

GENE NOTE

A sucrose transporter, *LjSUT4*, is up-regulated during *Lotus japonicus* nodule development*

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Abstract

LjSUT4, encoding a putative sucrose transporter, was identified in a *Lotus japonicus* nodule cDNA library. The deduced amino acid sequence showed a high degree of identity with sucrose transporters from other plants. Semi-quantitative RT-PCR analysis demonstrated that the *L. japonicus* SUT4 gene was expressed at high levels in both roots and nodules. *In situ* hybridization revealed that, in young nodules, SUT4 mRNA transcripts are present in vascular bundles, inner cortex and both infected and uninfected cells while, in mature nodules, accumulation of transcripts was restricted only in vascular bundles and the inner cortex. The results indicated that *LjSUT4* codes for a putative sucrose transporter, and its expression pattern suggests a possible shift in the mechanism of sugar transport during nodule development. The role of this polypeptide in sucrose transport and metabolism is discussed.

Key words: *In situ* hybridization, *Lotus japonicus*, root nodules, sucrose transporter, symbiosis.

Effective nitrogen fixation involves the complex interaction of legume plants with soil bacteria, *Rhizobium*, *Bradyrhizobium*, *Sinorhizobium*, *Mesorhizobium*, and *Azorhizobium*. A new organ is formed from this interaction, the root nodule. Within the nodule, the bacteria reduce atmospheric nitrogen to ammonia, which the plant assimilates in glutamine and glutamate. In turn, the plant provides the bacteria with carbon for growth and energy production. The carbon cost for this process is high, rendering the nodule a strong sink organ. Sucrose produced in photosynthetic tissues is the main carbohydrate translocated through the phloem to the nodules where it is rapidly metabolized (Vance *et al.*, 1997).

In sink tissues sucrose transport is mediated by specific transporters, a family of highly hydrophobic proteins consisting of 12 transmembrane domains, which function as sucrose/H⁺ co-transporters (Delrot *et al.*, 2001). Sink loading by sucrose transporters have been characterized in *Plantago major*, *Vicia faba*, *Pisum sativum*, and barley (Gahrtz *et al.*, 1996; Lemoine, 2000; Weschke *et al.*, 2000). In addition, enhanced expression of *Arabidopsis*

sucrose transporters *AtSUC2* and *AtSUT4* was observed in various sink tissues including roots, flower, green fruit, sink leaves, and ovaries (Weise *et al.*, 2000). Furthermore, a pollen-specific sucrose transporter-like protein (*NtSUT3*) has also been identified in tobacco (Lemoine *et al.*, 1999). Moreover, gene expression analyses revealed that leaf sucrose transporters are also expressed in sink tissues (Lemoine, 2000).

In the present study, the spatial and temporal expression of a *Lotus japonicus* putative sucrose transporter gene whose expression is highly enhanced in nodules was investigated.

Recently, large numbers of expressed sequences tags (ESTs) from *L. japonicus* nodules have been deposited in public databases and analysed by DNA arrays for transcriptome analysis (Colebatch *et al.*, 2002). Further analysis by digital northern revealed the presence of ESTs coding for polypeptides involved in monosaccharide and disaccharide transport. The complete nucleotide sequence of an EST clone showing high homology to previously characterized plant sucrose transporters was determined and designated as *LjSUT4*. The deduced amino acids of the *LjSUT4* sequence revealed the presence of an open reading frame of 511 amino acids. The multiple amino acid sequence alignment of *LjSUT4* with other known plant sucrose transporters expressed in sink tissues, revealed that the *LjSUT4* exhibits 70%, 67.1%, 65.7%, and 49.7% similarity to *Lycopersicon esculentum* SUT4, *Arabidopsis thaliana* SUT4, *Daucus carota* SUT1, and *Vicia faba* SUT, respectively (Fig. 1). *In silico* analysis of the hydrophobic regions in *LjSUT4* revealed the presence of 12 putative transmembrane domains, a characteristic of this family of membrane transporters (data not shown).

