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# ISOLATION, CLONING AND CHARACTERIZATION OF PROMOTER OF RUBISCO SMALL SUBUNIT 2B (*rbcS2B*) GENE OF *Arabidopsis thaliana*

Research Article

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**ABSTRACT**

**Cloning and characterization of tissue-specific or developmentally-regulated promoters is of paramount importance for the development of transgenics and for basic studies requiring ectopic gene expression such as activation tagging. In this study, we characterized the expression patterns conferred by promoters of *rbcS2B* genes of *Arabidopsis thaliana.* Transgenic approach was followed to characterize the promoter of *rbcS2B* gene. *In silico* analysis of the 5′ upstream intergenic region of *rbcS2B* gene showed mainly light-regulated elements. A 1.8 kb fragment upstream to the coding sequence of *rbcS2B* gene was PCR amplified and cloned into the pORE-R2 vector upstream to the β-*glucuronidase* (*GUS*) reporter gene. Histochemical assay of stably transformed *Arabidopsis* plants for *GUS* expression indicated that A*trbcS2B* promoter has wider expression pattern including in roots. qRT-PCR showed the presence of higher level of *GUS* transcripts than the endogenous *AtrbcS2B* in transgenic *Arabidopsis* harbouring pAtrbcS2B::GUS, indicating cloned upstream fragment (1.8 kb) of *rbcS2B* gene has stronger promoter activity than the native promoter. This strong promoter of *AtrbcS2B* will find application in activation tagging as well as in development of GM crops.**

## Introduction

RUBP carboxylase/oxygenase is a bi-functional enzyme which catalyses $CO_2$ fixation in Calvin cycle under high $CO_2$ concentration and acts as oxygenase under low $CO_2$ concentration (Izumi *et al.,* 2012). In higher plants, this is a multimeric enzyme consisting of eight large and eight small subunits. The large subunit gene is located in plastid as a single copy whereas the small subunit genes is located in nucleus as a gene family with four to ten copies (Dean *et al.,* 1989). In *Arabidopsis*, *rbcS* gene family consists of four members and based on linkage and sequence similarities the gene family has been subdivided into two subfamilies *i.e.* A and B (Krebbers *et al.,* 1988). The 'B' subfamily comprises three members (*rbcS1B, 2B, 3B*) and arranged in a head-to-tail fashion on chromosome 5 while the 'A' subfamily has only one member (*rbcS1A*) located on chromosome 1. Although *rbcS 'A'* members is present on a different chromosome than the *rbcS* 'B' genes and has the most divergent protein sequence among the four but its expression pattern is comparable to other *rbcS 'B'* genes (Krebbers *et al.,* 1988). *rbcS1B* which has similar coding sequence among 'B' subfamily, but shows deviation in response to red light from the other three genes. *rbcS2B* gene expression across the entire plant is chiefly or absolutely associated with subepidermal, photosynthetic cells whereas each of other genes has specialized expression domain such as root tip, leaf abaxial surface etc. (Sawchuk *et al.,* 2008).

Rubisco is the most abundant protein in the plants (Izumi *et al.,* 2012) and the abundance of nuclear encoded small subunits determines the accumulation of large subunits of Rubisco present in the chloroplast (Rodermel *et al.,* 1999). Rubisco is involved in the

photosynthetic process which is largely occurs in the leaf tissue of plants. Therefore, these nuclear genes are expected to have strong leaf tissue specific promoters and to some extent promoters could be green tissue specific because chloroplast is present in every green part of the plants. In plants, *rbcS* gene family members are one of the best characterized light inducible genes. Promoters of these genes are known to contain the complex arrangement of negative and positive regulatory elements that confers light and tissue specific expression in transgenic plants. Detailed characterization of pea *rbc3A* promoter reported the presence of redundant light regulated elements whose removal greatly reduced the light induced expression (Bakhsh *et al.,* 2012). Further, pea *rbc3A* promoter showed the presence of two highly conserved sequences (Box I and Box II) around -150 bp relative to the transcription start site which are required for *rbcs3A* expression and sequences upstream and downstream of -170 bp relative to transcription start site are required for light regulated and organ specific expression (Bakhsh *et al.,* 2012). Analysis of promoters of *rbcS* gene family of tomato reported the presence of I-box and G-box *cis*-acting elements which are important for tissue specific expression. In these genes upstream region relative to transcription start site ranging from 0.6 to 3.0 kb in which I-box and G-box are located within -600 to -100 bp were sufficient to confer the organ-specific expression pattern (Bakhsh *et al.*, 2012). A study on cotton *rbcS* promoter reported that 560 bp upstream fragment containing putative I-box and G-box was sufficient to drive GUS expression comparable to CaMV35S promoter (Song *et al.,* 2000). Study on rice *rbcS* promoter reported that it could control not only the tissue but also the cell-specific expression of foreign genes in transgenic rice (Bakhsh *et al.,* 2012). Promoter from *Arabidopsis thaliana* ats1A gene has been used in development of pod borer resistance chickpea carrying *cry2Aa* gene. This promoter led to organ specific expression of *cry2Aa* gene including leaves, floral parts, pods and developing cotyledons at 45 days-after-flowering as well as in mature dry seeds (Acharjee *et al.,* 2010). Although, a large number of *rbcS* promoters from different plant species have been characterized, promoters of *Arabidopsis rbcS* and 'B' sub family have not been characterized so far. Therefore, in the present study we attempted to determine the expression pattern of *rbcS2B* gene by analysis of its promoter linked to the *GUS* reporter gene. We generated transgenic *Arabidopsis* lines with pAtrbcS2B::GUS construct and analyzed the *GUS* expression histochemically in different tissues. Also, the comparative expression of *GUS* and the native *rbcS2B* gene in different organs was assessed through qRT-PCR.

