GMR Genetics and Molecular Research
Online Journal
ISSN:1676-5680

*rbc*S SRS4 promoter from *Glycine max* and its expression activity in transgenic tobacco

X.Y. Cui[1], Z.Y. Chen[2], L. Wu[1], X.Q. Liu[1], Y.Y. Dong[3], F.W. Wang[3] and H.Y. Li[1,3]

[1]College of Life Sciences, Jilin Agricultural University, Changchun, China
[2]College of Agronomy, Jilin Agricultural University, Changchun, China
[3]Ministry of Education Engineering Research Center of Bioreactor and Pharmaceutical Development, Jilin Agricultural University, Changchun, China

Corresponding author: H.Y. Li
E-mail: hyli99@163.com

Genet. Mol. Res. 14 (3): 7395-7405 (2015)
Received August 20, 2014
Accepted February 6, 2015
Published July 3, 2015
DOI http://dx.doi.org/10.4238/2015.July.3.15

ABSTRACT. The regulatory region of the ribulose-1,5-bisphosphate carboxylase small subunit gene *SRS4* from soybean (*Glycine max*) was cloned using TAIL-PCR and general PCR, and named the *rbc*S promoter. The promoter was fused with the *GUS* gene and introduced into *Nicotiana tabacum* via *Agrobacterium*-mediated leaf disk transformation. In 4-week-old transgenic tobacco plants, the highest GUS expression levels were observed in the leaves, GUS activity was 7.13- and 7.40-fold higher in leaves than in stems and roots, respectively. Moreover, *GUS* activity was stimulated by light. In conclusion, spatial and light regulation of the soybean *rbc*S promoter was observed in *N. tabacum*, thus illustrating a leaf-specific and light-induced promoter.

Key words: Soybean; *rbc*S promoter; Transgenic tobacco; GUS activity

 ©FUNPEC-RP www.funpecrp.com.br

INTRODUCTION

Soybean (*Glycine max*) is an important worldwide crop, and an important source of vegetable oil and protein (Cho et al., 1995). There is a large interest in improving soybean quality and production through genetic engineering (Wang and Xu, 2008). One strategy for regulating and improving the expression of foreign beneficial genes in transgenic plants is through the manipulation of gene promoters. This enables high expression of a gene of interest, allowing precise temporal and spatial regulation in specific plant parts (Khoudi et al., 1997). The promoter of the Rubisco small subunit is a useful tool to target expression of foreign genes specifically to plant leaves, and is regulated by light (Bakhsh et al., 2011).

Ribulose-1,5-bisphosphate carboxylase/oxygenase (*rbc*S; EC.4.1.1.39) is the most prevalent enzyme in higher plants, accounting for 30-50% of total soluble protein in the chloroplast. It catalyzes the primary step in photosynthetic carbon dioxide fixation, and is severely limited by oxygenase activity. The ratio of carboxylation and oxygenation reactions governs plant productivity (Dhingra et al., 2004). The structural composition of Rubisco consists of eight large and eight small subunits, encoded by nuclear gene families and chloroplast genomes, respectively (Dean et al., 1989). Members of the *rbc*S gene family encode the small subunits of Rubisco, their expression is regulated by light, and regulation occurs primarily at the transcriptional level (Kyozuka et al., 1993). *rbc*S transcripts are distributed in a tissue-specific manner, especially to photosynthetic organs; this is because transit peptides target proteins to chloroplasts and mitochondria (Spreitzer, 2003; Zhong et al., 2003).

The *rbc*S promoter has been cloned, sequenced, and studied in various plants, including pea (*Pisum sativum*; Nagy et al., 1985), cotton (*Gossypium* spp; Song et al., 2000), *Chrysanthemum* (Outchkourov et al., 2003), coffee (*Coffea arabica*; Marraccini et al., 2003), and potato (*Solanum tuberosum* L.; Qu et al., 2011). In soybean, the *rbc*S gene family comprises 6-10 members (Dean et al., 1989). To date, only two genes, *SRS1* and *SRS4*, have been investigated (Berry-Lowe et al., 1982; Berry-Lowe and Meagher, 1985; Grandbastien et al., 1986). Reporter genes are reported to be regulated by the soybean *rbc*S SRS1 promoter in a tissue-specific and light-induced manner in heterologous plants (Quandt et al., 1992; Gittins et al., 2000; Marraccini et al., 2003).

In this study, the 5'-flanking sequence of the soybean *rbc*S promoter was initially cloned using Thermal Asymmetric Interlaced PCR (TAIL-PCR), and the full-length sequence obtained using general PCR; the sequence, structure, and functions were then analyzed.

