Indian J. Genet., 74(4): 529-531 (2014)
DOI: 10.5958/0975-6906.2014.00884.0

Short Communication

Cloning and differential expression analysis of a new *rbcS* gene from *Lemna gibba*

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(Received: March 2014; Revised: July 2014; Accepted: August 2014)

Abstract

A novel Ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) small subunit gene (named *ssu4d*) was cloned from *Lemna gibba* by a novel chromosome walking technology.The full-length of *ssu4d* cDNA (named *ssu4dc*), contained a 522 bp open reading frame encoding a protein of 174 amino acids. Sequence analysis of *ssu4dc* and *ssu4d* showed that *ssu4d* contained an intron between +355 nt to +1125 nt downstream of transcriptional iniative site. *ssu4dc* contained 54 bp of 5' untranslated region (UTR), and an open reading frame of 174 amino acids consisting of a chloroplast transit peptide with 57 amino acids and a mature protein of 117 amino acids. The deduced amino acid sequence of *ssu4dc* shared 95-96% identity with *L. gibba* RbcS protein. Real time-PCR analysis showed differential expression of individual *rbcS* genes in light-grown *Lemna gibba*. And the levels of SSU4dc mRNA was regulated by the action of phytochrome, there was variability in the amount of expression of SSU4dc RNA comprared to the SSU1 and SSU5B from *Lemna gibba*.

Key words: Cloning, new *rbcS* gene, *lemna gibba*, transcriptional analysis, differential Expression

Ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) catalyzes both the carboxylation of ribulose bisphosphate during CO_2 fixation and the oxygenation of the same substrate during photorespiration [1]. The small subunits of Rubisco are produced by *rbcS* gene family in the nuclear genome [2], the 55-kDa large subunits are encoded by a single *rbcL* gene in the chloroplast genome [3].

The duckweed *Lemna gibba* is an aquatic monocot in which the *rbcS* is encoded by a 12-to 14-member gene family [4]. Genomic clones for six members of the gene family and a cDNA clone for a seventh have been isolated and characterized [5, 6]. The expression of individual *rbcS* sequences in total steady state RNA was shown to be under the control of phytochrome [5]. The most extreme differences between transcription rates versus steady state mRNA levels were measured for SSU1 and SSU5B [6].

Conserved regions were found in *Lemna gibba rbcS* genes (GenBank accession No. X17231.1, X17230.1, X17232.1, X17235.1, X17234.1 X17233.1, and X00137.1). A pair of generacy primers were designed based on this region. A novel *rbcS* gene fragment (about 400 bp) was obtained and sequenced. Sequence analysis revealed that it shared 82-85 % identity with the known *L. gibba rbcS* genes. Eventually, a new *rbcS* gene with a ful length of 1346 bp (designated as *ssu4d*) was cloned from *L. gibba* genomic DNA by SEFA-PCR. Sequence blast analysis revealed that *ssu4d* shared 95-96 % identity with the known *L. gibba rbcS* genes. According to the sequence of this putative novel *rbcS* gene, specific primers were designed to amplify the *rbcS* gene cDNA. Transcriptional origin site was confirmed by designing a series of 5’ forward primers based on the putative transcriptional site which was inferred by the analysis of known *rbcS* genes from *L gibba*. A 579 bp *rbcS* gene cDNA fragment was first generated by RT-PCR.

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Published by the Indian Society of Genetics & Plant Breeding, F2, First Floor, NASC Complex, PB#11312, IARI, New Delhi 110 012
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The integrity of the genomic DNA (1346 bp in length) and cDNA (579 bp in length) sequences of *ssu4d* was confirmed by nested PCR.