## Materials and methods

### *In-silico* analysis of upstream sequences

*Arabidopsis thaliana rbcS3B* and *rbcS2B* genes are present in tandem and are arranged in head-to-tail fashion on chromosome 5. The two genes are separated by 2038 bp intergenic region. Previously, study on *rbcS2B* promoter identified expression of *GFP* in the leaf mesophyll and guard cells of the leaf tissue (Kim *et al.,* 2003). Hence, in the present study we used 1817 bp *rbcS2B* upstream sequence for promoter analysis. For *in silico* study of *rbcS2B* promoter, 1817 bp region encompassing 1760 bp upstream intergenic region and complete 5′ UTR (57 bp) of the *rbcS2B* gene was downloaded from the TAIR database and analysed for the presence of various promoter elements using PLACE (Higo *et al.,* 1999), PlantCARE (Lescot *et al.,* 2002) and AGRIS AtcisDB.

### Cloning of *rbcS2B* upstream sequences

Total genomic DNA was isolated from *A. thaliana* leaves by CTAB method (Doyle *et al.,* 1990) and PCR primers were designed to amplify upstream sequences of *rbcS2B* (1817 bp) (Table 1.). The promoter fragment was PCR amplified using high fidelity DNA polymerase. The reaction mixture comprised 10 µL 5X buffer, 1µL 10 mM dNTP, 0.5 µL of each primer (10 µM), 0.5 µL Phusion polymerase, 1µL DNA (100 ng/µl), 36.5 µL $H_2O$. PCR conditions were: initial denaturation 98°C for 1 min followed by 36 cycles of denaturation at 98°C for 30 seconds, annealing at 52°C for 30 seconds and extension at 72°C for 90 seconds and a final extension at 72°C for 10 min. PCR product was cloned into pGEMT-easy vector (Promega, Wisconsin, USA). Clone identity was confirmed by restriction digestion and sequencing.

**Construction of *rbcS2B* promoter-reporter gene cassette**

Cloned *rbcS2B* fragments were released from the pGEMT-easy vector using *NotI* restriction enzyme, and subcloned into the plasmid pORE-R2 (Coutu *et al.*, 2007) predigested with *NotI* restriction enzyme. PCR and restriction digestion (using *NotI* restriction enzyme) were employed to identify recombinant clones. Since, *rbcs2B* promoter fragments was cloned non-directionally into pORE-R2 vector, the restriction enzymes whose recognition sequence were present internal to cloned fragments (e.g. *HindIII*) were chosen for checking the orientation of the insert. The resulting plasmid was named pAtrbcS2B::GUS and mobilized into *Agrobacterium tumefaciencs* strain GV3101.

***In planta* transformation of *Arabidopsis***

Plants of *A. thaliana* ecotype Columbia were grown under controlled conditions 23 ± 2°C day temperature / 18°C night temperature under long day condition (16 h) and used for *in planta* transformation as per the floral dip protocol (Clough and Bent, 1998). $T_1$ seedlings were selected on semi-solid MS medium (Murashige and Skoog, 1962) containing kanamycin (50 mg/L). Kanamycin resistant putative transformants were transferred to pots for further growth and molecular analysis.

**Histochemical GUS Assay**

Tissues from various stages starting from 4-day-old seedlings to fully mature stage were analyzed for histochemical GUS staining. GUS expression pattern was visualized by soaking tissues in GUS assay buffer [50 mM phosphate buffer (pH 7.0), 10 mM EDTA, 0.1% Triton X-100, 1mM potassium ferrocyanide, 1 mM potassium ferricyanide, 1 mM X-Gluc and 20% Methanol] at 37°C for overnight (Jefferson *et al.*, 1987) and then washed in 70% ethanol to remove chlorophyll and other pigments. Samples were observed and photographed under LEICA MZ16FA stereomicroscope.

**qRT-PCR analysis**

qRT-PCR was done to relatively quantify the expression level of native *rbcS2B* and *uidA* gene. Total RNA was isolated using Spectrum™ Plant Total RNA Kit (Sigma-Aldrich, Saint Louis, USA) from transgenic *Arabidopsis thaliana* plants carrying pAtrbcS2B::GUS construct. First-strand cDNA synthesis was performed using SuperScript® III First-Strand Synthesis System for RT-PCR (Invitrogen, Life Technologies, USA). qRT-PCR was performed with KAPA SYBR® FAST Universal 2X qPCR Master Mix (KAPA Biosystems, Woburn, USA) in a Stratagene MX3000P qPCR instrument. PCR reaction was performed as per following programme: 95°C for 10 sec, 45 cycles of 95°C for 30 sec, 60°C for 45 sec, and 72°C for 45 sec followed by dissociation curve programme: 95°C for 1 min, 55°C for 30 sec, 95°C for 30 sec to check for multiple amplicons. Primers for qRT-PCR are listed in Table 1. Transcript level in different tissue was normalized using *Actin8* (At1g49240) as the internal control. qRT-PCR data were as per $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001). Considering endogenous *rbcS2B* gene as calibrator in each tissue, $\log_2$ (fold change) of GUS with respect to *rbcS2B* was calculated. qRT-PCR experiment was conducted with six technical replicates and two biological replicates.