MATERIAL AND METHODS

Isolation of genomic DNA and cloning of the *rbc*S promoter

Glycine max cv. Jinong 13 plants were grown in an incubator stimulating normal growth conditions, as described by Bustamante et al. (2009). Genomic DNA was extracted from leaf tissue collected from 4-week-old plants. DNA concentration and purity was analyzed using gel electrophoresis and UV-spectrophotometry.

The 5'-flanking sequence of the soybean *rbc*S promoter was amplified by TAIL-PCR, as described by Liu and Whittier (1995), with slight modifications. Three nested gene-specific primers (SP1, SP2, and SP3) were designed according to the published RuBPcase (ribulose-1,5-bisphosphate carboxylase) small subunit gene sequence (GenBank accession No.

M16889), a further three arbitrary degenerate primers (AD1, AD2, and AD3) were provided by the Academy of Agricultural Sciences in Jilin Province, China. Resultant PCR products were analyzed using agarose gel electrophoresis. A target fragment (TAIL1) of approximately 900 bp was obtained and sequenced. The specific primer SS was synthesized according to the TAIL-PCR product sequence, while the SA primer was obtained from the sequence of the *rbc*S gene. PCR products, now named *rbc*SP, were cloned using primers SS and SA, with genomic Soybean Jinong 13 DNA as template. Following electrophoreses, the *rbc*SP product was extracted from gels and cleaned using an AxyPrep PCR Clean-up kit (Corning, China). Next, the *rbc*SP fragment was ligated into a pMD18-T vector (Takara, China) to generate the recombinant plasmid pT-*rbc*SP, and sequenced. All primers used and the TAIL-PCR procedure are shown in Tables 1 and 2, respectively.

Table 1. Primers used in TAIL-PCR and general PCR of the soybean *rbc*S SRS4 promoter.

Name	Sequences
AD1[a]	5'-NGTCGASWGANAWGAA-3'
AD2[a]	5'-WGTGNAGWANCANAGA-3'
AD3[a]	5'-AGWGNAGWANCAWAGG-3'
SP1[a]	5'-AATGTGTGATGGTGGTGGAGTTT-3'
SP2[a]	5'-CACTCTTATCCATTTTGTTGGGTAG-3'
SP3[a]	5'-ATAGAAGGGGATGGTAGGTGATGGG-3'
SS[b]	5'-GTGGATGACTCAAGTGCTGG-3'
SA[b]	5'-TGCATTGCACTCTTCCACCG-3'

[a]Primers for TAIL-PCR; [b]primers for general PCR. N: A/C/G/T; S: C/G; W: A/T.

Table 2. TAIL-PCR procedure used to analyze the soybean *rbc*S SRS4 promoter.

Reaction	Regent (μL)	Thermal cycling conditions	Cycle No.
Primary	10X LA PCR Buffer: 2.5	94°C 1 min, 98°C 1 min	1
	dNTP mixture (2.5 mM): 4	94°C 30 s, 55°C 1 min, 72°C 2 min	5
	AD(1-3) (100 μM): 0.5	94°C 30 s, 25°C 3 min, 72°C 2 min	1
	SP1 (10 μM): 0.5	94°C 30 s, 55°C 1 min, 72°C 2 min	15
	Genomic DNA: 0.5	94°C 30 s, 55°C 1 min, 72°C 2 min	
	TaKaRa LA Taq (5 U/μL): 0.5	94°C 30 s, 43°C 1 min, 72°C 2 min	
	ddH_2O: 16.5	72°C 10 min	1
Secondary	10X LA PCR Buffer: 2.5	94°C 30 s, 55°C 1 min, 72°C 2 min	15
	dNTP mixture (2.5 mM): 4	94°C 30 s, 55°C 1 min, 72°C 2 min	
	AD(1-3) (100 μM): 0.5	94°C 30 s, 43°C 1 min, 72°C 2 min	
	SP2 (10 μM): 0.5	72°C 10 min	1
	1/50 primary PCR product: 1		
	TaKaRa LA Taq (5 U/μL): 0.5		
	ddH_2O: 16		
Tertiary	10X LA PCR Buffer: 2.5	94°C 30 s, 55°C 1 min, 72°C 2 min	15
	dNTP mixture (2.5 mM): 4	94°C 30 s, 55°C 1 min, 72°C 2 min	
	AD(1-3) (100 μM): 0.5	94°C 30 s, 43°C 1 min, 72°C 2 min	
	SP3 (10 μM): 0.5	72°C 10 min	1
	1/100 primary PCR product: 1		
	TaKaRa LA Taq (5 U/μL): 0.5		
	ddH_2O: 16		

DNA sequencing and bioinformatic analysis of the *rbc*S promoter

The pT-*rbc*SP plasmid was sequenced by Takara Bio (China) using M13 primers. Sequences were retrieved from the GenBank database using the BLASTn program. Sequence analysis was performed using the DNAman 5.2.2 software. Putative *cis*-acting elements in

the promoter were identified using PlantCARE (http://bioinformatics.psb.ugent.be/webtools/plantcare/html/) and PLACE (http://www.dna.affrc.go.jp/PLACE/) softwares.