The accumulation of *LjSUT4* transcripts in different *L. japonicus* tissues (nodules, roots, leaves, stems, flowers, seedpods, cotyledons, hypocotyls, and apical meristems) was examined using a semi-quantitative reverse-transcription (RT)-PCR approach (Fig. 2A). The highest levels of *LjSUT4* transcripts were observed in sink tissues such as roots and nodules. Relatively lower levels were found in green seedpods and hypocotyls, whereas, no detectable expression was found in leaves, stems, flowers, cotyledons, and apical meristems. Accumulation of *LjSUT4* transcripts was also examined during nodule development. *LjSUT4* transcripts were detectable at

* The nucleotide sequence appeared in the DDBJ/EMBL/GenBank database with the accession number AJ538041.

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Abbreviations: dpi, days post-infection.

relatively low levels in emerging nodules 10 d post-infection (dpi), showed a maximum accumulation in young nodules (14 dpi), while

at consequent developmental stages the transcript levels decreased (Fig. 2B).

The spatial localization of *LjSUT4* gene transcripts during nodule development was examined using an *in situ* hybridization approach. Sections of *L. japonicus* nodules at various stages of development were hybridized with 11-digoxigenin-rUTP-labelled RNA probes transcribed from *LjSUT4* cDNA clone. Both antisense and sense labelled RNA transcripts were used as probes. At 14 dpi with *Mesorhizobium loti*, high levels of *LjSUT4* transcripts were observed

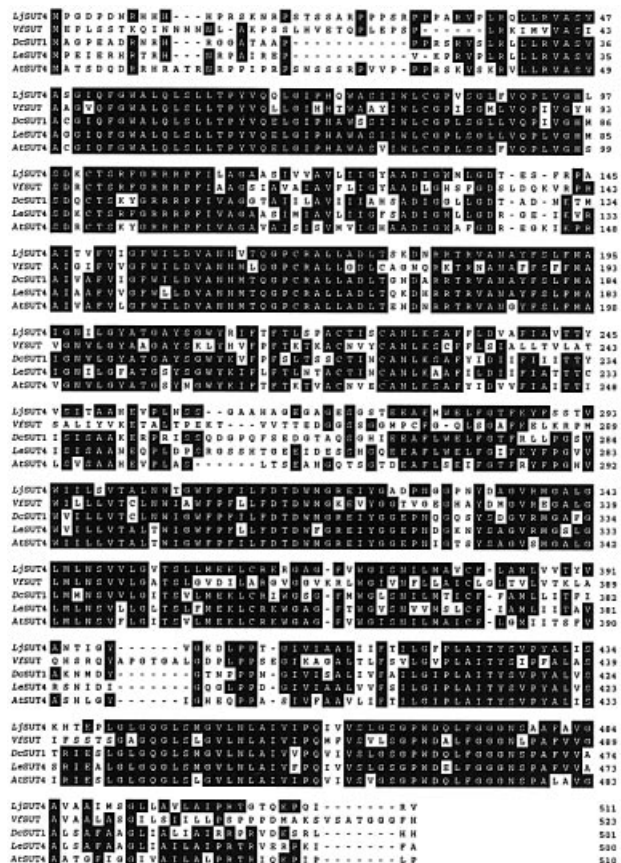


Fig. 1. Comparison of the deduced amino acid sequences of *L. japonicus* SUT4 with sucrose carriers from various plants. Organism symbols and GenBank database accession numbers are as follows: *LjSUT4*, *L. japonicus* SUT4 (AJ538041); *VjSUT*, *Vicia faba* SUT (T12198); *DcSUT1*, *Daucus carota* SUT1 (T14339); *LeSUT4*, *Lycopersicon esculentum* SUT4 (AAG09270); and *AtSUT4*, *Arabidopsis thaliana* SUT4 (AAG09191). Black-shaded boxes represent conserved amino acids, while dashes represent gaps in the alignment. The analysis was carried out using the CLUSTAL method with PAM250 residue weight table.