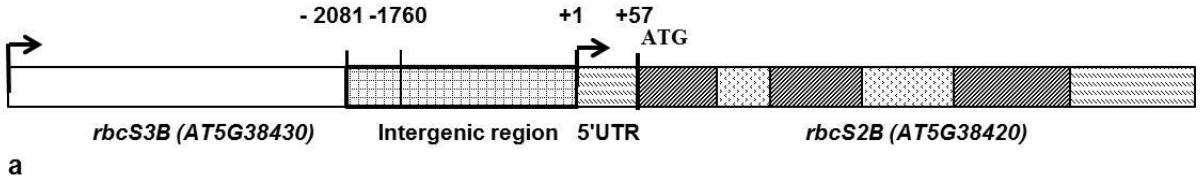
**Table 1. Sequences of primers used in the study**

| Sl. No. | Name of primer | Sequence (5′-3′) |
|---|---|---|
| 1. | *rbcS2B* F | CTTTACCCTAACTACTCCTTTCTCAGTTGGC3 |
| 2. | *rbcS2B* R | ACTTCTTCTTGTTGTTTCTCTTCTTCTTTT3' |
| 3. | *GUS* F | GGTGGGAAAGCGCGTTACAAGA |
| 4. | *GUS* R | TTGCCGTAATGAGTGACCGCATC |
| 5. | *rbcS2B* F2 | GACTTAACAGTTGAGGAACTATTGTTTG |
| 6. | *rbcS2B* R | AGCAAGAATTATGAGGATAATTTAAAG |
| 7. | *Act8* F | TGGCACCACCCGAGAGGAAGT |
| 8. | *Act8* R | AATGTGATCCCGTCATGGAAACG |

## Results and discussion

### *In silico* analysis of *AtrbcS2B* gene and its upstream intergenic region

As per The Arabidopsis Information Resource (TAIR) annotation, the *rbcS2B* gene (*AT5g38420*) has a coding sequence (CDS) of 546 bp, the 5' and 3' UTR of 57 bp and 258 bp, respectively. *rbcS2B* coding region has three exons of 171, 135 and 240 bp and two introns of 90 and 140 bp (Fig 1a.). As regulatory sequences are usually located upstream of the coding sequences therefore the fragment spanning the 5′UTR and intergenic region was tested for promoter activity.




a


b

**Fig 1. *In silico* analysis of *AtrbcS2B* gene and its upstream intergenic region. a.** *rbcS2B* gene organization, +1 indicates putative transcription start site (TSS), arrows indicate direction of *rbcS2B*. Numbers indicate positions with respect to TSS. Boxes with dotted grid, dashed downward diagonal lines, dark upward diagonal lines and divot shape represent intergenic region, UTR, exon and intron, respectively.

**b.** Physical location of various *cis*-acting elements identified through *in silico* analysis of the *rbcS2B* promoter region. TSS (+1) is indicated in red and various *cis*-acting elements of the promoter are highlighted with different colours.

*In silico* investigation of an *rbcS2B* upstream region encompassing 1760 bp intergenic region and 57 bp 5′UTR (-1760/+57) showed the presence of TATA box (-18, -609, -953) and CCAAT box (-473) which are necessary for transcription (Hernandez, 1993). Several CAATBOX1 (-92,-273,-671,-934,-1345) known for tissue specific expression (Shirsat *et al.*, 1989) are also present. Light specific *cis* regulatory elements like Conserved Modular Array 5 (CMA5) (-150~-190), the shortest native light responsive unit of photosynthetic gene promoter which are known to function as light responsive enhancer (Lo´pez-Ochoa *et al.*, 2007), two IBOX 'GATAAG' (-125, -73) which are known to confer photosynthetic tissue-specific expression (Marti´nez-Herna´ndez *et al*., 2002; Maclean *et al.*, 2002), BOX I 'TTTCAAA' (-1244), rbcS consensus sequence 'AATCCAAC' (-1151) and GATA box (-290, -977, -987,-1556) are also present in the upstream region of *rbcS2B.* In addition to these, RAV1A 'CAACA' (+11, -2, -115, -1675) elements are present which are known to confer high level gene expression in roots and rosette leaves (Kagaya *et al.*, 1999). The ROOTMOTIFTAPOX1'ATATT' found in *rolD* promoter of *Agrobacterium rhizogenes* (Elmayan *et al.*, 1995) which are known for regulating gene expression in roots are present at -392, -612, -1022, -1143,-1541 positions. GTGANTG10 elements known for regulating gene expression in pollen (Rogers et al., 2001) are located at -544, -1424, -1513 positions. SORLIP1AT 'GCCAC' elements known to be found in light induced promoters (Hudson *et al.*, 2003) are located at -682, -701, -1705bp upstream of transcription start site of *rbcS2B* gene (Fig 1b.).