Expression vector construction and tobacco transformation

The G*rbc*SP DNA fragment containing the soybean *rbc*S promoter was amplified from the recombination plasmid pT-*rbc*SP using a 5'-oligonucleotide (5'-AACTGCAGGTGGATG ACTCAAGTGCTGG-3') with a *Pst*I site and a 3'-oligonucleotide (5'-CATGCCATGGTGCAT TGCACTCTTCCACCG-3') with an *Nco*I site as primers. The PCR product and the plant expression vector pCAMBIA-1301 were double-digested with *Pst*I and *Nco*I, respectively, and then ligated to construct the pCAMBIA-G*rbc*SP vector (Figure 1); this was sequenced to confirm presence of the *rbc*S promoter.

Vectors pCAMBIA-G*rbc*SP and pCAMBIA-1301 were introduced into *Agrobacterium tumefaciens* strain LBA4404 using the tri-parental hybrid method. Positive recombined *Agrobacterium* LBA4404 colonies were selected and transformed into tobacco (*Nicotiana tabacum* cv. NC89) using the *Agrobacterium*-mediated leaf-disc method (Horsch et al., 1985). Transformed plants (named as p*rbc*SP and p35SP plants, for pCAMBIA-G*rbc*SP and pCAMBIA-1301 vectors, respectively) were screened on MS medium supplemented with 50 mg/L kanamycin and 500 mg/L cephaloglycin. Plantlets were verified by PCR, using the primers 5'-TTCCTGATTAACCACAAACC-3' and 5'-CGGTTCGTTGGCAATACTCC-3'. All positive plants were transferred to soil and grown in a greenhouse at 25°C under a 16/8-h light/dark regime. Tissues were collected, immediately frozen in liquid nitrogen, and stored at -80°C until required.

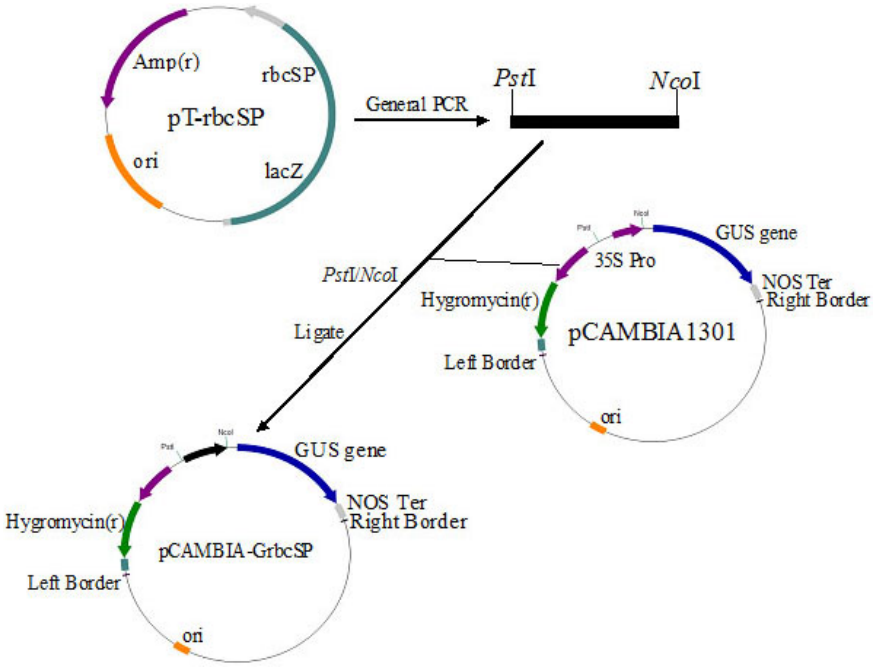

Figure 1. Construction of the expression vector pCAMBIA-G*rbc*SP.

Histochemical staining and fluorometric quantification of GUS activity

To characterize the function of the *rbc*S promoter, histochemical staining and fluorometric quantification were performed as described previously (Jefferson, 1987). Small sections of fresh tissues (leaves and roots) were immersed in GUS-staining solution [50 mM phosphate buffer, pH 7.0, containing 1 mM X-Gluc, 0.01 M disodium ethylenediaminetetraacetic acid (EDTA-Na_2), 0.1 M potassium ferricyanide, 0.1 M potassium ferrocyanide, and 1.0% Triton X-100 (v/v)] and incubated at 37°C for durations from a few hours to overnight. Stained samples were cleaned with 70% ethanol and observed.

