Comparison of Non-Mutant and Mutant *waxy* Genes in Rice and Maize

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ABSTRACT

The *waxy* gene, which is responsible for the synthesis of amylose in endosperm and pollen, is genetically well characterized in many grasses including maize and rice. Homology between the previously cloned maize *waxy* gene and the rice gene has facilitated our cloning of a 15-kb *Hin*dIII fragment that contains the entire rice gene. A comparison of the restriction maps of the maize and rice genes indicates that many restriction sites within translated exons are conserved. In addition, the rice gene encodes a 2.4-kb transcript that programs the *in vitro* synthesis of a 64-kD pre-protein which is efficiently precipitated with maize *waxy* antisera. We demonstrate that these gene products are altered in rice strains containing mutant *waxy* genes. Southern blot analysis of 16 rice strains, ten containing *waxy* mutations, reveals that the *waxy* gene and flanking restriction fragments are virtually identical. These results contrast dramatically with the high level of insertions and deletions associated with restriction fragment length polymorphism and spontaneous mutations among the *waxy* alleles of maize.

THE *waxy* (*wx*, also called *glutinous*) locus of rice (*Oryza sativa*), like the *waxy* locus of other grasses, is responsible for the synthesis of amylose in the triploid endosperm of the developing grain and in the haploid pollen (PARNELL 1921). The starch of wild-type endosperm tissue consists of between 15–30% amylose and 70–85% amylopectin whereas endosperm starch is 100% amylopectin in most *waxy* mutants (SANO, KATSUMATA and OKUNO 1986). The locus has been a favorite of plant geneticists since the turn of the century because mutants are fully viable and the mutant phenotype can be easily scored in thousands of progeny by a quick visual inspection (the endosperm has a waxy appearance) or by staining for the presence of amylose in either endosperm or pollen with I/KI.

Rice *waxy* mutants are not only of interest to geneticists; they are also of agronomic importance. The altered starch composition of *waxy* mutants changes the cooking properties of the rice grain making the starch valuable in the production of certain food products. In several countries, this type of rice accounts for over 10% of total rice production (GRIST 1986). For this reason over one hundred *waxy* mutants have been isolated and collected and are available for molecular analysis. This represents one of the largest collections of mutant alleles for any plant gene.

The molecular biology of the rice *waxy* gene is poorly understood despite the availability of numerous mutant alleles and a fine structure genetic map comprised of some of these mutations (LI, WANG and YEH 1965). In contrast, the maize *waxy* gene has been cloned (SHURE, WESSLER and FEDOROFF 1983) and several *waxy* alleles have been characterized. Structural analysis of these alleles has revealed the important role of DNA insertions and deletions in the generation of restriction fragment length polymorphism (RFLP) and spontaneous *wx* mutations (WESSLER and VARAGONA 1985; SPELL, BARAN and WESSLER 1988). In view of the paucity of genetic systems accessible to molecular techniques in higher plants and the importance of rice as a crop plant, we undertook the cloning of the rice *waxy* gene and the characterization of its non-mutant and mutant gene products. In this report we also present a structural analysis of some of the rice *waxy* alleles. Taken together, these results provide a unique opportunity to contrast the molecular mechanisms underlying RFLP and mutation in the diverse grasses maize and rice.

MATERIALS AND METHODS

Plant strains: The source of maize *Wx* DNA and RNA was the inbred HY (WESSLER and VARAGONA 1985). The source of rice *Wx* alleles were: Nato, Bluebonnet 50 (M. MISHKIND), IR 36 (R. COFFMAN), Labelle, Pecos (R. DILDAY), and M101 (N. RUTGER). The source of rice *wx* alleles were: *wx*CI 9972, *wx*PI 260662, *wx*PI 291667, *wx*PI 291656 (USDA ARS National Small Grain Collection, Beltsville, Maryland), *wx*IR29, *wx*PI 224836, *wx*PI 248486, *wx*PI 434619 (International Rice Research Institute, Los Banos, Philippines), M101*wx* (N. RUTGER), and *wx*Glut Znth 205 (R. DILDAY). Mutations *wx*CI 9972 and M101*wx* were induced with cobalt-60; others are believed to be spontaneous. The varieties of millet and sorghum are unknown, wheat was cv. Chinese Spring.

