of the subunit than did T65 (Fig. 2).

The Wx protein

SDS extracts of starch granules from nonwaxy strains contained a major protein in addition to one or two

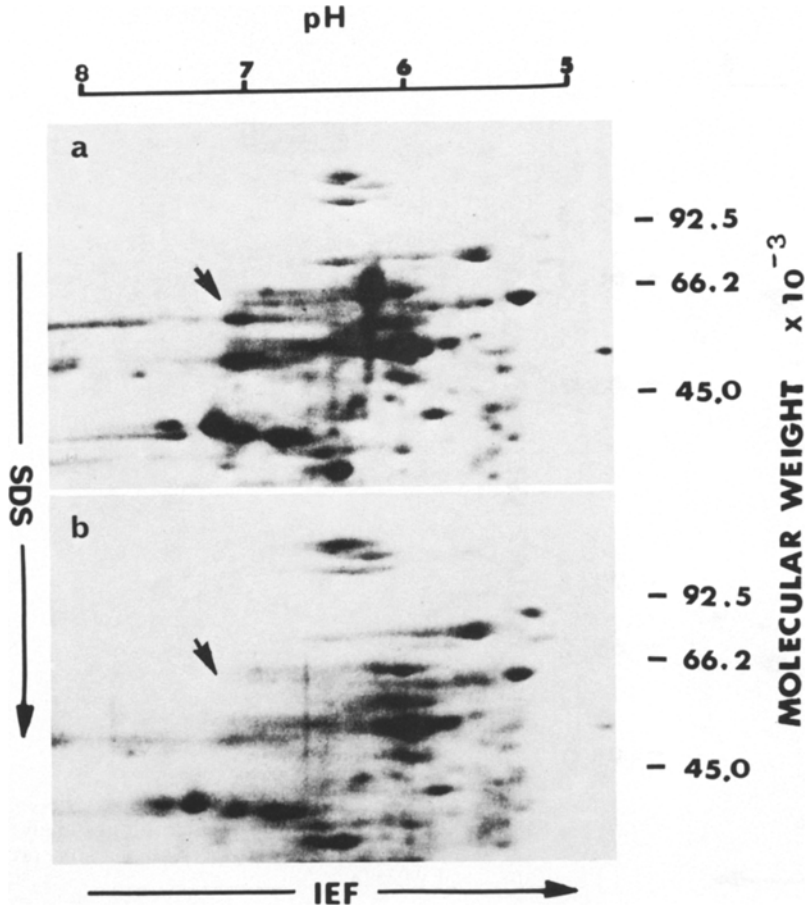

Fig. 1. Two-dimensional patterns of seed polypeptides from T65 (**a**) and T65*wx* (**b**)

minor proteins with higher molecular weight, as visualised on polyacrylamide gels (Figs. 3 and 5). On the other hand, SDS extracts from T65*wx* completely lacked the major protein (Fig. 3h). Out of 23 *wx* (or glutinous) strains examined, 22 lacked the protein as did T65*wx*. However, IKI staining of a strain from Nepal (C9078) showed a faint band corresponding to the protein although this strain was waxy. Thus, the absence or trace of the major protein was well correlated with waxy phenotypes. By mobility comparisons with molecular weight markers, the size of the major protein was estimated to be about 60,000 daltons. This indicated that the major protein from SDS extracts is the same as the protein specific to nonwaxy strains detected by two-dimensional electrophoresis.

SDS extracts from whole endosperms of T65 and T65*wx* were examined. When the extracts from T65 were heated to swell the starch granules, only the band of 60,000 daltons became clear (Fig. 4), suggesting a major starch-granule-bound protein associated with the *Wx* allele is similar to the one called the *Wx* protein in maize (Echt and Schwartz 1981). T65*wx* had the same patterns in SDS extracts of whole endosperms as that found in T65 except that the band of 60,000 daltons was completely missing even after heating.

The amount of *Wx* protein greatly varied among nonwaxy strains (Fig. 3). Two strains, T65 and N8 (Japonica type of *O. sativa*), both showed a smaller amount of *Wx* protein than the other nonwaxy strains (Indica type of *O. sativa* and *O. glaberrima*), being consistent with the results in two-dimensional patterns. Amylose content in starch granules also varied among nonwaxy strains (Table 1). Amylose content appeared to be correlated with the amount of *Wx* protein since T65 and N8 had lower amylose contents than did the other nonwaxy strains.