**Isolation and cloning of *rbcS2B* promoter in plant transformation vector**

A 1817 bp fragment containing upstream intergenic region (1760 bp) and 5′ UTR (57 bp) was PCR amplified (Fig 2a.) through *rbcS2B* upstream region specific primers and cloned upstream of *GUS* reporter gene in a plant transformation vector pORE-R2. Integration and orientation of promoter sequence in pORE-R2 vector was confirmed by PCR and plasmid restriction digestion (Fig. 2b, c). Plasmid was named pAtrbcs2B::GUS. Schematic representation of pAtrbcs2B::GUS vector map is shown in Fig. 2d.

**Expression pattern of *rbcS2B* promoter in light grown plants**

*Arabidopsis thaliana* seeds were obtained following floral dip transformation and transgenic $T_1$ seedlings were selected on kanamycin. We examined *GUS* activity in $T_2$ generation plants. To determine the expression pattern of *rbcS2B* promoter, we analysed the promoter activity right from 2-days-old seedling to mature plant stage. In 2-days-old seedling, dark blue *GUS* staining was detected in cotyledon, hypocotyl (Fig 3a.). In 4- days-old seedling, uniform dark blue GUS staining was detected in cotyledon, hypocotyl and first juvenile leaf (Fig 3b.). However, in root *GUS* staining was detected only in maturation zone of root (Fig 3b.). In 15-day-old seedlings, GUS staining was recorded in the entire rosette. However, *GUS* expression in the root remained randomly scattered and detected only in the primary root (Fig. 3c,d). Mature plants leaves showed deep *GUS* staining evenly in the entire lamina including trichomes (Fig. 3e, f.). Histological study of leaf showed that GUS staining was restricted to the mesophyll cells (Fig 3g.). In stem tissue, deep *GUS* staining was recorded uniformly (Fig 3h.). Transverse section of the stem showed *GUS* expression in the cortex region (Fig 3i.). In the reproductive tissue, *GUS* staining was recorded in green sepals, anther filaments and pollens but not in petals, anther sac or ovule (Figs. 3 j, k, l, m, n, o). *GUS* activity was also recorded in the siliques and seeds (Fig. 3p, q). Histochemical study suggests that *rbcS2B* promoter has wider expression pattern including in roots. Study of *rbcS2B* promoter using *YFP* reporter also reported its wider expression (Sawchuk *et al.*, 2008) but unlike to our finding, they didn't reported any expression in roots. However, eFP Browser (Winter *et al.*, 2007) data support our results (Suppl. Fig 1.). Further, we wanted to know whether the cloned 1.8 kb upstream fragment of *rbcS2B* gene has promoter activity similar to that of native *rbcS2B* promoter. For this, we performed qRT-PCR to analyse the relative expression level of the endogenous *rbcS2B* and *uidA* transcripts in tissues like root, leaf,

stem, bud and siliques of transgenic *Arabidopsis* harbouring pAtrbcS2B::GUS cassettes using gene specific primers of *rbcS2B* and *uidA* genes. qRT-PCR results showed several fold higher *uidA* transcripts than *rbcS2B* transcripts in different tissues of transgenic *Arabidopsis* (Fig 4.), indicating cloned fragment has stronger promoter activity than the native *rbcS2B* promoter.This suggests presence of negative regulatory elements beyond the cloned region. It has been reported that *rbcS* gene family in *Arabidopsis* consists of four members *rbcS1A, rbcS3B, rbcS2B, rbcS1B* (Krebbers *et al.,* 1988). Among these *rbcS1A* and *rbcS3B* mRNA account for 80% of the total *rbcS* mRNA and contribution of *rbcS2B* mRNA to total *rbcS* mRNA is less than 15% (Izumi *et al.*, 2012). Although, *rbcS2B* and *rbcS3B* genes have identical coding sequences and first 324 nucleotide from the translation start site are also similar (data not shown), however they differ in the level of transcript abundance. This indicates that there are some elements in the promoter region of *rbcS2B* gene which limits the accumulation of *rbcS2B* transcript in the wild type leaves of *Arabidopsis*. Present study indicates that cloned fragment might miss the important negative regulatory elements which limit its accumulation in the wild type *Arabidopsis* plant organs.