Genomic DNA and RNA preparation and genomic blot

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analysis: DNA was purified from 2–4-week-old maize plantlets or from tillering rice by methods described previously (SHURE, WESSLER and FEDOROFF 1983). For Southern blot analysis, six μg of restricted DNA was fractionated by electrophoresis through 1% agarose, blotted in an alkaline solution to GeneScreen Plus (CHOMCZYNSKI and QASBA 1984), and hybridized in 6X SSC, 1% SDS, 10 mM EDTA, 5% dextran sulfate and 0.5 mg/ml heparin. Washes were in 0.5X SSC, 0.5% SDS at 65° for heterologous probes and in 0.1X SSC, 0.5% SDS at 65° for homologous probes.

RNA was isolated from immature maize seed (harvested 18–22 days after pollination), immature rice seed at the milky stage, and immature millet, wheat and sorghum seed by method described previously for maize tissue (SHURE, WESSLER and FEDOROFF 1983). Poly(A) RNA was fractionated in formaldehyde-containing agarose gels as described in WESSLER *et al.* (1986), and transferred to Magnagraph (Fisher Scientific) according to the method of THOMAS (1980). DNA probes used for Southern and Northern blot analysis were labeled by nick translation (RIGBY *et al.* 1977).

***In vitro* translations and immunoprecipitations:** Poly(A) RNA was used to program the synthesis of [^{35}S]methionine-labeled polypeptides in a rabbit reticulocyte lysate system (Bethesda Research Labs). A polyclonal antiserum raised against the maize *Wx* protein was used to immunoprecipitate polypeptides from the total translation products. *In vitro* translations and immunoprecipitations were as described (SHURE, WESSLER and FEDOROFF 1983). Samples were separated on a 10–20% (w/v) SDS-polyacrylamide gradient gel with a 5% polyacrylamide stacking gel. Electrophoresis was carried out at 4° for 16 hr at 20 mA using the discontinuous buffer system of LAEMMLI (1970). Gels were trimmed, fixed, impregnated with PPO and POPOP in DMSO and exposed to Kodak X-Omat film overnight (LASKEY and MILLS 1975).

Genomic cloning of the wild-type rice *waxy* gene: A rice library was constructed using the lambda vector 2001 (Stratagene) and genomic DNA from the Labelle variety. One-half microgram of *Hin*dIII digested DNA was ligated with one μg of Lambda 2001 arms derived from a *Hin*dIII digestion. Ligated molecules were packaged with GigaPack Plus (Stratagene), plated on LE392 cells (ENQUIST and WEISBERG 1977) and filter lifts were hybridized with probe 2 from the maize *Wx* gene (see Figure 3). From a library of 200,000 primary recombinant phage, eight phage hybridized with the probe and were shown to be identical following digestion with *Hin*dIII, *Eco*RI or *Sal*I.

A 6-kb *Eco*RI fragment, determined by Southern blot analysis to contain all sequences that hybridize with the maize *Wx* gene, was subcloned into pUC 18 (YANISCH-PERRON, VIERA and MESSING 1985) and a detailed restriction map was prepared.

RESULTS

Homologous *Wx* transcripts in several grasses: Previous genetic and biochemical studies indicate that the *Wx* genes of maize and rice are very similar. Both genes encode starch granule bound UDP-glucosyl transferases that are responsible for the synthesis of amylose in endosperm and pollen (NELSON and RINES 1962; MURATA, SUGIYAMA and AKAZAWA 1965). The *Wx* proteins have been isolated from endosperm starch granules in maize (SHURE, WESSLER and FEDOROFF 1983) and rice (SANO 1984) and have apparent M_r of 58 kD and 60 kD, respectively.

To determine if this similarity extended to the DNA sequences of the two genes, we used Northern blot analysis to detect transcripts homologous with the cloned maize *Wx* gene among poly(A) RNA isolated from the immature seed of rice and other grasses. The results of this analysis are presented in Figure 1. Under conditions of high stringency (see MATERIALS AND METHODS), maize *Wx* probes 2, 3, and 4 (see Figure 3) hybridize with a 2.4-kb transcript from maize, rice, millet and wheat (Figure 1A, lanes 1, 2, 4, 5, respectively and a 2.6-kb transcript from sorghum (lane 3).

The fact that the 2.4-kb rice transcript is altered in two rice strains containing *waxy* mutations demonstrates that this transcript is the product of the *Wx* gene (Figure 1B). Strain *wx*CI 9972 is null for *waxy* expression (as assayed by I/KI staining of endosperm and pollen) and contains a *wx* transcript that is 200 bp shorter than the non-mutant strain (Figure 1B, lanes 1 and 2), whereas the null allele harbored by strain *wx*PI 260662 does not encode a distinct transcript (Figure 1B, lane 3).