Dosage effect of the Wx gene

Since the triploid endosperm has one dose of the paternal and two doses of the maternal allele, it is possible to examine the effect of *Wx* gene dosage on the level of the gene product by using reciprocally crossed seeds of waxy and nonwaxy strains. Three kinds of crosses, T65×T65*wx*, C8669×221, and GM1×GM2, were made and the hybrid seeds were analysed as to

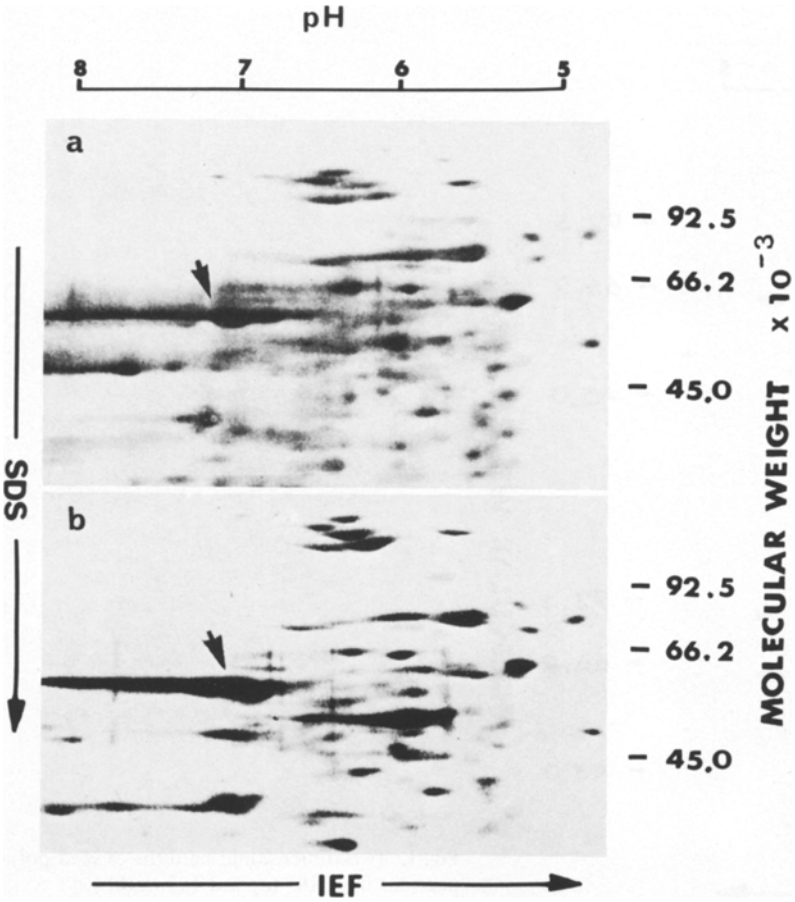

Fig. 2. Two-dimensional patterns of seed polypeptides from two nonwaxy strains, 108 (**a**) and W025 (**b**)

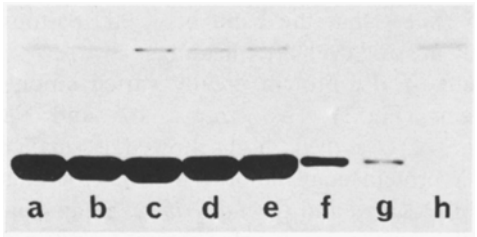

Fig. 3. Proteins extracted from starch granules of mature endosperms. *a* 108; *b* C8669; *c* C8005; *d* W025; *e* GM1; *f* T65; *g* C9078; *h* T65*wx*

the level of *Wx* protein. The amount of *Wx* protein was well correlated with the *Wx* gene dosage (Fig. 5). Thus, one, two and three doses of *Wx* alleles in the endosperm revealed a progressive increase in *Wx* protein levels, although the protein level in T65 × T65*wx* was lower than those in the other crosses. Two additional minor proteins with higher molecular weights did not appear to vary with the dosage.