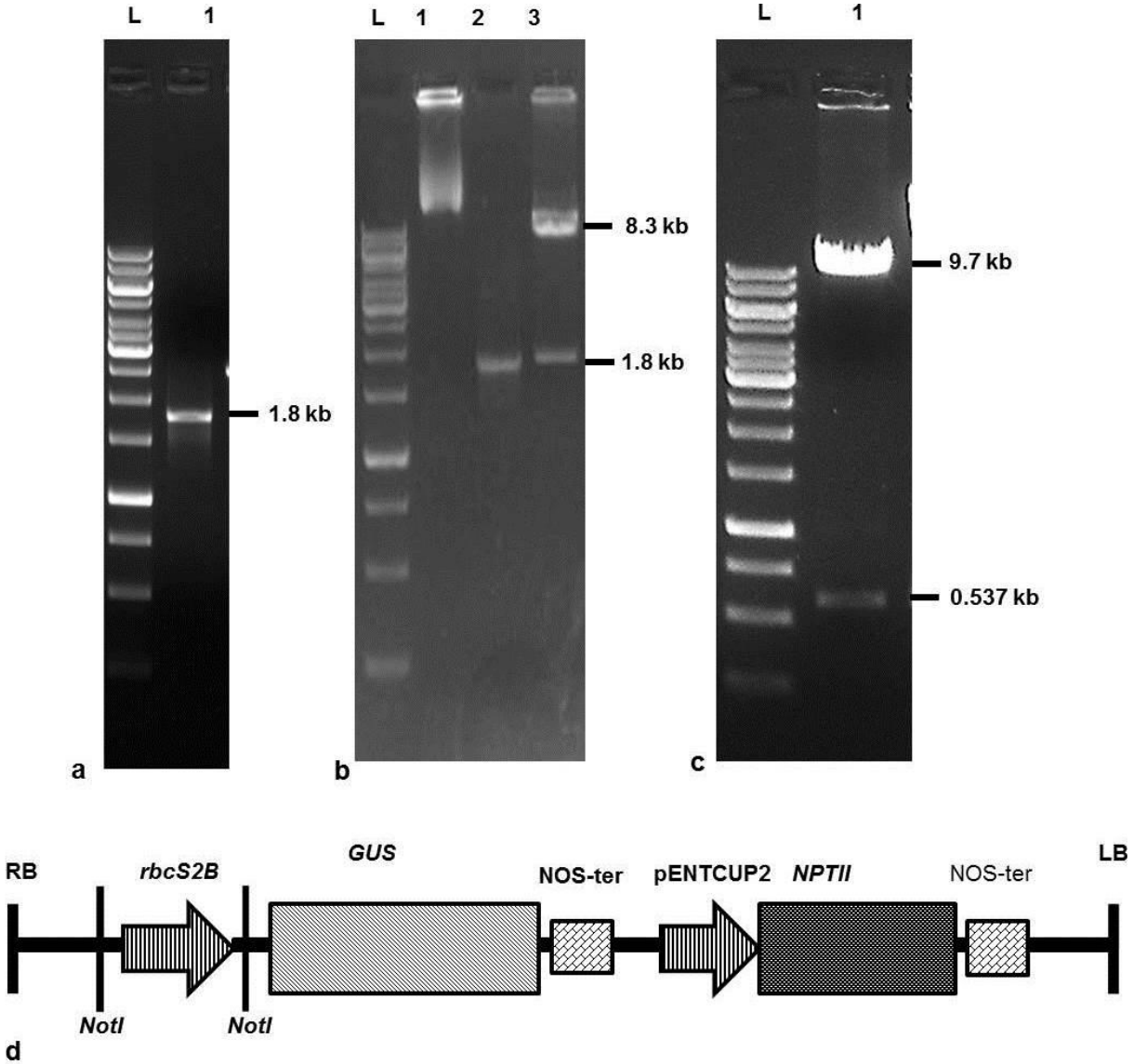


**Fig 2. Construction of plant transformation vector. a.** gel photograph showing PCR amplified product of *rbcS2B* genepromoter. Lane 1. PCR amplicon of 1.8 kb, Lane L. 1 kb ladder; **b.** Confirmation of pAtrbcS2B::GUS expression vector with PCR and restriction digestion, lane 1.Unrestricted plasmid, Lane 2.PCR amplicon of *rbcS2B* promoter from pAtrbcS2B::GUS plasmid, lane 3.pAtrbcS2B::GUS plasmid was restricted with *NotI*, lane L. 1 kb ladder; **c.** detection of orientation of pAtrbcS2B::GUS plasmid by restriction digestion, lane 1. pAtrbcS2B::GUS plasmid restricted with *HindIII*, lane L. 1 kb ladder; **d.** linear map of pAtrbcS2B::GUS transformation vector showing T-DNA region.