The rice *Wx* gene encodes a preprotein of the expected size: The 58-kD maize *Wx* protein is synthesized as a 65-kD precursor that is processed to its mature size during transport into amyloplasts (SHURE, WESSLER and FEDOROFF 1983). A polyclonal antiserum raised against the maize *Wx* protein (SHURE, WESSLER and FEDOROFF 1983) was used to identify antigenically related polypeptides among the *in vitro* translation products programmed by rice poly(A) RNA isolated from immature seed. The maize antiserum specifically precipitates a 64-kD polypeptide from the total translation products of rice *Wx* RNA (Figure 2, compare lanes 2 and 6). This polypeptide comigrates with the maize polypeptide (Figure 2, lane 5). Strain *wx*PI 260662, which has no distinct *wx* transcript (Figure 1B, lane 3), produces no antigenically-related polypeptide (Figure 2, compare lanes 3 and 7) whereas strain *wx*CI 9972, which has a shorter *wx* transcript (Figure 1B, lane 2), produces a 61-kD polypeptide (Figure 2, compare lanes 4 and 8). Thus, the rice *Wx* protein is immunologically related to the maize protein and is synthesized as a precursor polypeptide of 64 kD that is probably processed to its mature size of 60 kD (SANO 1984) during transport into amyloplasts. This is similar to the maize precursor polypeptide of 65 kD that is processed to 58 kD during transport.

Cloning and characterization of the rice *Wx* gene: Southern blot analysis revealed that all rice sequences that hybridize with maize *Wx* probes 2, 3, and 4 (Figure 3) reside on a 15-kb *Hin*dIII fragment in several rice strains containing *Wx* alleles (data not shown). This fragment was cloned from the Labelle strain by ligating a *Hin*dIII digest of genomic DNA into the lambda vector 2001 and identifying clones

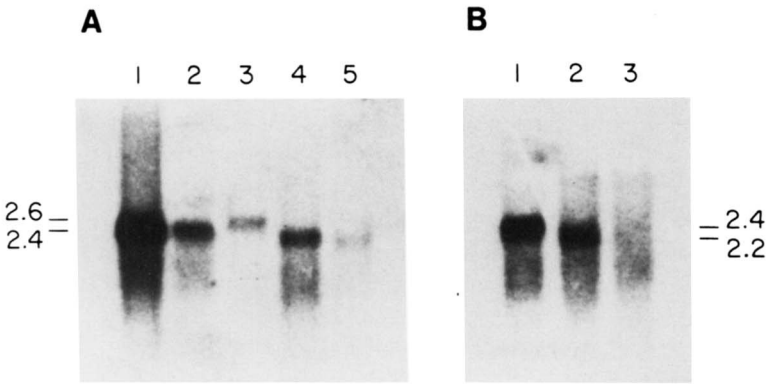

FIGURE 1.—Northern blot analysis of transcripts that hybridize with maize *Wx* probe 2 (Figure 3) in rice and other grasses. Identical results were obtained when maize *Wx* probes 3 or 4 were used. (A) Poly(A) RNA isolated from the immature seed of maize (1 μg, lane 1), rice (1 μg, lane 2), sorghum (1 μg, lane 3), millet (2 μg, lane 4) and wheat (1 μg, lane 5) are displayed. (B) One microgram of poly(A) RNA from immature seed of rice strains with a *Wx* allele (lane 1) or the *wx* alleles *wx*CI 9972 (lane 2) or *wx*PI 260662 are displayed. All blots were probed under conditions described in the Methods and washed in 0.1× SSC at 65°. The size of RNAs are in kilobases and were determined by comparison with RNA standards that are not shown.