Endosperm starch was waxy in the F_1 hybrids of T65*wx* × 221 and T65*wx* × GM2, indicating that the three waxy strains have the same recessive gene. Selfed seeds from heterozygous *Wx*/*wx* plants are expected to segregate endosperms with different doses of *Wx* alleles. In the F_2, endosperms with *Wx Wx Wx*, *Wx Wx wx*, *Wx wx wx* and *wx wx wx* segregate into a ratio of 1 : 1 : 1 : 1. Only endosperms with the genotype of *wx wx wx* are distinguishable from others by IKI staining. The three F_1 hybrids, T65 × T65*wx*, C8669 × 221 and GM1 × GM2, all showed seed fertility higher than 85% and a good fit to 3 *Wx*:1 *wx* ratio in F_2, although hybrids between distantly related strains of rice often result in a distorted segregation at the *wx* locus. *Wx* proteins in F_2 endosperms were examined individually in the three crosses in order to know if four types of endosperms are distinguished from intensities of *Wx* protein levels. SDS extracts from individual endosperms showed that the four different intensity types were distinguishable. Expectedly, they segregated into a 1 : 1 : 1 : 1 in the three crosses examined (Table 2).

Differentiation of Wx alleles

As shown in Figs. 3 and 5, *Wx* alleles of Indica type of *O. sativa* and *O. glaberrima* had a high efficiency in the

Table 2. Segregation for the *Wx* protein level in F_2 seeds of crosses between nonwaxy (*WxWx*) and waxy (*wxwx*) strains

Cross	No. of seeds examined	No. of endosperms with[a]				χ^2 (1 : 1 : 1 : 1)	*P*
		WxWxWx	*WxWxwx*	*Wxwxwx*	*wxwxwx*		
T65 × T65*wx*	35	9	11	7	8	1.089	> 0.75
C8669 × 221	25	9	4	7	5	2.360	> 0.25
GM1 × GM2	20	5	7	4	4	1.200	> 0.75

[a] Genotypes were estimated from the intensity of the *Wx* protein level as shown in Fig. 5

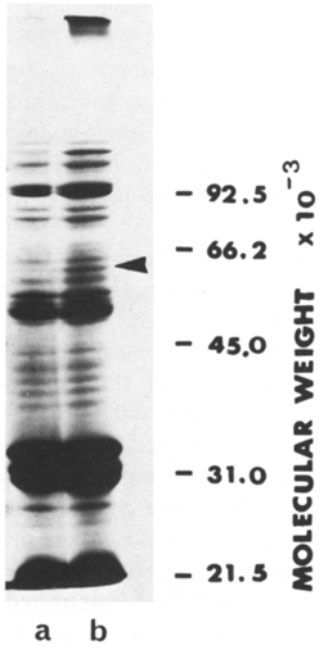

Fig. 4. The effect of heating on extraction of whole endosperm proteins in T65. *a* heated; *b* not heated

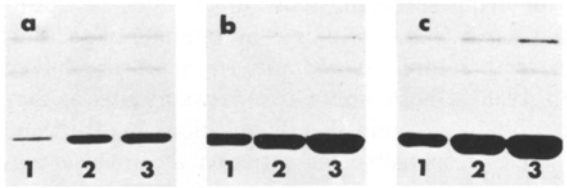

Fig. 5a–c. Dosage effect of *Wx* gene on the *Wx* protein level. Numerals indicate the number of *Wx* alleles in the endosperm. **a** T65 × T65*wx* (two-fold amounts of samples were loaded); **b** C8669 × 221; **c** GM1 × GM2

production of *Wx* protein than those of Japonica type of *O. sativa*. This strongly suggests that at least two different *Wx* alleles exist among rice strains. If there were different *Wx* alleles in addition to recessive *wx* alleles at the locus, the hybrids between two nonwaxy strains, one having a full level of *Wx* protein as found in C8669 and GM1 and the other having a lower level as in T65, would be expected to produce four different endosperms in F_2 with regard to the amount of *Wx* protein formed in starch granules although all F_2 seeds are phenotypically nonwaxy by IKI staining. The F_1 hybrids between T65 and C8669 or GM1, however, were highly sterile. Therefore, C8005, which showed a full level of *Wx* protein in starch granules (Fig. 3c) and gave completely fertile F_1 plants in the cross with T65, was used for this purpose.

