Light-Regulated and Cell-Specific Expression of Tomato rbcS-gusA and Rice rbcS-gusA Fusion Genes in Transgenic Rice

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A previously isolated rice (Oryza sativa) rbcS gene was further characterized. This analysis revealed specific sequences in the 5' regulatory region of the rice rbcS gene that are conserved in rbcS genes of other monocotyledonous species. In transgenic rice plants, we examined the expression of the β-glucuronidase (gusA) reporter gene directed by the 2.8-kb promoter region of the rice rbcS gene. To examine differences in the regulation of monocotyledonous and dicotyledonous rbcS promoters, the activity of a tomato rbcS promoter was also investigated in transgenic rice plants. Our results indicated that both rice and tomato rbcS promoters confer mesophyll-specific expression of the gusA reporter gene in transgenic rice plants and that this expression is induced by light. However, the expression level of the rice rbcS-gusA gene was higher than that of the tomato rbcS-gusA gene, suggesting the presence of quantitative differences in the activity of these particular monocotyledonous and dicotyledonous rbcS promoters in transgenic rice. Histochemical analysis of rbcS-gusA gene expression showed that the observed light induction was only found in mesophyll cells. Furthermore, it was demonstrated that the light regulation of rice rbcS-gusA gene expression was primarily at the level of mRNA accumulation. We show that the rice rbcS gene promoter should be useful for expression of agronomically important genes for genetic engineering of monocotyledonous species.

Expression of the Rubisco small subunit genes (rbcS), which are regulated both developmentally and by light (for review, see Tobin and Silverthorne, 1985; Edwards and Coruzzi, 1990; Gilmartin et al., 1990), is one of the best models for studies of gene expression in plants (for review, see Gilmartin et al., 1990). However, compared with dicotyledonous rbcS genes, the regulation of rbcS genes in monocotyledonous species is not well understood. Part of the reason for this is the lack of a well-defined in vivo assay system. Transgenic monocots have not been available until recently, and to date monocotyledonous rbcS gene expression has been studied in transient assays of transformed mesophyll protoplasts (Schafrner and Scheen, 1991) or with particle-bombarded leaf tissues (Rolfe and Tobin, 1991; Bansal et al., 1992). However, developmental regulation of gene expression is not easily analyzed in such transient assay systems.

In other studies, transgenic tobacco has been used to examine the expression of light-regulated photosynthetic genes from monocotyledonous species (Lloyd et al., 1991; Matsuoka and Sanada, 1991). In these studies, anatomical differences between the leaf tissues of monocots and dicots have prevented an accurate evaluation of the observed tissue and cell specificity of gene expression. Moreover, monocotyledonous genes are not always expressed in an expected manner when introduced into transgenic dicotyledonous plants (Lamppa et al., 1985; Keith and Chua, 1986). In contrast, highly regulated expression of reporter genes directed by monocotyledonous promoters in transgenic rice (Oryza sativa) plants (Kyozuka et al., 1991; Tada et al., 1991; Zhang et al., 1991; Terada et al., 1993) has indicated that this in vivo assay system can be used for the analysis of promoters not only of rice genes but also of genes from other monocotyledonous species.

It has been suggested that the molecular mechanisms underlying gene expression are not the same for monocots and dicots. The promoters from rbcS genes of monocots, but not of dicots, are active in maize mesophyll protoplasts. In these protoplasts, promoter elements from dicotyledonous rbcS genes failed to replace functionally those from a monocotyledonous rbcS promoter (Schafrner and Scheen, 1991). It has also been found that important regulatory sequences in rbcS promoter regions are not conserved between monocotyledonous and dicotyledonous species. Monocotyledonous rbcS

Abbreviations: GUS, β-glucuronidase; gusA, β-glucuronidase gene; Hm', hygromycin resistance; hph, hygromycin phosphotransferase gene; MUG, 4-methylumbelliferyl-β-D-glucuronide; nos, nopaline synthase gene; rbcS, gene for small subunit of Rubisco; X-glu, 5-bromo-4-chloro-3-indolyl-β-glucuronic acid.
genes share a monocotyledonous consensus sequence that is not found in dicotyledonous species (Schaffner and Scheen, 1991).