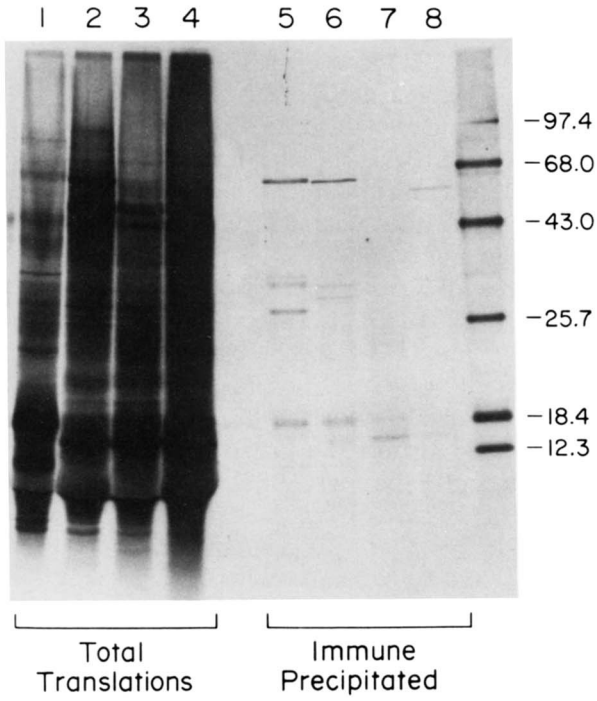

FIGURE 2.—*In vitro* translations programmed by poly(A) RNA isolated from immature seeds of maize and rice. [^{35}S]Methionine-labeled polypeptides synthesized in a rabbit reticulocyte translation system were fractionated by gel electrophoresis either before (lanes 1–4) or after precipitation with maize *Wx* antiserum (lanes 5–8). The source of RNA was: maize *Wx* (lanes 1 and 5), rice *Wx* (Nato) (lanes 2 and 6), rice *wx*PI 260662 (lanes 3 and 7) and rice *wx*CI 9972 (lanes 4 and 8). The sizes of protein standards are in kilodaltons.

containing sequences that hybridize with maize *Wx* probes. From a primary library of 200,000 clones, eight positive plaques were recovered. One recombinant phage was selected for subcloning after initial restriction mapping showed that the eight phage were identical.

A 6-kb *Eco*RI fragment, identified by hybridization with maize *Wx* probes as containing most, if not all, of the *Wx* gene, was subcloned into the plasmid pUC 18 and used to generate a restriction map that includes all the sites for the enzymes *Ava*I, *Bam*HI, *Pst*I, *Sal*I and *Sst*I. A comparison between the rice and maize genes (Figure 3) indicates that several restriction sites within exonic sequences that encode the mature maize *Wx* protein are apparently conserved (Figure 3). Two of the *Sal*I sites and two of the *Sst*I sites within the maize gene (indicated by stars in Figure 3) are less than 50 bp apart and difficult to resolve by gel analysis and, therefore, might also be present in the rice gene. We were able to confirm the alignment of sites displayed in Figure 3 by probing restriction digests of the rice gene with the appropriate labeled maize subclones (data not shown). These Southern blot analyses also revealed that probe #1, which contains the untranslated first exon of the maize *Wx* gene and 5′ flanking sequences, does not hybridize with the 6-kb *Eco*RI rice subclone or the 15-kb *Hin*dIII fragment from which it was derived (data not shown).

Genomic organization of wild-type and mutant *waxy* genes: Insertions and deletions within and flanking the maize *waxy* gene are the predominant causative agents underlying RFLP and spontaneous mutations (WESSLER and VARAGONA 1985; SPELL, BARAN and WESSLER 1988). In order to see if this finding is applicable to other grasses, the restriction fragments that comprise the *waxy* gene and flanking DNA were compared among six *Wx* and ten *wx* alleles.

Digestion of the cloned *Wx* gene isolated from the strain Labelle with either *Pst*I, *Eco*RI, *Hin*dIII, *Sst*I, *Bam*HI or *Sal*I produces the fragments displayed in Figure 4. These fragments span a region of approximately 25 kb which includes the 4 kb of the *Wx* transcription unit and 7 kb and 14 kb flanking, respectively, the 5′ and 3′ ends of the transcription unit. From the restriction maps shown in Figure 4, it can be seen that digestion with either *Eco*RI, *Pst*I or *Sal*I (group I) produces fragments that contain predominantly the transcription unit whereas digestion with *Hin*dIII, *Sst*I or *Bam*HI (group II) produces larger fragments extending into adjacent sequences.