By examining SDS extracts from starch granules in individual F_2 seeds of the T65 × C8005 cross, four different types of endosperms were recognized from intensities of *Wx* protein levels, as visualized in Fig. 6. Assuming that C8005 and T65 have Wx^a and Wx^b alleles, respectively, and that Wx^a produces a higher level of the gene product, four different endosperms with $Wx^aWx^aWx^a$, $Wx^aWx^aWx^b$, $Wx^aWx^bWx^b$ and $Wx^bWx^bWx^b$ are expected to occur in F_2 and contain different levels of *Wx* protein segregating into a 1 : 1 : 1 : 1 ratio. In fact, the four types estimated from the intensities agreed with a 1 : 1 : 1 : 1 ratio (Table 3). This indicates the presence of two *Wx* alleles which control different levels of *Wx* protein in starch granules.

Discussion

The waxy locus on the first linkage group (Chromosome 6) in rice controls the production of amylose in the endosperm (Ikeno 1914; Nagao and Takahashi 1963; Iwata and Omura 1971). Starch produced in the

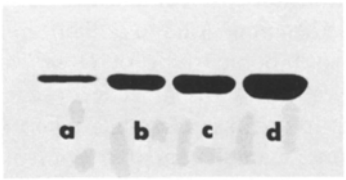

Fig. 6. Gene dosage effect of Wx^a and Wx^b on the *Wx* protein level in F_2 of C8005 (Wx^aWx^a) × T65 (Wx^bWx^b). *a* $Wx^aWx^aWx^a$ *b* $Wx^aWx^aWx^b$; *c* $Wx^aWx^bWx^b$; *d* $Wx^bWx^bWx^b$

Table 3. Segregation for the *Wx* protein level in F_2 seeds of a cross between two strains showing different level of the *Wx* protein, C8005 (Wx^aWx^a) and T65 (Wx^bWx^b)

No. of seeds examined	No. of endosperms with[a]				χ^2 (1 : 1 : 1 : 1)	*P*
	$Wx^aWx^aWx^a$	$Wx^aWx^aWx^b$	$Wx^aWx^bWx^b$	$Wx^bWx^bWx^b$		
36	6	9	11	9	1.457	> 0.50

[a] Genotypes were estimated from the intensity of the *Wx* protein level as shown in Fig. 6

homozygous *Wx* endosperm is composed of about 15–30% amylose and 85–70% amylopectin while the *wx* endosperm lacks amylose and contains primarily amylopectin (IRRI 1974). The published data in maize show that the *Wx* gene product is almost certainly the starch-granule-bound NDP sugar-starch glucosyl transferase whose activity is nearly absent in *wx* endosperm (Nelson and Rines 1962; Nelson et al. 1978) and varies linearly with *Wx* gene dosage (Tsai 1974). In addition, analysis in the protein level of the gene product was successfully carried out by Echt and Schwartz (1981). They reported that the amount of the gene product called *Wx* protein also increases with *Wx* gene dosage and *wx* alleles result in the inactivation, absence or drastic reduction of *Wx* protein.

The present results show that a major protein tightly bound to starch-granules in rice endosperm had same characteristics as the *Wx* protein found in maize. The major protein called *Wx* protein in rice seems to be the *Wx* gene product although enzymatic activity was not examined in rice. Out of 23 waxy strains examined, 22 lacked *Wx* protein completely in spite of the fact that they could have an inactivated protein instead of the absence of *Wx* protein. The reason why most of waxy strains lack *Wx* protein in rice remains unknown, but this suggests a possibility that the level of *Wx* protein partly controls amylose content in rice endosperm.