For the fluorometric assay, 0.1 g fresh tissue was first ground to a fine powder in liquid nitrogen, and then ground into a homogenate in 1 mL GUS extraction buffer (100 mM phosphate buffer, pH 8.0, with 1% PVP and 10 mM β-mercaptoethanol). The homogenate was placed on ice for 1 h, and centrifuged at 13,000 rpm for 10 min at 4°C. The supernatant was transferred to GUS assay buffer and incubated at 37°C for 20 min; the reaction was stopped using 0.2 M Na_2CO_3 and fluorescence measured using a 970 CRT fluorescence spectrophotometer (Shanghai Analytical Instrument Factory). Meanwhile, the total protein content of the extracts was determined using the Bradford method (1976). Each sample was measured three times and the mean GUS activity for each independent line determined.

Light-dependent development of GUS expression in transgenic tobacco plants

To examine GUS activity in leaf tissue induced by light, transgenic plants were first grown in complete darkness at 25°C for 1 week. Plants were further cultured under a 16/8-h light/dark regime. After 24, 48, 72, and 96 h of light treatment, GUS activity of young and old leaf blades was analyzed.

RESULTS

Cloning of the soybean *rbc*S promoter

The 5'-flanking sequence of the soybean *rbc*S promoter (TAIL1) was obtained using TAIL-PCR amplification with primers AD2, SP1, SP2, and SP3 (Figure 2, lanes 1-9). The fragment was ligated into pMD18-T. The fragment *rbc*SP was cloned through PCR using primers SS and SA with genomic Soybean Jinong 13 DNA as template (Figure 2, lane 10). *rbc*SP was then cloned into the pMD18-T vector to generate the pT-*rbc*SP recombinant plasmid (Figure 2, lanes 11 and 12).

Sequence analysis of the soybean *rbc*S promoter

The cis-acting elements of the soybean *rbc*S promoter (*rbc*SP) were analyzed using the PLACE and PlantCARE software. Several motif types were present in the promoter (Figure 3): 1) A/T rich and CAAT boxes upstream of the transcription start site; these serve as basal promoter elements for transcription. 2) GT binding site Box II (GGTTAA); this light-specific regulatory module was first identified in the pea *RBCS*-3A promoter, GT-elements evolved from Box II have been found in the promoter regions of many other genes and encode diverse functions (Green et al., 1987; Zhou, 1999). 3) Light-induced sequence G box (A/CCACGTG)

or I box (GATAAG); mutations in the context of *Arabidopsis rbc*S-1A promoter substantially reduce the expression of *Adh* and *GUS* reporter genes (Donald and Cashmore, 1990). 4) GATA motifs (WGATAR); these are implicated to act in the light-dependent and nitrate-dependent control of transcription in plants (Reyes et al., 2004). 5) Motifs with a known relationship to light including H box (CCTACC), REalpha element (AACCAA), and CCAAT-box (Song et al., 2000; Outchkourov et al., 2003; Qu et al., 2011). The cis-acting elements identified in *rbc*SP strongly suggested that heterologous genes driven by *rbc*SP could be induced in a light-specific manner.

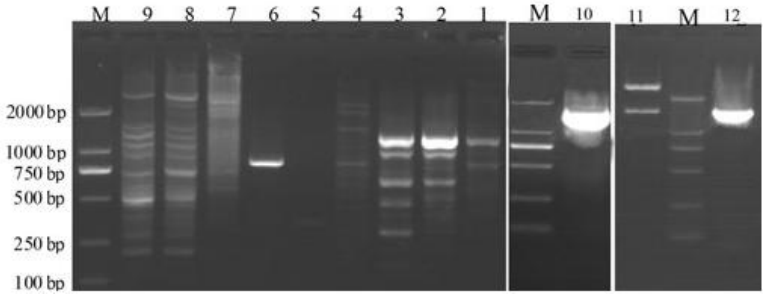

Figure 2. Analysis of TAIL-PCR products from the soybean *rbc*S SRS4 promoter following 1% agarose electrophoresis and restriction digestions. *Lane M*: DL2000; *lanes 1*, *2*, and *3*: products of AD1 in the primary, secondary, and tertiary reactions; *lanes 4*, *5*, and *6*: products of AD2 in the primary, secondary, and tertiary reactions; *lanes 7*, *8*, and *9*: products of AD3 in the primary, secondary, and tertiary reactions; *lane 10*: PCR product of the *rbc*S promoter from soybean; *lanes 11* and *12*: restriction digestion of pT-*rbc*S and PCR product of pT-*rbc*S.