To identify insertions or deletions larger than 50 bp, genomic DNA, isolated from each of the 16 strains, was first digested with at least one enzyme from group I and one from group II. Following gel electrophoresis and transfer to filters, Southern blots were probed with the rice 6-kb *Eco*RI fragment, which

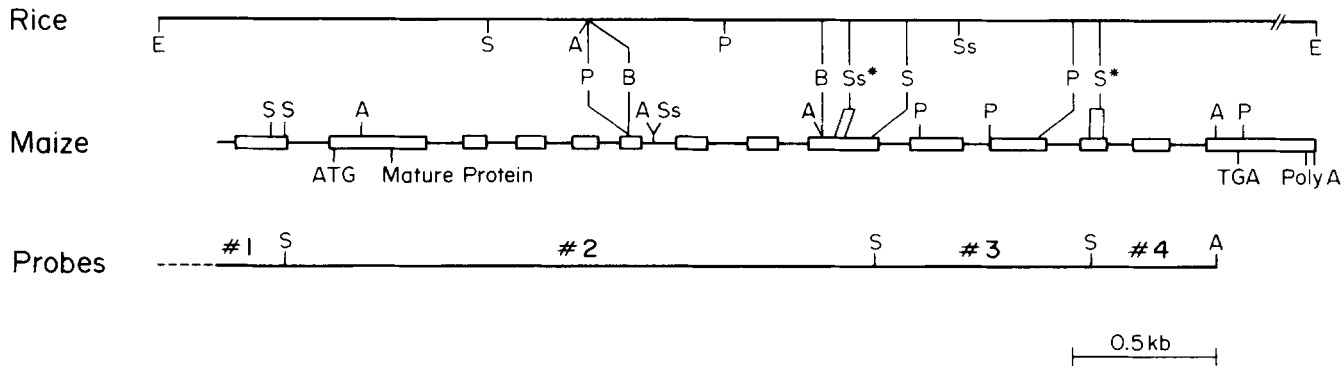

FIGURE 3.—Comparative restriction maps of the maize (HY) and rice (Labelle) *waxy* genes and the positions of the maize probes used in this study. The restriction map of a 6-kb *Eco*RI rice genomic fragment which contains most, or all, of the *Wx* transcription unit is aligned with the maize gene. Restriction sites in common between rice and maize are shown by the connecting lines. The position of exons and other landmarks within the maize *Wx* gene were determined by KLOSGEN *et al.* (1986). Asterisks indicate maize restriction sites separated by less than 50 bp. At our level of resolution we cannot determine whether there are one or two corresponding sites in the rice gene. The relative positions of the maize probes used in this study are also shown; probe 1 is a 3.2-kb *Sal*I fragment. Restriction sites are abbreviated as follows: A, *Ava*I; B, *Bam*HI; E, *Eco*RI; P, *Pst*I; S, *Sal*I; and Ss, *Sst*I.