With regard to amylose content, the *wx* allele is incompletely recessive to *Wx* allele since amylose content increases with the number of *Wx* alleles in triploid endosperms (IRRI 1976; Okuno 1978). Taking gene dosage effect on *Wx* protein into account, amylose content seems to be partly determined by the amount of *Wx* protein. In addition, the present study suggests that among nonwaxy rice strains there are different *Wx* alleles, Wx^a and Wx^b, which control not only the level of the gene product but also amylose content in endosperm starch. Okuno (1978) and Amano (1980) indicated that *Wx* alleles in Japonica type of *O. sativa* (T65 and N8) show different patterns of gene dosage effect on amylose content from those of Indica type of *O. sativa* and *O. glaberrima,* namely amylose content increased markedly by one dose of *Wx* allele in the latter compared to in the former. This is easily explained by assuming that Wx^a carried by the latter produces a higher level of *Wx* protein and amylose than Wx^b of the former and the alleles act additively in triploid endosperms as shown in the present study. Reciprocal hybridizations between high- and low-amylose lines showed some decrease in amylose with an increase in the low-amylose gene, also indicating partial dominance of high amylose over low amylose (IRRI 1976). This is in accordance with the above assumption if the hybrids were heterozygous for Wx^a and Wx^b.

To prove the above assumption, Wx^a of W025 has been introduced by successive backcrossing (BC_{10}) into the genetic background of T65*wx* which lacks *Wx* protein. The result will be published elsewhere, but the isogenic line with Wx^a showed a level of *Wx* protein and amylose as high as the donor parent W025, higher than T65 with Wx^b (in preparation). Hence, one of the major factors controlling the levels of *Wx* protein and amylose appears to be allelic difference at the *Wx* locus. However, the inheritance of amylose content seems to be rather complicated among nonwaxy rice strains. In general, high amylose content is controlled by one gene of major effect and several modifiers (Bollich and Webb 1973; IRRI 1976; Satoh and Omura 1981; McKenzie and Rutger 1983; Okuno et al. 1983). Recently, McKenzie and Rutger (1983) reported that a major gene controlling amylose content is linked with a gene for alkali spreading score (*alk*) which is an additional starch characteristics as an estimate of gelatinization temperature. As *alk* and *wx* genes are linked (Kudo 1968; Ghosh and Govindaswamy 1972), they most probably examined a segregation for Wx^a and Wx^b alleles responsible for variation in amylose content.

Of special interest is the presence of different alleles, Wx^a and Wx^b, which regulate the quantitative level of the gene product. Such a difference may be regarded as the result of a regulatory mutation (Scandalios and Baum 1982). This mutant acts in cis because dosage effect was observed in F_2 endosperms of the heterozygotes, Wx^a/Wx^b, Wx^a/wx and Wx^b/wx. In addition, all *wx* strains examined showed the absence or drastic reduction of *Wx* protein rather than the formation of an inactive protein which might result from a mutation within the structural gene. In the mutants where the

Wx protein is absent or reduced, possibly the transcription, translation or RNA processing may be defective so that no or little protein is synthesized as discussed by Echt and Schwartz (1981). Although little is known about the function of such regulatory information, similar *cis*-acting elements are assumed to exist in maize (Schwartz 1966; Schwartz and Endo 1966; Freeling and Cheng 1978), rice (Endo 1981) and *Petunia* (Berg et al. 1983). In rice, amylose content greatly varies among rice strains (IRRI 1974), however, it is suggested that the Japonica type of *O. sativa* has comparatively lower amylose content than the Indica type showing some overlapping. Further examinations are needed to know if differential regulation as found between Wx^a and Wx^b is related to intraspecific differentiation into the Japonica and Indica types. Thus, biochemical approaches to the study of *Wx* protein in rice may throw more light on not only an important grain quality trait but also regulatory mechanism of gene expression in higher plants.

Acknowledgements. I express my hearty thanks to Drs. H. I. Oka, H. Morishima, T. Endo for their helpful advice and suggestion and Drs. C. Cohen, S. G. Echt for their kind technical advice. I am also indebted to Dr. K. Okuno for analysis of amylose content, and Mrs. R. Sano, Miss M. Katsumata for their interest and technical assistance. This work was partly supported from the Ministry of Education, Science and Culture, Japan.