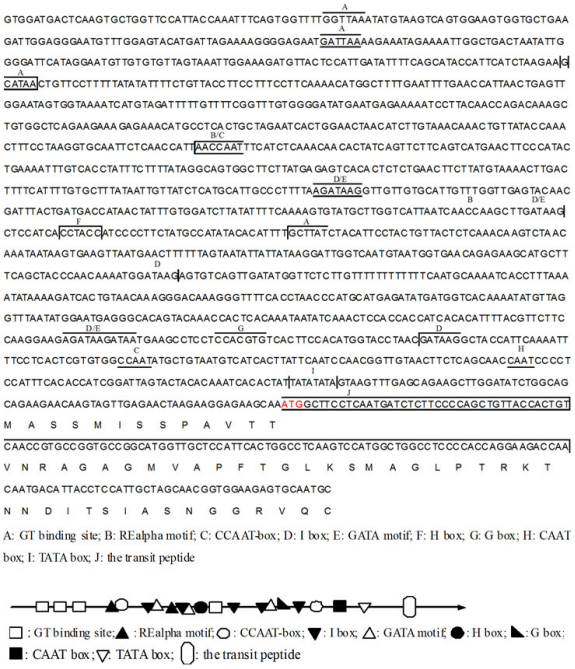

Figure 3. Sequence analysis of the soybean *rbc*S promoter and schematic diagram summarizing the distribution of light-specific promoter related motifs within the nucleotide sequences of *rbc*SP.

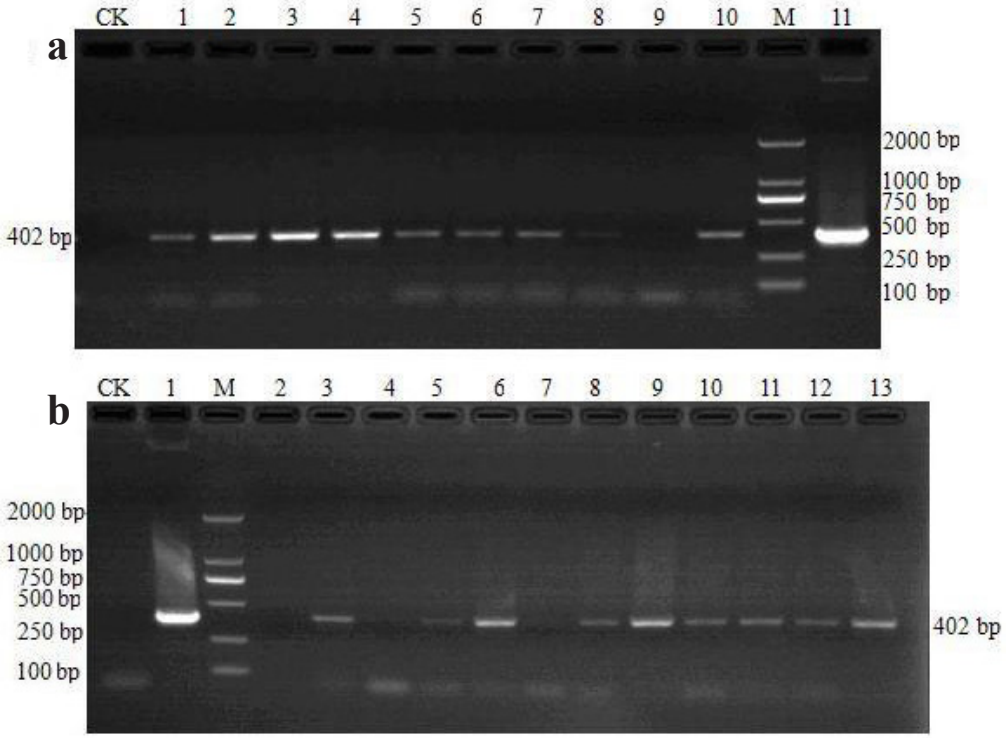

Figure 4. Identification of p*rbc*SP plants (**a**) and p35SP plants (**b**) using PCR. **a.** *Lane M*: DL2000; *lanes 1-10*: the p*rbc*SP plants; *lane 11*: pCAMBIA-G*rbc*SP vector; CK: the wild-type plant. **b.** *Lane M*: DL2000; *lanes 2-13*: the p35SP plants; *lane 1*: pCAMBIA1301 vector; CK: the wild-type plant.

Expression analysis of the *GUS* gene under control of the soybean *rbc*S promoter in transgenic tobacco

PCR analysis was performed to confirm that the promoter was integrated into the genomic DNA of transgenic tobacco (Figure 4).

Histochemistry of leaves and roots from transgenic and wild-type plants was conducted to reveal the spatial expression pattern driven by the soybean *rbcS* promoter (Figure 5a-c). In p*rbc*SP plants, the GUS staining of leaf tissue was deep, but was only slight in the root. In p35SP plants, GUS staining was observed in both leaf and root tissue. No GUS activity was detected in the wild-type plants. Therefore, the *GUS* reporter gene in transgenic tobacco was expressed in a leaf-specific manner under the control of the soybean *rbc*S promoter.