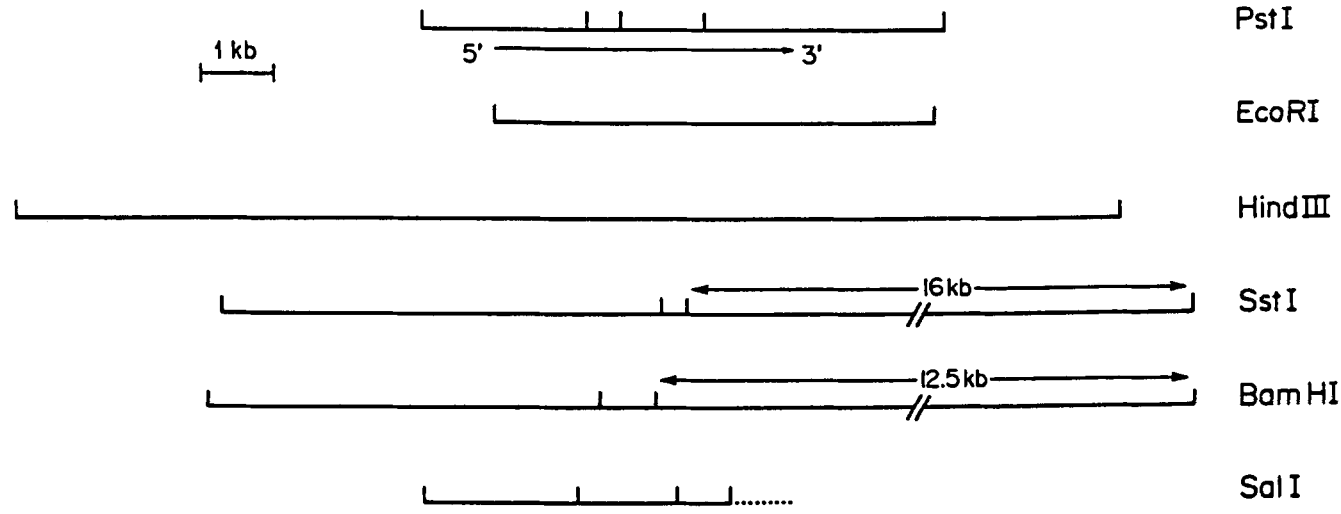

FIGURE 4.—Restriction fragments within and flanking the *Wx* gene of the rice strain Labelle. The presence or absence of these fragments in other strains is summarized in Table 1 and Figure 5. The 3' *Sal*I fragment is not resolved on gels and may be extremely large. The arrow indicates the approximate limits of the *Wx* transcription unit.

can detect all of the fragments displayed in Figure 4. Comparison of eight of the 16 strains digested with three enzymes is shown in Figure 5. These data and the results from additional Southern blots were compared to the Labelle fragments (Figure 4) and are summarized in Table 1.

Of the six strains containing *Wx* alleles that were analyzed in this manner, we detected only two restriction fragments that differed from those in Figure 4; a 6.8-kb *Sst*I fragment in IR36 (*vs.* 6.1 kb) (Figure 5, lanes 1 and 2) and a 15-kb *Bam*HI fragment in M101 (*vs.* 12.5 kb). In both cases insertions or deletions are not associated with the polymorphism because digestion with another enzyme that generates fragments containing these polymorphic regions reveals the standard pattern. Comparison of the ten strains containing *wx* alleles also reveals only two different fragments: a 2.0-kb *Pst*I fragment in *wx*Ci 9972 (*vs.* 2.3 kb) (Figure 5, lane 4) and a 15-kb *Bam*HI fragment in M101*wx* (*vs.* 12.5 kb). Again, insertions or deletions are ruled out as the causative agent of these mutations since digestion with other enzymes does not produce unique fragments. In addition, the 15-kb *Bam*HI fragment in M101*wx* is also present in its progenitor, M101.

DISCUSSION

The *waxy* locus is genetically well characterized in several diverse plant species. With the recent isolation and analysis of several normal and mutant maize *waxy* genes (SHURE, WESSLER and FEDOROFF 1983; FEDOROFF, WESSLER and SHURE 1983; SCHWARZ-SOMMER *et al.* 1984; BEHRENS *et al.* 1984; WESSLER and VARAGONA 1985; KLOSGEN *et al.* 1986; WESSLER *et al.* 1986; WESSLER, BARAN and VARAGONA 1987), the cloning of the rice gene provides a unique opportunity