References

Amano E (1980) Phenotypic expression of *Wx* gene in cereals. Annu Rep Natl Inst Genet, Misima 31:82–83

Berg BM van den, Wijsman HJW, Bianch F (1983) Genetics of the peroxidase isoenzyme in *Petunia*. 6. Differential temporal expression of *prx B* alleles. Theor Appl Genet 66: 173–178

Bollich CN, Webb BD (1973) Inheritance of amylose of two hybrid populations of rice. Cereal Chem 50:631–636

Echt CS, Schwartz D (1981) Evidence for the inclusion of controlling elements within the structural gene at the waxy locus in maize. Genetics 99:275–284

Endo T (1981) Differential regulation of peroxidase isozymes coded by *Px-1* locus in rice. Jpn J Genet 56:175–183

Freeling M, Cheng DSK (1978) Radiation-induced alcohol dehydrogenase mutants in maize following allyl alcohol selection of pollen. Genet Res 31:107–129

Ghosh AK, Govindaswamy S (1972) Inheritance of starch-iodine-blue value and alkali digestion value in rice and their genetic association. Il Riso 21:123–132

Ikeno S (1914) Über die Bestäubung und die Bastardierung von Reis. Z Pflanzenzücht 2:495–503

International Rice Research Institute (1974) Annu Rep 1973. Los Banos, Philippines, pp 3–9

International Rice Research Institute (1976) Annu Rep 1975. Los Banos, Philippines, pp 85–86

Iwata N, Omura T (1971) Linkage analysis by reciprocal translocation method in rice plants (*Oryza sativa* L.). 1. Linkage groups corresponding to the chromosome 1, 2, 3, and 4. Jpn J Breed 21:19–28

Kudo M (1968) Genetical and breeding studies on physiological and ecological characters in hybrids between ecological groups of rice. Bull Natl Inst Agric Sci, Ser D 19:1–84

Laemmli UK (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227: 680–685

Li HW, Wang S, Yeh PZ (1965) A preliminary note on the fine structure analysis of glutinous gene in rice. Bot Bull Acad Sin 6:101–105

Li HW, Wu PH, Wu L, Chu MY (1968) Further studies of the interlocus recombination of the glutinous gene in rice. Bot Bull Acad Sin 9:22–26

Lowry OH, Rosenbrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the Folin phenol reagent. J Biol Chem 193:265–274

McKenzie KS, Rutger JN (1983) Genetic analysis of amylose content, alkali spreading score, and grain dimensions in rice. Crop Sci 23:306–313

Nagao S, Takahashi ME (1963) Trial construction of twelve linkage groups in Japanese rice (Genetical studies on rice plant XXVII). J Fac Agric, Hokkaido Univ 53:72–130

Nelson OE, Chourey PS, Chang MT (1978) Nucleoside diphosphate sugar-starch glucosyl transferase activity of *wx* starch granules. Plant Physiol 62:383–386

Nelson OE, Rines HW (1962) The enzymatic deficiency in the *waxy* mutant of maize. Biochem Biophys Res Commun 9: 297–300

O'Farrell PH (1975) High resolution two-dimensional electrophoresis of proteins. J Biol Chem 250:4007–4021

Oka HI (1977) Genetic variations of *Oryza glaberrima,* their survey and evaluation. In: Meeting on African Rice Species, IRAT-ORSTOM, Paris, pp 77–82

Okuno K (1978) Gene dosage effect of waxy alleles on amylose content in endosperm starch of rice. Jpn J Genet 53:219–222

Okuno K, Fuwa H, Yano M (1983) A new mutant gene lowering amylose content in endosperm starch of rice. Jpn J Breed 33:387–394

Sano Y (1978) Gene markers obtained in *O. glaberrima.* Annu Rep Natl Inst Genet, Misima, 29:97–98

Satoh H, Omura T (1981) New endosperm mutations induced by chemical mutagens in rice, *Oryza sativa* L. Jpn J Breed 31:316–326

Scandalios JG, Baum JA (1982) Regulatory gene variation in higher plants. Adv Genet 21:347–370

Schwartz D (1966) The genetic control of alcohol dehydrogenase in maize: gene duplication and repression. Proc Natl Acad Sci USA 56:1431–1436

Schwartz D, Endo T (1966) Alcohol dehydrogenase polymorphisms in maize-simple and compound loci. Genetics 53: 709–715

Schwartz D, Echt CS (1982) The effect of *Ac* dosage on the production of multiple forms of *Wx* protein by the wx^{m-9} controlling element mutation in maize. Mol Gen Genet 187:410–413

Tsai CY (1974) The function of the waxy locus in starch synthesis in maize endosperm. Biochem Genet 11:83–96