Comparison of DNA sequences indicated that *ssu4d* gene contained an intron which is different from its homologous counterparts from *L. gibba.* The single intron in *ssu4d* gene is located from +355 nt to +1125 nt The intron, with the size of 770 nt, was quite different from other introns of *L. gibba rbcS* gene in length. A 579 bp cDNA fragment was analysed and searched against NCBI database to find the conserved domain and putative function, it was found that the *L. gibba* cDNA (*ssu4dc*) possessed a 522 bp open reading frame (ORF) from 55 bp to 579 bp of the sequence with a 54 bp 5'untranslated region (5'-UTR) (Fig. 1). This cDNA encoded a protein with domains which were similar to other *L. gibba* RbcS (Fig. 2). The deduced amino acid sequences of *ssu4d* gene are shown Fig. 2. The transit peptide is less conserved than the mature SSU polypeptide when the deduced amino acid sequence of *ssu4dc* from *L. gibba* was compared with those sequences from other organisms in GenBank database using BLASTX search. The result indicated

AGAGACCCCAGAGCTTCCGAGAAGAAGAGGAAGAGAGAGAGAAGGAGAGTGACCAT

GGCTGCCTCCATGATGAGCTCCACCGCCGCCGTGGCCAGCGTTGCCAAGACCAGCATG

GTCGCACCCTTCAACGGGCTGAGGTCCGCCGTCGCCTTCCCGGCGACCAGGAAGGCC

AACGATCTGTCGACTCTGCCCAGCAATGGCGGGAGGGTGAGCTGCATGCAGGTGTGGC

CGCCGGAGGGGCTGAAGAAGTTCGAGACCCTCTCCTACCTCCCTCCCCTCTCCGTCGA

GGCTCTCTCCAAGGAGGTCGACTACCTCCTCCGCAACGGCTGGATTCCCTGCGTTGAG

TTCTCCAAGG*TATAACAAAACCCATTCTTATCGGTTATCGGATCCTTATCGAATATCATACCCT*

TTTTGGAATTCTCCAAGGCGAAAATCTTTCCGATTTTGGTCATCCTTATTGGTACCATTTTCGA

TAACAATTGGGCCCCGTTTCAGTTAATGGGTCCCATTTTGATACCGAATCCGTATTGATATCTC

CTTCAGTACCGATTGTGTTGTCGAAACGAGTGTTTCTATACTTGATTGATCAGACCCATGTTTT

GGTTGCTAAAATTGATTGGTATCGGATGCCGAAACGGAAACTTTTGATTTTGCGGTTCTTATC

GGTACCATTTTCGACCAAAAGTTTCCGTTAACGGGTCCCGCTTTGATACCACTATCCGTTATC

GGTTACACGATCCAATTCTGATATTGGACCTGTACCAATATCTCTTCTAGTAACGATGGCGATA

TAGATTGCTATCCAATTGCGATATCCATATCAAAATGACTAGTTCGATAGTCGATATCCAGACC

CATGTTTGGTATCCAATATTGATTGATATTGAATACCAAAAGAGAAAGTATCAGTATTCAATTCT

TATCGGTATTGCTACTAGATGCGATGTCGGTACCTGGATGGGCGAACCCGCAAAGGATTATG

AAGGGAAGTGGTGCCGTATCCTTATCGATACCGGTTCAATACCGGTATCCAATACCGATAACA

GCTCCACTATCCAAAGTCAATCGAAGCCGCAAAACCCAAGTGTGTATTAGTATCTAAAATCCG

*ATATTCGTTAAC*AAAAGGGTTCGTGTACCGCCAATACCACGCCTCCCCCGGGTACTACGA

TGGGCGCTACTGGACCATGTGGAAGCTGCCCATGTTCGGGTGCACCGACGCCAGCCAG

GTGATCGCCGAGGTGGAGGAGGCCAAGAAGGCCTACCCCGAGTATTTCGTCAGAATCA

TCGGCTTCGACAACAAGCGCCAAGTCCAGTGCATCAGCTTCATCGCCTACAAGCCCAC

CTAA

Fig. 1. DNA sequence *ssud* gene from *L.gibba.*The intron of *ssud,* which was italic, was located from +355 nt to +1125 nt. 54 bp of 5' untranslated region (UTR) was underlined