Fluorescence analysis of GUS activity in transgenic tobacco

Histochemical analysis does not provide a precise measure of GUS expression levels in different tissues. Therefore, GUS activity was assessed using a quantitative GUS assay in the transgenic plants. In p*rbc*SP plants, GUS activity was mainly detected in the leaves, and was minimal in shoots and roots (Figure 5d); while in p35SP plants, GUS activity was consistent across all tissues. This result further confirms the leaf specificity of the *rbc*S promoter.

Light induction of *rbc*SP-gusA in transgenic tobacco plants

*rbc*SP plants were germinated in the dark for 7 days and then exposed to light for 24, 48, 72, or 96 h. Old and young leaf blades from each plant were assayed for GUS activity (Figure 5e). GUS activity did not decrease in old blades transferred to the dark, but increased slightly, and then remained invariant following exposure to light. In younger blades, GUS activity decreased after being kept in darkness for 7 days, but continued to increase after light induction.

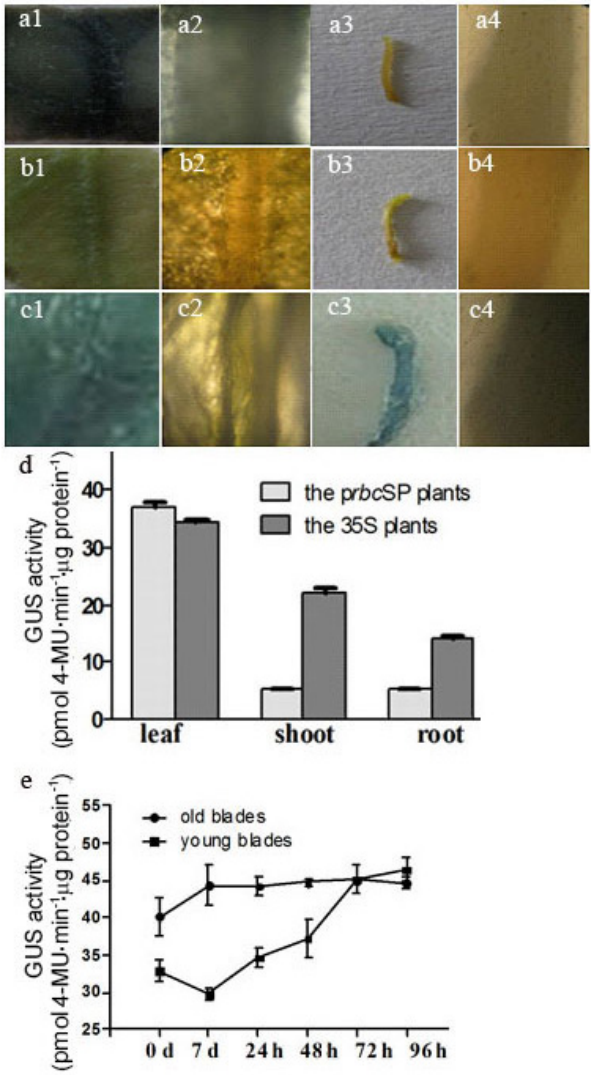

Figure 5. Analysis of GUS in transgenic and wild-type plants. **a. b.** and **c.** Expression of the *gus* gene in different tissues of the p*rbc*S, wild-type, and p35S plants, respectively (1 and 2: leaf; 3 and 4: root; 1 and 3 were macroscopic; 2 and 4 were observed under the microscope); **d.** GUS activity in different tissues of transgenic plants; **e.** Light induction of *gus* expression in the p*rbc*SP plants.

DISCUSSION

TAIL-PCR enables convenient, quick, efficient, and specific cloning, and can therefore be used to obtain long DNA sequences proximal to a known gene. This technique has proven to be suitable for isolating powerful promoters (Terauchi and Kahl, 2000; Li et al., 2007). In this study, TAIL-PCR was successfully applied to clone the soybean *rbc*S promoter. TAIL-PCR products can be achieved in a 0.2-2-kb range (Liu and Whittier, 1995). The product generated in this study was only approximately 900 bp, despite the PCR procedure taking about 10 h. A possible reason for the shortness of the TAIL1 sequence was the quantity of the arbitrary degenerate primers (only three primers), therefore further AD primers are required for future research.

TAIL-PCR provides an effective and convenient way to clone an unknown sequence adjacent to a known sequence. In this study, the soybean *rbc*S promoter was cloned using both TAIL-PCR and general PCR. The length of the promoter was 1775 bp, and included several *cis*-acting elements implicated in transcriptional regulation by light in other higher plants.