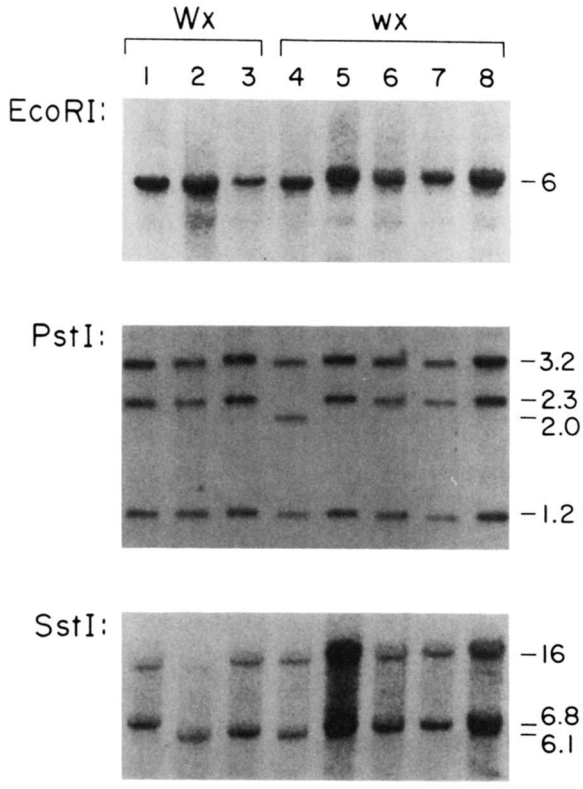

FIGURE 5.—Southern blot analysis of *waxy* genes in several strains of rice containing either *Wx* or *wx* alleles. For each lane, six micrograms of genomic DNA were digested with either *Eco*RI, *Pst*I, or *Sst*I, blotted and probed with the 6-bp *Eco*RI fragment shown in Figure 3. The large *Sst*I band in lane 2 is faint in this exposure, but is present in longer exposures. In these experiments the smallest fragments resulting from digestion with *Pst*I, *Sst*I, or *Bam*HI were run off the end of the gel to enhance resolution of the larger fragments. Small variations in band migration reflect differences in the actual amount of genomic DNA in each lane as revealed by ethidium bromide staining. Lane 1, IR 36; lane 2, Labelle; lane 3, Nato; lane 4, *wx*CI 9972; lane 5, *wx*PI 260662; lane 6, *wx*PI 291667; lane 7, *wx*PI 291656; lane 8, *wx*IR 29.

to compare non-mutant and mutant gene structure and gene expression in these distantly related grasses.

The *waxy* gene in maize and rice: In this report we have described the identification and cloning of a 15-kb *Hin*dIII fragment from the rice strain Labelle that contains a region that is homologous with the maize *Wx* gene. The following evidence leads us to conclude that this fragment contains the rice *Wx* gene: (1) under stringent hybridization conditions, this is the only rice DNA that hybridizes with maize *Wx* probes, (2) restriction maps of the two genes are very similar when exonic DNA is compared, and (3) when used as a probe of Northern blots, this fragment or subclones from it detect only a single transcript of 2.4 kb among poly(A) RNA isolated from immature seed of non-mutant maize or rice. A transcript of this length is not seen when RNA from two rice *wx* mutants is examined.

DNA sequence similarity between the maize and rice genes is reflected in the antigenic relatedness of

TABLE 1

Summary of restriction analysis of *waxy* alleles in rice[a]

waxy Allele	*Eco*RI	*Hin*dIII	*Sst*I	*Pst*I	*Sal*I	*Bam*HI
Wx:						
Labelle	1	1	1	1	1	
IR36	1	1	2	1	1	1
Bluebonnet 50	1				1	1
Pecos	1	1				
M101	1	1	1			4
Nato	1	1	1	1		
wx:						
M101*wx*	1	1			1	4
*wx*CI 9972	1	1	1	3		1
*wx*PI 260662	1	1	1	1	1	1
*wx*PI 291667	1	1	1	1	1	1
*wx*PI 291656	1	1	1	1	1	1
*wx*IR 29	1	1	1	1	1	1
*wx*PI 224836					1	1
*wx*PI 248486					1	1
*wx*PI 434619					1	1
*wx*Glut Znth 205					1	1

[a] The numbers indicate the following: 1, This strain, with this enzyme, has the same fragments as Labelle; 2, IR36 has a 6.8-kb *Sst*I band replacing the 6.1-kb band; 3, *wx*CI 9972 has a 2.0-kb *Pst*I band replacing the 2.3-kb band; 4, M101 and M101*wx* have a 15-kb *Bam*HI band replacing the 12.5-kb band.

the *Wx* proteins. Antiserum raised against the maize *Wx* protein cross-reacts with a single rice polypeptide synthesized *in vitro* from *Wx* poly(A) RNA. This polypeptide is approximately the same size as the maize *Wx* preprotein and was either absent or altered when the *in vitro* translation products encoded by the poly(A) RNA isolated from two rice strains containing *wx* alleles were analyzed.

Despite the similarities between the maize and rice genes in the regions that encode the mature *Wx* protein (see Figure 3), other regions of the gene do not appear to be highly conserved. First, probe 1 (Figure 3) which contains the maize *Wx* promoter region and the first (untranslated) exon does not hybridize with the rice 6-kb *Eco*RI fragment or any part of the 15-kb *Hin*dIII fragment under conditions that would detect up to 30% sequence mismatch (Figure 4). Second, preliminary DNA sequence analysis of selected regions of the rice gene indicate that although there is between 85% and 90% nucleotide identity when regions that encode the mature proteins are compared, identity between the putative transit peptides (encoded by part of exon 2, Figure 3) is limited to a few short blocks of sequence (R. J. OKAGAKI and S. R. WESSLER, unpublished results). This peptide is probably responsible for targeting the *Wx* protein to its final destination in the amyloplasts of the developing endosperm (SHURE, WESSLER and FEDOROFF 1983, KLOSGEN *et al.* 1986). A comparison of nuclear-encoded chloroplast proteins from diverse plant species, such as the small subunit of ribulose 1,5-biphosphate carboxylase/oxygenase, also reveals highly conserved

mature proteins and transit sequences that are less conserved (KARLIN-NEUMANN and TOBIN 1986).