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X17235.1    ----MMVSTAAVARVRPAQTNMVGAFNGCRSSVAFPATRKANNDLSTLPSSGGRVSCMQV
X00137      ---------------------------------------------------------MQV
SSU4dc      MAASMMSSTAAVASV-_AKTSMVAPFNGLRSAVAFPATRKAN-DLSTLPSNGGRVSCMQV
                                                                     ***

X17235.1    WPPEGLKKFETLSYLPPLSVEDLAKEVDYLLRNDWVPCIEFSKEGFVYRENNASPGYYDG
X00137      WPPEGLKKFETLSYFPLSSVEDLAKEVDYLLRNDWVPCIEFSKEGFVYRENNASPGYYDG
SSU4dc      WPPEGLKKFETLSYLPPLSVEALSKEVDYLLRNGWIPCVEFSKEGFVYRQYHASPGYYDG
            **************: *  ****:*********.*:**:*********::*********

X17235.1    RYWTMWKLPMFGCTDASQVIAEVEEAKKAYPEYFVRIIGFDNKRQVQCISFIAYKPT
X00137      RYWTMWKLPMFGCTDASQVIAEVEEAKKAYPEYFVRIIGFDNKRQVQCISFIAYKPT
SSU4dc      RYWTMWKLPMFGCTDASQVIAEVEEAKKAYPEYFVRIIGFDNKRQVQCISFIAYKPT
            *****************************************************
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Fig. 2. Comparison of *Lemna gibba rbcS* sequences encoding the mature SSU protein. The deduced amino acid sequence of X17235.1, X00137 from *L.gibba* are shown. The deduced transient peptide of Ssu4dc was underlined with boldfaced letters. An asterisk(*) denotes the position of residues identical with X17235.1

that *ssu4d* conserved segments have broad homology to the cloned *L. gibba* RbcS with the similarity and identity.

It was demonstrated that the *ssu1* is the most highly represented, followed by *ssu5B* in *L. gibba* [5]. To examine the expression of *ssu4dc*, *L. gibba* total RNA was isolated from cultures grown under different illumination conditions. Real-time PCR analysis of the cDNA clone were performed using primers designed based on the 5'-UTR and 3 '-UTR. The results indicated that the SSU1 was the most highly represented, followed by *ssu4dc* and SSU5B, the expression level of *ssu4dc* gene was higher than that of SSU5B in light condition (Fig. 3).

The expression levels of the SSU1, SSU5B and *ssu4dc* mRNAs varied widely. So it is likely that the differential expression of individual *rbcS* genes is the result of more than one regulatory events. The analysis of these regulatory steps may provide a clue as to the mechanisms of phytochrome regulation of *rbcS* gene expression.

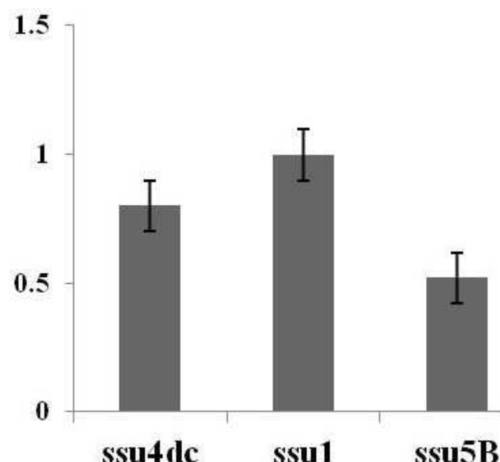

Fig. 3. Differential expression of individual *rbcS* genes in light-grown plants. The amount of expression of each gene is normalized to the expression of SSUI. The mean of triplicate determinations is shown

Acknowledgements

This research was supported by a grant from National Natural Science Foundation of China (# 30771239) and open grant from Hubei Key Laboratory Polutant Analysis and Resue Technology, Hubei Normal University, Huangshi (#KY2010G16)

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