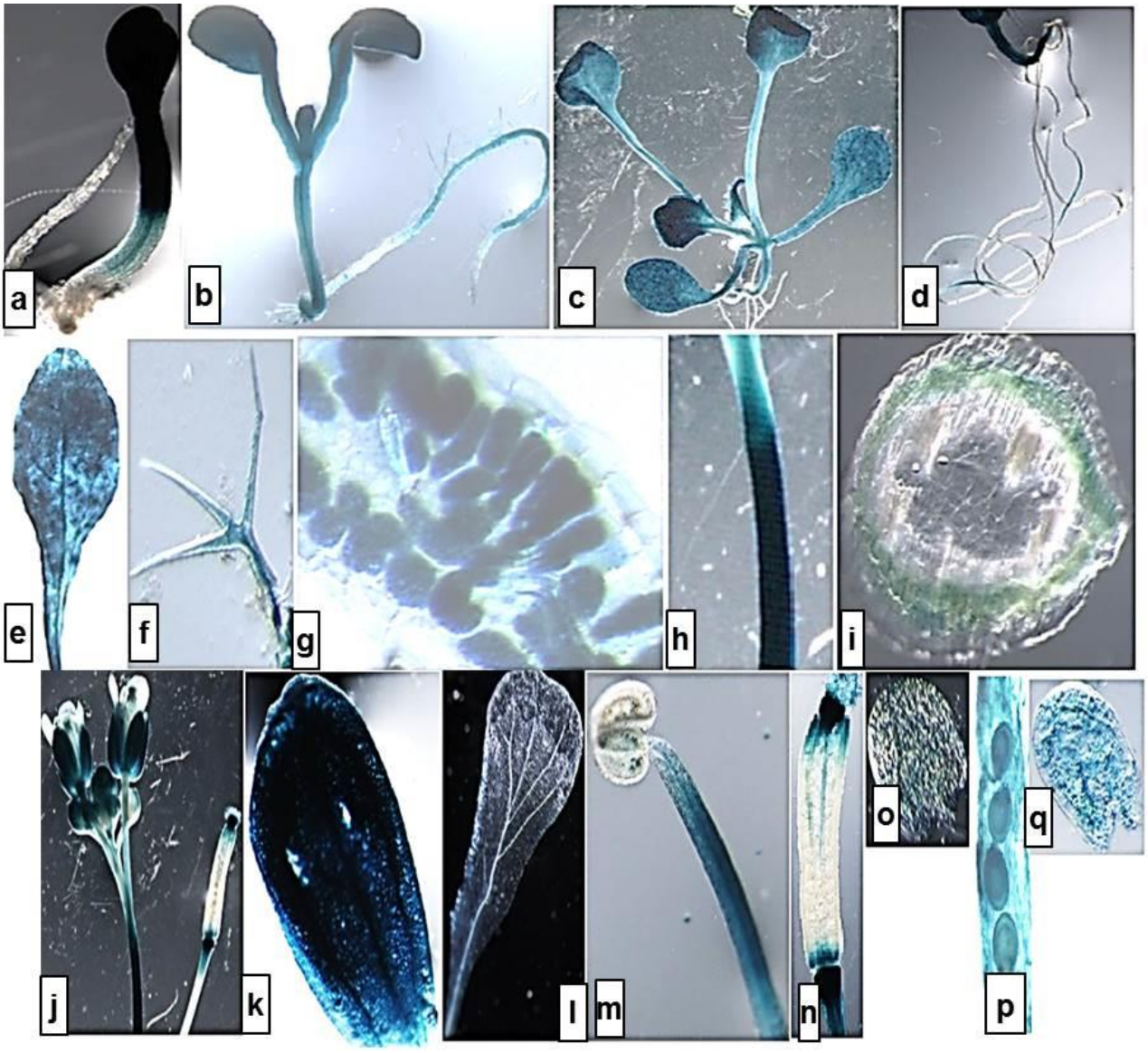



**Fig 3. Histochemical localization of *GUS* activity in pAtrbcS2B::GUS transgenic plants. a.** 2-day-old seedling; **b.** 4-day-old seedling; **c.** 15-day-old seedling; **d.** roots of 15-day-old seedling; **e.** rosetteleaf; **f.** trichome; **g.** transverse section of leaf; **h.** stem; **i**. transverse section of stem; **j.** flower buds; **k.** sepal; **l.** petal; **m.** stamen; **n.** gynoecium; **o.** ovule; **p.** opened green siliques showing developing seeds; **q.** close-up view of developing seed.