In this study, to begin to understand the regulation of rice rbcS gene expression, the promoter region of a rice rbcS gene was fused to the gusA coding region and the expression of the fusion gene was examined in transgenic rice plants. Furthermore, to gain insights into the different mechanisms of gene regulation in dicots and monocots, the expression of the gusA gene directed by the tomato rbcS3C promoter was compared with that of the rice rbcS-gusA fusion gene.

**MATERIALS AND METHODS**

**Sequence Characterization of the Rice rbcS Clone**

A 2.8-kb PstI restriction fragment from pRR1 containing the 5' flanking region of the rice (Oryza sativa) rbcS gene (Xie et al., 1987) was cloned into pBlueScriptII-KS (Stratagene) plasmids in both orientations to produce the vectors pRR5'53P and pRR5'35P for sequence determination.

**Genomic DNA Preparation and Analysis**

Rice genomic DNA was isolated from young greenhouse-grown plants (cv IR26) as described by Dellaporta et al. (1983) with the addition of a phenol-chloroform extraction prior to the final ethanol precipitation.

For DNA gel blot analysis, 10-µg samples of rice genomic DNA were restriction-enzyme digested, fractionated by electrophoresis using 0.9% agarose gels, and transferred to GeneScreen Plus membranes (New England Nuclear). A 0.6-kb PstI-SmaI restriction fragment spanning the rice rbcS coding region was utilized in the production of a 35P-labeled probe (Maniatis et al., 1982). Hybridization was carried out overnight at 42°C in 5 × SSC, 50% formamide, with a final wash at 65°C in 0.1% SDS and 0.1 × SSC, following the procedure of Broglio et al. (1983).

**Analysis of gusA RNA**

RNA was isolated by a published method (Chomczynski and Sacchi, 1987) from young leaves of dark-adapted mature R5 transgenic plants. For the dark adaptation, plants were kept in the dark for 4 d. Forty micrograms of total RNA were separated by electrophoresis on a 1.0% agarose gel and transferred to a Hybond-N membrane (Amersham). Hybridization with the gusA gene was carried out according to the manufacturer's protocol (Amersham).

**Rice rbcS-gusA Chimeric Gene Construction**

A 1.8-kb BamHI-SstI restriction fragment containing the Escherichia coli gusA coding region was isolated from the plasmid pAct1-F (McElroy et al., 1990) and cloned into the vector pNOS72, a pSP72 (Promega) derivative containing the transcription termination region of Agrobacterium tumefaciens nos, to create the gusA-nos-containing construct pGN72. Replacement of the 0.4-kb PstI-SmaI restriction fragment of pGN72 with the equivalent 0.4-kb PstI-SmaI restriction fragment of pRAJ275 (Clontech), which contains a eukaryotic translation initiation consensus sequence, resulted in the creation of the gusA-nos construct pGN73. Cloning of the 2.8-kb PstI restriction fragment from pRR1, containing the 5' transcribed and non-transcribed region of the rice rbcS gene, into the PstI site of pNG73 resulted in the creation of the rbcS-gusA-nos-containing pRGN73.

**Construction of the Tomato rbcS-gusA Chimeric Gene**

A 1.7-kb fragment containing the entire promoter region of the tomato rbcS3C (Sugita et al., 1987) was polymerase chain reaction-amplified with the primers 5'–CAGGAAA-CAGCTATGAC-3', corresponding to a part of pUC plasmid, and 5'–CCTCTAGAATAATTGGTTAAGA-3', corresponding to the transcription start site of the rbcS3C gene. The latter primer contained an XbaI site at position +14 relative to the transcription initiation site of the tomato rbcS3C. A HindIII-XbaI fragment containing 1.7 kb of 5' transcribed and non-transcribed region isolated from the amplified fragment was replaced with the HindIII-XbaI fragment of pG221 (Ohta et al., 1990), resulting in pTRIGN, consisting of the 1.7-kb tomato rbcS3C promoter, the gusA coding region carrying the first intron of castor bean catalase 1 gene, and the nos polyadenylation region. Translation of the gusA mRNA of pTRIGN starts at the ATG derived from the castor bean catalase 1 gene.