To identify the expression pattern of the soybean *rbcS* promoter, *rbc*S promoter-GUS and *CaMV* 35S promoter-GUS fusion genes were transformed into tobacco. GUS staining and fluorescence analysis revealed that *GUS* reporter gene expression driven by the soybean *rbcS* promoter was only found in the leaves of transgenic tobacco plants (Figure 5a-d). The small quantity of GUS staining observed in the stems could be due to there being few chloroplasts. However, the unexpected GUS detection in the roots may be a result of leakage. In all transgenic tobacco plants, *GUS* driven by the *CaMV* 35S promoter expressed constitutively. These results are comparable with previous studies (Baranski and Puddephat 2004; Baba-Kasai et al., 2005).

Meanwhile, *Gus* expression levels driven by the soybean *rbcS* promoter in leaves were the same as those driven by the *CaMV* 35S promoter (Figure 5d). Therefore, the soybean *rbc*S promoter is a perfect substitute for the 35S promoter in the leaf. The soybean *rbc*S promoter can induce the expression of foreign genes possessing leaf-specific patterns in transgenic plants, reducing the burden of plants and effects on the agronomic traits of crops, and improving the concentration of the foreign gene in the particular part, thus, increasing the expression level of the foreign gene.

When transgenic tobacco plants containing the soybean *rbc*S promoter were transferred between light and dark conditions, *Gus* expression levels controlled by the promoter were induced in younger leaves (raised in the presence of light, decreased in the absence of light). However, in older leaves, expression remained approximately the same across all time points (Figure 5e). Further investigation of this phenomenon is recommended for future study.

In conclusion, the isolation and sequence analysis of the soybean *rbc*S promoter provides an important insight into the elements controlling foreign gene expression. This study indicates that the *rbc*S promoter confers a high level of GUS expression in leaves, and is under light-regulation. Thus, the *rbc*S promoter can be applied in transgenic research for the modification of crop quality.

ACKNOWLEDGMENTS

Research supported by grants from the Special Program for Research of Transgenic Plants (#2014ZX08010-002), the National Science Foundation of China (#31271746, #31201144, #31101091, and #31171459), and the National Science Foundation of Jilin Province (#201215178).

REFERENCES

Baba-Kasai A, Hara N and Takano M (2014). Tissue-specific and light-dependent regulation of phytochrome gene expression in rice. *Plant Cell Environ*. 37: 2654-2666.

Bakhsh A, Qayyum RA, Shamim Z and Husnain T (2011). A mini review: RuBisCo small subunit as a strong, green tissue-specific promoter. *Arch. Biol. Sci.* 63: 299-307.

Baranski R and Puddephat IJ (2004). Tissue specific expression of β-glucuronidase gene driven by heterologous promoters in transgenic cauliflower plants. *Acta Physiol. Plant.* 26: 307-315.

Berry-Lowe SL and Meagher RB (1985). Transcriptional regulation of a gene encoding the small subunit of ribulose-1,5-bisphosphate carboxylase in soybean tissue is linked to the phytochrome response. *Mol. Cell. Biol.* 5: 1910-1917.

Berry-Lowe SL, Mc Knight TD, Shah DM and Meagher RB (1982). The nucleotide sequence, expression, and evolution of one member of a multigene family encoding the small subunit of ribulose-1,5-bisphosphate carboxylase in soybean. *J. Mol. Appl. Genet.* 1: 483-498.

Bradford MM (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem*. 72: 248-254.

Bustamante CA, Civello PM and Martínez GA (2009). Cloning of the promoter region of β-xylosidase (*FaXyl1*) gene and effect of plant growth regulators on the expression of *FaXyl1* in strawberry fruit. *Plant Sci.* 177: 49-56.

Cho MJ, Widholm JM and Vodkin LO (1995). Cassettes for seed-specific expression tested in transformed embryogenic cultures of soybean. *Plant Mol. Biol. Rep.* 13: 255-269.

Dean C, Pichersky E and Dunsmuir P (1989). Structure, evolution, and regulation of *Rbc*S genes in higher plants. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 40: 415-439.

Dhingra A, Portis AR and Daniell H (2004). Enhanced translation of a chloroplast-expressed *Rbc*S gene restores small subunit levels and photosynthesis in nuclear *Rbc*S antisense plants. *Proc. Nat. Acad. Sci. U.S.A.* 101: 6315-6320.

Donald R and Cashmore AR (1990). Mutation of either G box or I box sequences profoundly affects expression from the *Arabidopsis* rbcS-1A promoter. *EMBO J.* 9: 1717-1726.