***waxy* mutations and RFLP:** One striking difference between the rice and maize *waxy* genes revealed by this study is the level of genetic variation observed when six *Wx* alleles and ten *wx* alleles are compared by Southern blot analysis. Whereas insertions and deletions account for three-fourths of the spontaneous maize *waxy* lesions analyzed in one study (WESSLER and VARAGONA 1985), we could not detect a single clear-cut example of insertion or deletion among the rice *wx* alleles examined in this study. Similarly, comparison of maize *Wx* alleles identified four different 5′ flanking regions when 12 inbred lines were compared. These differences resulted from insertions and deletions outside of the transcription unit (WESSLER and VARAGONA 1985). Differences in the 3′ flanking regions were even more complicated and could not be deciphered by genomic Southern blot analysis. It is conceivable that each of the 12 lines is unique for the region of the chromosome containing the maize *Wx* gene and flanking sequences (M. J. VARAGONA and S. R. WESSLER, unpublished results). In contrast, the rice *waxy* gene is nearly invariant among the six *Wx* alleles examined. The limited RFLP detected is consistent with single base changes or very small insertions or deletions that add or remove restriction sites rather than the gross changes found in maize.

There are several possible reasons for the observed difference in genomic diversity among the *waxy* genes from rice and maize:

1. The *waxy* gene may be unusual and not representative of the types of changes that occur at other genes. In maize this is clearly not the case since the diversity observed among *waxy* alleles is also seen when alleles of *adh1* (JOHNS, STROMMER and FREELING 1983, SACHS *et al.* 1986), *sh* (BURR *et al.* 1983; ZACK, FERL and HANNAH 1986), and *bz* (RALSTON, ENGLISH and DOONER 1988) are compared.

2. The variation in rice may not have been adequately sampled. Since the pedigrees of most of the strains analyzed in this study are not known, it is conceivable that some strains had common ancestors and now contain identical *waxy* alleles. Although this is possible for a few of the strains, it cannot be true for most of them because differences in the *waxy* gene or its gene products are observed. For example, we have detected a few instances of RFLP among the 16 strains examined. In addition, Northern blot analysis of RNA from four of the *waxy* mutants listed in Table 1 reveals *wx* messages that differ in size and abundance, suggesting that they are independent mutations (Figure 2; M101*wx*, 2.4 kb, PI291667, 3.5 kb) (R. J. OKAGAKI and S. R. WESSLER, unpublished results). These mRNAs are encoded by genes that are indistinguishable from *Wx* alleles at the level of Southern blot analysis. The variability of sizes may result from mutations that alter normal splicing or normal transcription initiation or termination. Finally, unlike the maize *waxy* alleles analyzed previously, which represent a narrow geographical sampling (North American inbreds), the rice lines examined in this study were collected throughout the Asian continent.

3. The differences may reflect a real distinction between maize and rice. The examples of gross genomic differences observed when maize genes are compared generally results from transposable elements or DNA insertions (SACHS *et al.* 1986; SPELL, BARAN and WESSLER 1988; RALSTON, ENGLISH and DOONER 1988). The absence of this diversity among the *waxy* alleles of rice may indicate either that rice has fewer transposable elements or that the elements it has transpose less frequently. In this regard it is interesting to note that three rice *wx* alleles that have a high frequency of germinal reversion to a nonmutant phenotype do not have detectable transposable elements within or near the *wx* gene (T. BUREAU, G. KHUSH, R. J. OKAGAKI and S. R. WESSLER, unpublished results). This is in contrast to all other unstable alleles analyzed to date in maize and other plants where transposable elements have been shown to be responsible for gene instability.

Recently, MCCOUCH *et al.* (1988) reported that 78% of random rice genomic probes detected polymorphism between two rice cultivars. They attribute a significant proportion of these polymorphisms to insertions/deletions. Although these results appear to be at odds with our findings, the differences may reflect the fact that they, for the purposes of their study, selected cultivars that displayed the highest levels of polymorphism and discarded those that did not. In addition, it is likely that the vast majority of probes used in their study were not genes (G. KOCHERT, personal communication) and thus, the regions of the genome that were assayed may be subjected to different selection pressures than those exerted on active genes such as *waxy*. Finally, consistent with our results was their finding that the level of polymorphism detected in rice was lower than that found in maize.

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