**Production of Transgenic Rice Plants**

Protoplasts were isolated from embryogenic suspension cultures of rice (cv Nipponbare) according to Kyozuka et al. (1987). Electroporation, selection of Hm' calli, and plant regeneration were carried out as described previously (Shimamoto et al., 1989). The rbcS-gusA chimeric genes were
introduced into rice protoplasts by cotransformation with a hygromycin-resistance gene, hph. Hm' calli were selected, introduced into rice protoplasts by cotransformation with a hygromycin-resistance gene, hph. Hm' calli were selected, and plants regenerated from them were screened by staining the shoots with X-gluc.

**Analysis of GUS Enzyme Activity**

GUS activity was analyzed by both histochemical analysis with X-gluc and fluorometric analysis with MUG as described previously (Kyozuka et al., 1991). Incubation of tissue samples with X-gluc was carried out for 18 h at 37°C except for leaf sections of transgenic plants carrying the rice rbcS-gusA gene, which were incubated for 3 h at 37°C. Statistical analysis of the GUS specific activity results was done using the StatView SE+ program (Abacus Concepts Inc., Berkeley, CA).

Dark-grown seedlings (6- to 7-d-old) were used to examine distribution of GUS activity in the leaf tissue induced by light. The upper part of a seedling leaf was cut into sections and incubated with X-gluc. Then the rest of the seedling was grown further under light conditions (16 h light, 8 h dark). After 24, 48, or 96 h of light treatment, sections were prepared from the seedling leaves and stained with X-gluc.

**RESULTS**

**Sequence Characterization of the Rice rbcS Gene**

Partial restriction maps and the complete nucleotide and derived amino acid sequence of the rice rbcS gene are shown in Figure 1, A and B, respectively. Sequence analysis revealed that the rbcS sequence of pRRI showed 98.8% homology with the rice rbcS cDNA clone OSRUBPC1 (Matsuoka et al., 1988). The rice rbcS genomic clone is predicted to code for a processed transcript of at least 830 nucleotides. The potential coding region of the rbcS processed transcript, if translated in vivo, would code for 175 amino acids and a precursor rbcS protein of 19.6 kD. Removal of a putative N\textsubscript{\text{H}}\text{I}-terminal transit peptide by cleavage between the Cys (residue 47) and Met (residue 48) would yield a mature rbcS protein of 128 amino acids with a 14.7-kD estimated molecular mass.

An inspection of the region 5' of the putative TATA box of the rice rbcS gene identified sequences (Fig. 1C) that have previously been found to be conserved among monocotyledonous rbcS genes but that are absent from dicotyledonous rbcS promoters (Schaffner and Scheen, 1991). Analysis of the 3' noncoding region of the rice rbcS gene revealed the presence of a GT-rich sequence downstream of a putative polyadenylation region (Fig. 1B). Such GT-rich sequences have been reported to be required for efficient gene expression in plant cells (Ingelbrecht et al., 1989). The 3' noncoding region of the rice rbcS gene was also found to contain a 245-nucleotide sequence that represents an imperfect repeat of part of the second exon of the rbcS coding region (Fig. 1A). Alignment of the repeated sequences shows that they have 75.5% sequence similarity (data not shown).

**RNA and DNA Gel Blot Analysis of the Rice rbcS Gene**

Prior to carrying out RNA gel blot analysis, gel blots of rice genomic DNA digested with EcoRI, BamHI, HindIII, and XhoI were hybridized with a 32P-labeled probe from the coding region of the rice rbcS gene (Fig. 2A). Because this rice rbcS gene is known to be a member of a gene family (Matsuoka et al., 1988), relatively high hybridization stringency (42°C, 5 x SSC, 50% formamide) was used to obtain gene-specific hybridization conditions. As can be seen from Figure 2A, the rice rbcS probe hybridized to only one band from each digestion, confirming that these DNA-DNA hybridization conditions are gene specific.

The steady-state levels of transcripts from the rice rbcS gene under investigation were examined by gel blot hybridization analysis of RNA isolated from 6-d-old etiolated rice seedlings before and after a 2-h light treatment. To ensure that no cross-hybridization occurred between rice rbcS genes, hybridization was carried out at 48°C rather than 42°C to compensate for the increased stability of DNA-RNA hybrids over DNA-DNA hybrids. The abundance of the transcripts from the rice rbcS gene increased by an estimated 30-fold upon illumination of etiolated rice seedlings (Fig. 2B).