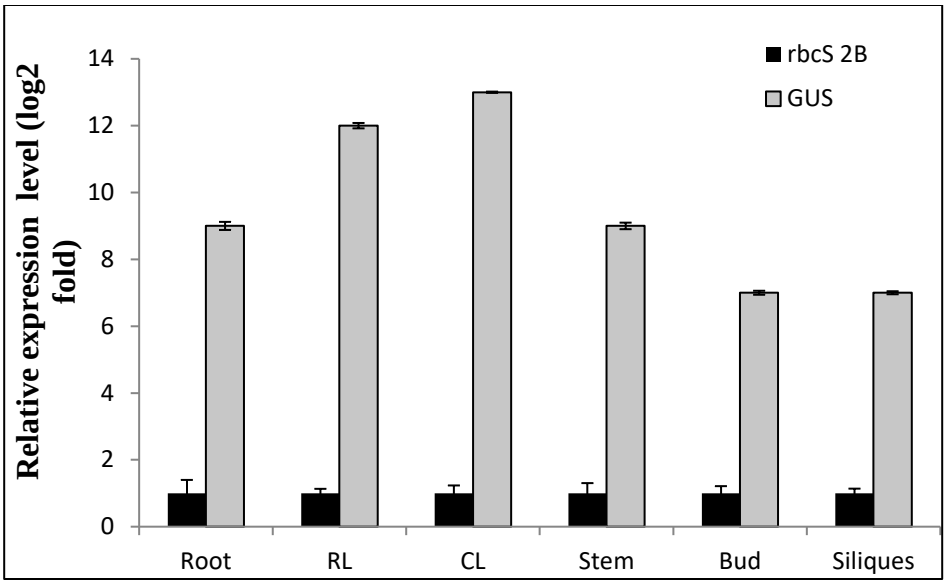
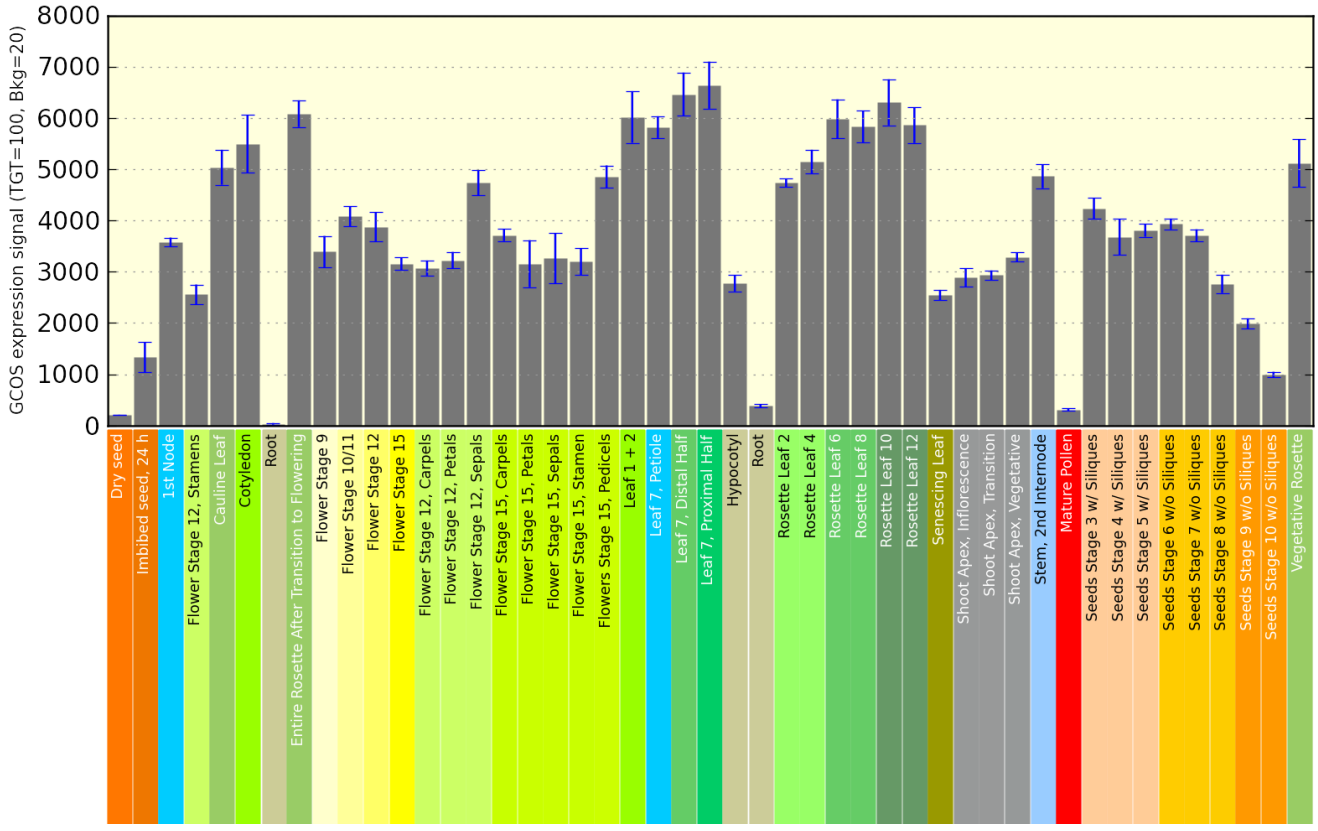



**Fig 4. Analysis of *GUS* and native *rbcS2B* gene transcripts.** Bar chart showing relative expression of *GUS* and *rbcS2B* genes in various organs of *Arabidopsis* transgenic harbouring pAtrbcS2B::GUS. Error bar represents S.E. of six replicates. *GUS* expression of *rbcS2B* is taken as calibrator in each organ.




**Supplementary Fig 1. Expression pattern of *rbcS2B* as per eFP browser.**

**Conclusion**

Our results show that regulatory sequences of *AtrbcS2B* gene lie in the intergenic region between *AtrbcS3B* and *AtrbcS2B.* Far upstream intergenic region or 3′UTR of gene might be carrying some of the important regulatory elements which lower the accumulation of *rbcS2B* transcript in various organs and inhibit its expression in root. Thus the results of the present study call for further investigation of such negative regulatory elements located in the *rbcS2B* promoter.

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**References**

Acharjee, S., B.K. Sarmah., P.A. Kumar, K. Olsen, R. Mahon, W.J. Moar, A. Moore, T.J.V. Higgins. 2010. Transgenic chickpeas (*Cicer arietinum* L.) expressing a sequence-modified cry2Aa gene. Plant Science, **178**: 333–339.

Bakhsh, A. and T. Husnain. 2012. Endeavours of Rubisco small subunit promoter as a tool of green tissue specific expression. Czech J. Genet. Plant Breed 48: 1–9.

Clough, S. J, A. F. Bent. 1998. Floral dip: A simplified method for Agrobacterium-mediated transformation of Arabidopsis thaliana. Plant J. **16:**735-43.

Coutu, C., J. Brandle, D. Brown, K. Brown. 2007. pORE: A modular binary vector series suited for both monocot and dicot plant transformation. Transgenic Res. **16**:771-81.

Dean, C., E. Pichersky, P. Dunsmuir. 1989. Structure, evolution, and regulation of rbcS genes in higher plants. Annu. Rev. Plant. Physiol. Plant. Mol. Biol., 40: 415–439.