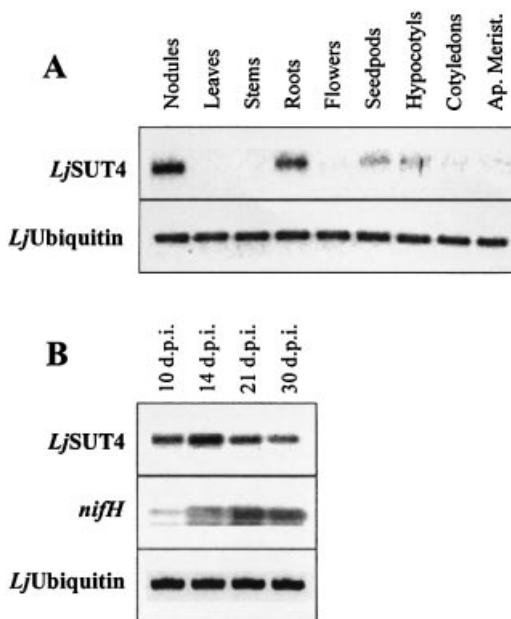


Fig. 2. Accumulation of *LjSUT4* mRNA in *L. japonicus* nodules and non-symbiotic tissues. Total RNA was isolated from various tissues and developmental stages as indicated and subjected to semi-quantitative reverse-transcription polymerase chain reaction (RT-PCR) analysis using *L. japonicus* ubiquitin as an internal control. (A) The expression levels at 21 dpi nodules, mature leaves, stems, young roots from 3-d-old seedlings, flowers, green seedpods, hypocotyls, cotyledons, and apical meristems. (B) The expression levels in root nodules at 10, 14, 21, and 30 dpi. The onset of nitrogen fixation was determined by the amplification of *M. loti* *nifH* gene transcripts.

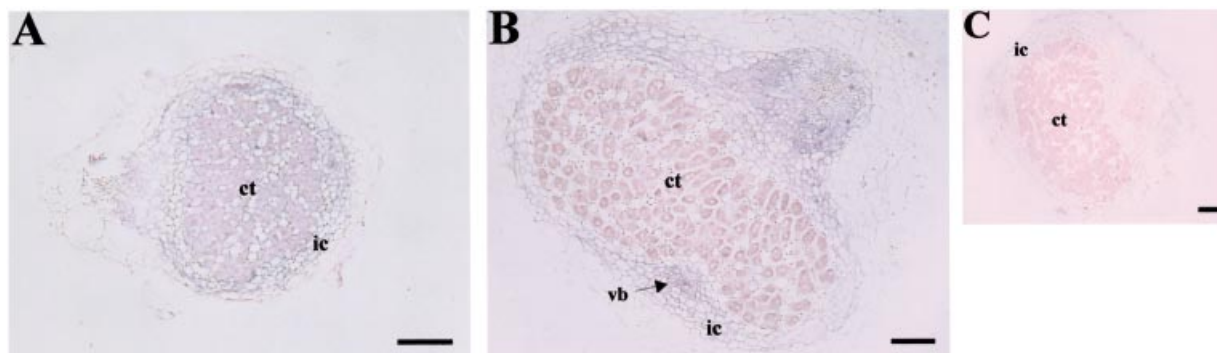


Fig. 3. *In situ* localization of *LjSUT4* gene transcripts in *L. japonicus* root nodules. Transverse 7–10 μ m thin sections of nodules at 14 (A) and 28 (B) dpi with *Mesorhizobium loti* were hybridized with digoxigenin-11-rUTP labelled RNA probe *in vitro* transcribed from a *LjSUT4* cDNA clone. The hybridization signal is visible as a blue-purple precipitate. As a negative control, transverse sections of 28 dpi (C) were hybridized to sense digoxigenin-11-rUTP labelled RNA transcribed from a *LjSUT4* cDNA clone. In this case, no significant hybridization signal was visible. Abbreviations: ct, central tissue; ic, inner cortex; vb, vascular bundle. Bars represent 100 μ m.