**Expression of Both Rice and Tomato rbcS-gusA Chimeric Genes Is Mesophyll Cell Specific in Transgenic Rice Plants**

We chose the promoter of the tomato rbcS3C gene (Sugita et al., 1987) to examine whether a dicotyledonous rbcS promoter could confer regulated expression of the gusA gene in transgenic rice. Expression of the tomato rbcS3C gene, which is one of five genes in the tomato rbcS gene family, is known to be strictly light dependent and is detected only in shoots and leaves (Sugita et al., 1987; Wanner and Gruissem, 1991). To construct the tomato rbcS-gusA fusion gene, we inserted the first intron of castor bean catalase 1 gene, which is known to increase the foreign gene expression in transgenic rice (Tanaka et al., 1990), into the NH\textsubscript{2}-terminal coding sequence of the gusA region (Fig. 3).

To investigate the activity of the rice and tomato rbcS promoters in transgenic rice, the rbcS-gusA fusion genes (Fig. 3) were introduced into rice. Five transgenic plants carrying pRGN73 (rice rbcS-gusA) and three carrying pTRIGN (tomato rbcS-gusA) were obtained. The number of integrated rbcS-gusA genes in each plant was estimated by DNA gel blot hybridization (Table I). Although six of the seven plants examined were found to contain one rbcS-gusA gene, one plant transformed with pTRIGN was estimated to contain two to three copies of the tomato rbcS-gusA fusion gene.

We analyzed the tissue and cell-type expression specificity of the chimeric gusA genes by histochemical GUS analysis using X-gluc as a substrate. In transgenic rice plants, the rice rbcS promoter directed high-level expression of the gusA gene in leaf blade (Fig. 4A) and sheath (Fig. 4B) mesophyll cells. However, in these plants, GUS activity was not observed in leaf epidermal cells, vascular cells, roots (Fig. 4C), flower organs (Fig. 4D), or seeds (Fig. 4E). The results clearly indicate that expression of the rice rbcS-gusA gene is restricted to the mesophyll cells of transgenic rice plants. A similar pattern of GUS expression was observed in transgenic rice plants containing the tomato rbcS3C-gusA gene. GUS activity was present in mesophyll cells of both leaf blades (Fig. 4F) and leaf sheaths (Fig. 4G), but not in roots (Fig. 4H) or flowers (data not shown). These results
Figure 1. A, Restriction map of the pRR1 insert. Coding region exons of the rbcS gene are depicted as open boxes. The position of the second exon duplication is indicated by a closed box. The region that was subcloned for sequence determination is indicated by a double-headed arrow below the pRR1 restriction map. The region that was subcloned for use as a probe in DNA and RNA gel blot hybridization is indicated by a line above the pRR1 restriction map. Restriction sites are abbreviated as follows: B, BamHI; C, BglII; E, EcoRI; R, EcoRV; H, HindIII; P, PstI; S, SstI; Sa, SalI; Sm, Smal; Xb, XbaI. B, Nucleotide and deduced amino acid sequence of rice rbcS. An alignment between the nucleotide sequence of the rice rbcS cDNA clone OSRUBPC1 (Matsuoka et al., 1988) and that of the rbcS genomic clone was used to identify the number and location of coding region exons within the rbcS sequence. The end points of the rbcS cDNA sequence are indicated by closed arrows. Differences between the rbcS genomic and cDNA sequences are indicated above the genomic sequence. The nucleotide sequence is numbered with the presumptive translation initiation site as +1. The predicted site of rbcS transit peptide cleavage is indicated by an open arrow. Lowercase letters represent intron sequence. The exon-intron junctions within the rbcS transcribed sequence are indicated by slashed lines. The putative TATA box.
show that both rice and tomato \textit{rbcS} promoters confer mesophyll-specific gene expression in transgenic rice plants.

To compare the rice and tomato \textit{rbcS} promoter activities, GUS enzyme levels in leaves (blade and sheath) of mature transgenic plants were examined by fluorometric analysis, using MUG as a substrate (Table 1). Both the rice \textit{rbcS-gusA} and the tomato \textit{rbcS-gusA} genes showed no statistically significant difference between the activity in leaf blades and sheaths ($P = 0.05$). However, we did calculate a statistically significant difference ($P = 0.05$) in transgenic rice leaves (blade plus sheath) between the rice and tomato \textit{rbcS-gusA} fusion genes, with mean activities of 63,351 and 4,277 pmol methylumbelliferone mg$^{-1}$ min$^{-1}$, respectively.