Gittins JR, Pellny TK, Hiles ER, Rosa C, et al. (2000). Transgene expression driven by heterologous ribulose-1,5-bisphosphate carboxylase/oxygenase small-subunit gene promoters in the vegetative tissues of apple (*Malus pumila* Mill.). *Planta* 210: 232-240.

Grandbastien MA, Berry-Lowe SL, Shirley BW and Meagher RB (1986). Two soybean ribulose-1,5-bisphosphate carboxylase small subunit genes share extensive homology even in distant flanking sequences. *Plant Mol. Biol.* 7: 451-465.

Green PJ, Kay SA and Chua NH (1987). Sequence-specific interactions of a pea nuclear factor with light-responsive elements upstream of the *rbcS-3A* gene. *EMBO J.* 6: 2543-2549.

Horsch RB, Fry JE, Hoffmann NL, Eichholtz D, et al. (1985). A simple and general method for transferring genes into plants. *Science* 227: 1229-1231.

Jefferson RA (1987). Assaying chimeric genes in plants: the GUS gene fusion system. *Plant Mol. Biol. Rep.* 5: 387-405.

Khoudi H, Vézina LP, Mercier J, Castonguay Y, et al. (1997). An alfalfa rubisco small subunit homologue shares cis-acting elements with the regulatory sequences of the *RbcS-3A* gene from pea. *Gene* 197: 343-351.

Kyozuka J, McElroy D, Hayakawa T, Xie Y, et al. (1993). Light-regulated and cell-specific expression of tomato *rbcS-gusA* and rice *rbcS-gusA* fusion genes in transgenic rice. *Plant Physiol.* 102: 991-1000.

Li Q, Yin H, Li D, Zhu H, et al. (2007). Isolation and characterization of CMO gene promoter from halophyte *Suaeda liaotungensis* K. *J. Genet. Genomics* 34: 355-361.

Liu YG and Whittier RF (1995). Thermal asymmetric interlaced PCR: automatable amplification and sequencing of insert end fragments from P1 and YAC clones for chromosome walking. *Genomics* 25: 674-681.

Marraccini P, Courjault C, Caillet V, Lausanne F, et al. (2003). Rubisco small subunit of *Coffea arabica*: cDNA sequence, gene cloning and promoter analysis in transgenic tobacco plants. *Plant Physiol. Biochem.* 41: 17-25.

Nagy F, Morelli G, Fraley RT, Rogers SG, et al. (1985). Photoregulated expression of a pea *rbc*S gene in leaves of transgenic plants. *EMBO J.* 4: 3063-3068.

Outchkourov NS, Peters J, de Jong J, Rademakers W, et al. (2003). The promoter-terminator of chrysanthemum rbcS1 directs very high expression levels in plants. *Planta* 216: 1003-1012.

Qu D, Song Y, Li WM, Pei XW, et al. (2011). Isolation and characterization of the organ-specific and light-inducible promoter of the gene encoding rubisco activase in potato (*Solanum tuberosum*). *Genet. Mol. Res.* 10: 621-631.

Quandt HJ, Broer I and Puhler A (1992). Tissue-specific activity and light-dependent regulation of a soybean *rbc*S promoter in transgenic tobacco plants monitored with the firefly luciferase gene. *Plant Sci.* 82: 59-70.

Reyes JC, Muro-Pastor MI and Florencio FJ (2004). The GATA family of transcription factors in *Arabidopsis* and rice. *Plant Physiol.* 134: 1718-1732.

Song P, Heinen JL, Burns TH and Allen RD (2000). Expression of two tissue-specific promoters in transgenic cotton

plants. *J. Cotton Sci.* 4: 217-223.

Spreitzer RJ (2003). Role of the small subunit in ribulose-1,5-bisphosphate carboxylase/oxygenase. *Arch. Biochem. Biophys.* 414: 141-149.

Terauchi R and Kahl G (2000). Rapid isolation of promoter sequences by TAIL-PCR: the 5′-flanking regions of *Pal* and *Pgi* genes from yams (*Dioscorea*). *Mol. Gen. Genet.* 263: 554-560.

Wang G and Xu Y (2008). Hypocotyl-based *Agrobacterium*-mediated transformation of soybean (*Glycine max*) and application for RNA interference. *Plant Cell Rep.* 27: 1177-1184.

Zhong H, Teymouri F, Chapman B, Maqbool SB, et al. (2003). The pea (*Pisum sativum* L.) *rbc*S transit peptide directs the *Alcaligenes eutrophus* polyhydroxybutyrate enzymes into the maize (*Zea mays* L.) chloroplasts. *Plant Sci.* 165: 455-462.

Zhou DX (1999). Regulatory mechanism of plant gene transcription by GT-elements and GT-factors. *Trends Plant Sci.* 4: 210-214.

 ©FUNPEC-RP www.funpecrp.com.br