in the nodule parenchymatous cells, vascular bundles and in the infected and uninfected cells of the central tissue (Fig. 3A). In mature nodules at 28 dpi a strong signal was present mainly in the vascular bundles and nodule parenchymatous cells, while no hybridization signal could be detected in the cells of the central tissue (Fig. 3B). As a negative control, sections of *L. japonicus* nodules at 14 dpi with *M. loti*, were hybridized to sense digoxigenin-labelled RNA probes transcribed from a *LjSUT4* clone (Fig. 3C). In this case no significant hybridization signal was detected.

The results indicate that *LjSUT4* codes for a putative sucrose transporter, which accumulates in various sink tissues of *L. japonicus* including root nodules. The differences of *LjSUT4* spatial expression patterns during nodule development suggest that there is a possible shift in the transport and consequent metabolism of sugars associated with nodule maturation. This shift in the transport mechanism remains to be elucidated, especially with respect to the characterization and localization of additional transporters involved in sucrose transport. A developmentally similar spatial expression pattern of sucrose synthase transcripts was observed in soybean nodules (Kavroulakis *et al.*, 2000). These data taken together suggest that sucrose may not be the immediate carbon source for cells located in the central tissue of mature nodules, but phosphorylated derivatives of sucrose catabolism (trioses or hexoses) are translocated from the inner cortex.

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References

- Colebatch G, Kloska S, Trevaskis B, Freund S, Altmann T, Udvardi M. 2002. Novel aspects of symbiotic nitrogen fixation uncovered by transcript profiling with cDNA arrays. *Molecular Plant-Microbe Interactions* **15**, 411–420.
- Delrot S, Atanassova R, Gomes E, Coutos-Thevenot P. 2001. Plasma membrane transporters: a machinery for uptake of organic solutes and stress resistance. *Biochimica et Biophysica Acta* **1465**, 281–306.
- Gahrtz M, Schmelzer E, Stolz J, Sauer N. 1996. Expression of the *PmSUC1* sucrose carrier from *Plantago major* L. is induced during seed development. *The Plant Journal* **9**, 93–100.
- Kavroulakis N, Flietakis E, Aivalakis G, Katinakis P. 2000. Carbon metabolism in developing soybean root nodules: the role of carbonic anhydrase. *Molecular Plant-Microbe Interactions* **13**, 14–22.
- Lemoine R. 2000. Sucrose transporters in plants: update on function and structure. *Biochimica et Biophysica Acta* **1465**, 246–262.
- Lemoine R, Burkle L, Barker L, Sakr S, Kuhn C, Regnacq M, Gaillard C, Delrot S, Frommer WB. 1999. Identification of a pollen specific sucrose transporter-like protein *NtSUT3* from tobacco. *FEBS Letters* **454**, 325–330.
- Vance CP, Miller SS, Driscoll BT, Robinson DL, Trepp G, Gantt JS, Samas DA. 1997. Nodule carbon metabolism: organic acids for N₂ fixation. In: Elmerich CE, Kondorosi A, Newton WE, eds. *Biological nitrogen fixation for the 21st century*. Dordrecht, The Netherlands: Kluwer Academic Publishers, 443–448.
- Weise A, Barker L, Kuhn C, Lalonde S, Bushmann H, Frommer WB, Ward JM. 2000. A new subfamily of sucrose transporters, SUT4, with low affinity/high capacity localized in enucleate sieve elements of plants. *The Plant Cell* **12**, 1345–1355.
- Weschke W, Panitz R, Sauer N, Wang Q, Neubohn B, Weber H. 2000. Sucrose transport into barley seeds: molecular characterization of two transporters and implications for seed development and starch accumulation. *The Plant Journal* **21**, 455–467.