### Expression of Both Rice and Tomato \textit{rbcS-gusA} Chimeric Genes Is Light Inducible in Mesophyll Cells of Transgenic Rice Plants

The light induction of \textit{rbcS-gusA} fusion gene expression was examined at the cellular level by histochemical GUS analysis of \textit{R$_i$} seedlings grown in the dark for 7 d and then illuminated for 24, 48, or 96 h (Fig. 5). GUS activity was observed in the mesophyll cells from young leaves of the dark-grown seedlings carrying the rice \textit{rbcS-gusA} before illumination (Fig. 5A), whereas the tomato \textit{rbcS-gusA} gene did not show any expression in such dark-grown shoots (Fig. 5D). After 24 h of light treatment, induction of rice \textit{rbcS-gusA} expression was evident in the mesophyll cells of transgenic rice plants (Fig. 5, B and C). However, no similar induction was found in \textit{R$_i$} seedlings containing the tomato \textit{rbcS-gusA} gene (data not shown). Induction of tomato \textit{rbcS-gusA} gene expression in the mesophyll cells of transgenic rice plants was first visible after 48 h of illumination (Fig. 5E), and expression continued for a further 48 h (Fig. 5F). These observations establish that light induction of the \textit{gusA} gene directed by both rice and tomato \textit{rbcS} promoters is mesophyll cell specific in transgenic rice plants.

To determine whether the observed light induction of GUS activity directed by the \textit{rbcS-gusA} genes is due to an increase in their respective steady-state mRNA levels, total RNA was isolated from leaves of dark-adapted primary transgenic rice plants before and after light treatment, and RNA gel blots were hybridized with the \textit{gusA} probe (Fig. 6). In this analysis, we used a rice \textit{rbcS-gusA} plant with an intermediate level of GUS specific activity (line 1) and a tomato \textit{rbcS-gusA} plant (line N6-4) with the highest GUS specific activity (see Table 1). A low level of \textit{gusA} transcripts was detected in the dark-adapted plant containing the rice \textit{rbcS-gusA} gene (Fig. 6, lane 5). The level of \textit{gusA} transcripts driven by the rice \textit{rbcS} promoter increased upon illumination for 6 h (Fig. 6, lane 6) and slightly decreased after 24 h of illumination (Fig. 6, lane 7). We do not know the cause of this decrease in the \textit{gusA} transcripts between 6 and 24 h of illumination. In contrast, in a rice plant containing the tomato \textit{rbcS-gusA} gene, the \textit{gusA} transcripts were completely absent in dark-adapted leaves (Fig. 6, lane 2) and became detectable only after 24 h of illumination (Fig. 6C).
Table 1. GUS specific activity of rice and tomato rbcS-gusA fusion genes in transgenic rice

<table>
<thead>
<tr>
<th>Fusion Gene</th>
<th>Plant</th>
<th>Copy No.</th>
<th>GUS Specific Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Leaf Blade</td>
</tr>
<tr>
<td>Rice rbcS-gusA</td>
<td>1</td>
<td>1</td>
<td>32,939</td>
</tr>
<tr>
<td></td>
<td>2-2</td>
<td>1</td>
<td>34,239</td>
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<tr>
<td></td>
<td>2-3</td>
<td>1</td>
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<tr>
<td></td>
<td>2-9</td>
<td>1</td>
<td>9,736</td>
</tr>
<tr>
<td>Tomato rbcS-gusA</td>
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</tr>
<tr>
<td></td>
<td>N1</td>
<td>NT</td>
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<td></td>
<td>N6-7</td>
<td>1</td>
<td>834</td>
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</table>

<table>
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<th>Paired t Test, Blade vs. Sheath</th>
<th>Modified Unpaired t Test, Rice vs. Tomato rbcS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Value</td>
<td>Mean</td>
<td>d.f.</td>
</tr>
<tr>
<td>Rice rbcS-gusA</td>
<td>4</td>
<td>0.91</td>
</tr>
</tbody>
</table>