Doyle, J.J., J.L. Doyle. 1990. Isolation of plant DNA from fresh tissue. Focus, **12**: 13–15.

Elmayan, T., M. Tepfer. 1995. Evaluation in tobacco of the organ specificity and strength of the *rolD* promoter, domain A of the *35S* promoter and the *35S* promoter. Transgenic Res. **4**: 388-396.

Hernandez, N. 1993. TBP, a universal eukaryotic transcription factor? Genes Dev., **7**: 1291–1308.

Higo, K., Y. Ugawa, M. Iwamoto, T. Korenaga. 1999. Plant *cis*-acting regulatory DNA elements (PLACE) database. *Nucleic Acids Res.,* **27**(1): 297-300.

Hudson, M.E., P.H. Quail. 2003. Identification of promoter motifs involved in the network ofphytochromeAregulated gene expression by combined analysis of genomic sequence and microarray data. Plant Physiol., **133**: 1605-1616.

Izumi, M., H. Tsunoda, Y. Suzuki, A. Makino, H. Ishida. 2012. RBCS1A and RBCS3B, two major members within the *Arabidopsis* RBCS multigene family, function to yield sufficient Rubisco content for leaf photosynthetic capacity. J. Exp. Bot., **63**: 2159–2170.

Jefferson, R.A., T. Kavanagh, M.W. Bevan. 1987. GUS fusion: ß-glucuronidase as a sensitive and versatile gene fusion marker in higher plants. EMBO J. **6**: 3901-3907.

Kagaya, Y., K. Ohmiya, T. Hattori. 1999. RAV1, a novel DNA-binding protein, binds to bipartite recognition sequence through two distinct DNA-binding domains uniquely found in higher plants. *Nucleic Acids Res.,* **15**: 470-478.

Kim, J.Y., Z. Yuan, D. Jackson. 2003. Developmental regulation and significance of KNOX protein trafficking in *Arabidopsis*. Dev., **130**: 4351-4362.

Krebbers, E., J. Seurinck, L. Herdies, A.R. Cashmore, M.P. Timko. 1988. Four genes in two diverged subfamilies encode the ribulose-1,5-bisphosphate carboxylase small subunit polypeptides of *Arabidopsis thaliana*. *Plant Mol. Biol.*, **11**: 745–759.

Lescot, M. 2002. Plant Care, a database of plant cis-acting regulatory elements and a portal to tools for in silico analysis of promoter sequences. Nucleic Acids Res. **30**(1): 325-327.

Livak, K.J., T.D. Schmittgen. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta DeltaC(T)) method. Methods, **25**: 402–408.

Lo´pez-Ochoa, L, G. Acevedo-Herna´ndez, A. Marti´nez-Herna´ndez, G.Argu¨ello-Astorga. 2007. Structural relationships between diverse *cis*-acting elements are critical for the functional properties of arbc S minimal light regulatory unit. *J. Exp. Bot*., **58**: 4397–4406.

Maclean, D., C.A. Jerome, A.P. Brown, J.C. Gray. 2008. Co-regulation of nuclear genes encoding plastid ribosomal proteins by light and plastid signals during seedling development in tobacco and Arabidopsis. *Plant Mol.Biol.*, **66**: 475–490.

Marti´nez-Herna´ndez, A., L. Lo´pez-Ochoa, G. Argu¨ello-Astorga, L. Herrera-Estrella. 2002. Functional properties and regulatory complexity of a minimal RBCS light-responsive unit activated by phytochrome, cryptochrome, and plastid signals. Plant Physiol., **128**:1223–1233.

Murashige, T.and F. Skoog. 1962. A revised medium for rapid growth and bio-assays with tobacco tissue cultures. *Physiol. Plant.*, **15**(3): 473-497.

Rodermel, S. 1999. Subunit control of Rubisco biosynthesis: a relic of an endosymbiotic past? Photosynth. Res., **59**: 105–123.

Rogers, H.J., N. Bate, J. Combe, J. Sullivan, J. Sweetman, C. Swan, D.M. Lonsdale, and D. Twell. 2001. Functional analysis of *cis*-regulatory elements within the promoter of the tobacco late pollen gene *g10*. *Plant Mol. Biol.*, **45**: 577-585.

Sawchuk, M.G., T.J. Donner, P. Head, E. Scarpella. 2008. Unique and overlapping expression patterns among members of photosynthesis-associated nuclear gene families in *Arabidopsis*. *Plant Physiol.*, **148**: 1908–1924.

Shirsat, A., N. Wilford, R. Croy, D. Boulter. 1989. Sequences responsible for the tissue specific promoter activity of a pea legumin gene in tobacco. *Mol. Gen. Genet.*, **215**: 326-331.

Song, P., J.L. Heinen, T.H. Burns, R.D. Allen. 2000. Expression of two tissue-specific promoters in transgenic cotton plants. *J. Cotton Sci.*, **4**: 217–223.

Winter, D. B.Vinegar, H. Nahal, R. Ammar, G.V. Wilson, N.J. Provart. 2007. An "Electronic Fluorescent Pictograph" browser for exploring and analyzing large-scale biological data sets, *PLoS ONE* **2**(8): e718.

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