<sup>a</sup> Estimated copy number of rbcS-gusA fusion genes from DNA gel blot hybridization analysis (data not shown).  
<sup>b</sup> Testing the hypothesis that the mean GUS specific activities in leaf blade and leaf sheath are equal for the rice or tomato rbcS-gusA fusion genes.  
<sup>c</sup> Degrees of freedom (n - 1).  
<sup>d</sup> Calculated value of t, assuming equal variance.  
<sup>e</sup> Tabulated value of t for P = 0.05.  
<sup>f</sup> Testing the hypothesis that the mean GUS specific activities in leaves (blade and sheath) expressing either rice or tomato rbcS-gusA fusion genes are equal.  
<sup>g</sup> Mean of leaf blade and leaf sheath GUS specific activity in transgenic plants expressing either rice or tomato rbcS-gusA fusion genes.  
<sup>h</sup> Calculated degrees of freedom for a modified unpaired t test.  
<sup>i</sup> Calculated value of t, assuming unequal variance.  
<sup>j</sup> Tabulated value of t for P = 0.05.

illuminated (Fig. 6, lane 4). The presence of approximately equivalent amounts of intact RNA in each lane was confirmed by detecting comparable levels at various time points of hph transcripts that are driven by a constitutively expressed cauliflower mosaic virus 35S promoter (data not shown). These results indicate that, in transgenic rice plants, the expression of both the rice and tomato rbcS-gusA genes is regulated by light at the level of mRNA abundance, although the rate and length of time required for induction appear to be distinct between the two rbcS genes.

**DISCUSSION**

In this work, we describe further characterization of a previously isolated rice rbcS gene (Xie et al., 1987; Xie and Wu, 1988). The 2.8-kb promoter region of the rice rbcS gene was fused to the gusA reporter gene and transferred into rice. For comparison, we also examined the promoter activity of a dicotyledonous rbcS gene, the tomato rbcS3C, in transgenic rice plants. Our analysis indicated that both rice and tomato rbcS promoters confer light-regulated and mesophyll cellspecific expression of the gusA gene in transgenic rice plants. However, our results suggest that the activity of the tomato rbcS promoter is at least 1 order of magnitude lower than that of the rice rbcS promoter.

A comparison between the rice rbcS coding region sequence presented in Figure 1B and that of the previously published sequence (Xie and Wu, 1988) revealed a number of single-nucleotide differences that result in shifts between their respective reading frames. The comparison of their predicted translation products with that of the wheat (Broglie et al., 1983) and maize (Lebrum et al., 1987) rbcS sequences clearly indicate that all the differences between the two rice rbcS genomic sequences are due to errors in the previously published sequence (data not shown).

Analysis of the 3' noncoding region of the rice rbcS gene allowed us to identify a region bearing partial homology to part of the second exon of the rice rbcS gene. We do not believe that the sequence from the 3' end of the rbcS genomic clone codes for any partial rbcS protein product, since its potential reading frame is interrupted by numerous nucleotide additions and deletions. We propose that the original duplication event that gave rise to the 3'-end rbcS repeat may have resulted from an illegitimate recombination event between two rice rbcS genes. However, until more rice rbcS genes are sequenced, we cannot tell if the source of the original duplication was the rice rbcS gene described here. The full extent of the duplication could not be investigated because the pRR1 genomic clone insert terminates at the 3' end of the rbcS repeat.

By RNA gel blot analysis, we were able to show that the rice rbcS gene codes for a transcript whose abundance increases over 30-fold upon the illumination of etiolated rice seedlings. This is in good correspondence with the observation that GUS activity is present at a detectable level in darkgrown seedlings of transgenic rice plants containing the rice rbcS-gusA fusion gene and increases rapidly upon light treatment. These results indicate that the light-responsive activation of rice rbcS gene expression occurs largely at the level of transcription.

Regulated gusA expression from the tomato rbcS3C promoter in transgenic rice plants suggests that the molecular mechanisms essential for the qualitative expression of photosynthetic genes are conserved between rice and tomato...
Figure 5. Cell specificity in the light-dependent induction of rice (A–C) and tomato (D and F) rbcS-gusA genes. A and D, Transverse section of a shoot grown in dark for 5 d. B and C, Transverse section of a dark-grown shoot after 24 h of light treatment. E, Transverse section of a dark-grown shoot after 48 h of light treatment. F, Transverse section of a dark-grown shoot after 96 h of light treatment.

irrespective of the low level of sequence conservation in their rbcS promoter regions. Sequence comparison revealed that two elements are conserved between the rice and tomato rbcS promoter regions; one is the I-box (GATAG, positions −115 to −111, in rice rbcS, and GATAAG, positions −168 to −163, in tomato rbcS3C), and the other is, unexpectedly, a part of a previously proposed monocotyledonous rbcS consensus sequence (GCGGCCAAT, positions −99 to −91, in rice rbcS, and GTGGCCAT, positions −120 to −113, in tomato rbcS3C). Both the I-box and the monocotyledonous consensus sequence have been reported to function as light-responsive elements in the promoter of maize rbcSZm1 (Schaffner and Scheen, 1991), tomato rbcS3C (Manzara et al., 1991), tobacco cab-E (Schindler and Cashmore, 1990), and Arabidopsis rbcSIA (Donald and Cashmore, 1990) genes. These observations, along with the results described here, imply that these two sequence elements may be involved in the regulation of rice rbcS gene expression.

Despite the apparent functional conservation of rbcS promoters from monocots and dicots, our results suggest that the dicotyledonous (tomato) rbcS promoter used in our analysis is less competent than the monocotyledonous (rice) rbcS
promoter in transgenic rice plants. Some points need to be considered, however, before drawing any firm conclusion. First, there is a difference in vector constructs; an intron is present in the tomato rbcS-gusA gene that is absent from the rice rbcS-gusA gene. We previously found that the castor bean catalase 1 intron used in this study increases expression of the gusA gene in transgenic rice more than 10-fold in combination with the cauliflower mosaic virus 35S promoter (Tanaka et al., 1990). Moreover, enhancement of foreign gene expression by introns has been observed from other plant promoters (Callis et al., 1987; Kyozuka et al., 1990; McElroy et al., 1990) in transformed monocot cells. These results suggest that it is likely that insertion of an intron in the tomato rbcS-gusA fusion gene increases its expression in transgenic rice. Second, the copy number of integrated genes could be an important factor determining expression levels of introduced rbcS-gusA genes. Our DNA gel blot analysis established that six of eight transgenic plants analyzed contained a single copy of the introduced gene (Table I), indicating that the different expression level detected in transgenic plants carrying the tomato rbcS-gusA gene and those carrying the rice rbcS-gusA gene is not caused by the difference in copy number of the introduced gusA genes. Finally, because rbcS is a gene family, the tomato rbcS5C gene may not represent the member of the gene family corresponding to its developmental and light regulation to the rice gene used in our study. However, previous studies with the tomato rbcS gene family indicated that, among the five tomato rbcS genes, the rbcS3C gene used in our study is expressed at the highest level in green leaves (Sugita et al., 1987; Wanner and Gruissem, 1991). Taken together, our results suggest that there are differences in the quantitative regulation of gene expression between monocotyledonous and dicotyledonous rbcS promoters in transgenic monocots. This notion was supported by previous studies analyzing expression of monocot genes in transgenic tobacco plants (Lamppa et al., 1985; Ellis et al., 1986; Keith and Chua, 1986). Our results support this view in the reciprocal sense that the activity of a dicotyledonous promoter is reduced in a transgenic monocotyledonous plant. Recently, virus-resistant rice plants expressing the coat protein gene of rice stripe virus have been produced (Hayakawa et al., 1992), suggesting that genetic engineering will be increasingly applied to monocotyledonous crops in the near future. The rice rbcS promoter described here should be useful in monocot transformation.

Figure 6. Light induction of gusA mRNA abundance in leaves of transgenic rice. Gel blots containing 40 μg of total RNA from nontransformed rice (lane 1), transgenic rice carrying tomato rbcS-gusA gene (lanes 2-4), and transgenic rice carrying the rice rbcS-gusA gene (lanes 5-7) were hybridized with a gusA probe. RNAs were isolated from leaves of dark-adapted plants after 0 h (lanes 2 and 5), 6 h (lanes 3 and 6), and 24 h (lanes 1, 4, and 7) of light treatment.

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