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Molecular cloning and characterization of two cDNAs encoding asparagine synthetase from *Astragalus sinicus*

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Astragalus sinicus (renge-sou) is a legume and forms indeterminate-type nodules with *Mesorhizobium huakuii* . The aim of our research is to isolate nodule specific genes from *A. sinicus* and characterize these genes to understand the mechanisms of nodule formation and symbiosis.

Two putative nodulin genes, c9 and c9-4, isolated from *A. sinicus* by differential screening were found to encode asparagine synthetase. In temperate legumes, asparagine is the primary assimilation product of N_2 fixation and the predominant transport product. c9 encoded an ORF of 584 aa and c9-4 encoded an ORF of 586 aa. The expression of two AS genes was investigated in various organs by Northern hybridization. The signal of c9-4 was detected in nodule and root. The signal of C9 was detected in nodule and root; weak signal of c9 was also detected in leaf. This difference of the expression suggests that both genes have different functions in plant. When 0.5mM NH_4SO_4 was added to the culture medium, the expression of both genes was reduced in nodules. This is the first report of the repression of asparagine synthetase gene expression by ammonium in root nodules.

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Promoter analysis of a soybean nodule-enhanced PEPC gene

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Phosphoenolpyruvate carboxylases (PEPCs) play essential roles in legume root nodules. Two soybean PEPC genes, *GmPEPC7* and *GmPEPC15*, were suggested to encode the nodule-enhanced form and a house-keeping isoform, respectively (Plant J. 13: 267 (1998)).

We constructed chimeric genes by fusing the 5'-flanking region of either *GmPEPC7* or *GmPEPC15* to the GUS reporter gene, transformed soybean roots by *Agrobacterium rhizogenes*-mediated hairy root method, and then inoculated *Bradyrhizobium japonicum*. High GUS activity was detected in the *GmPEPC7*-transformed nodules whereas *GmPEPC15*-transformed nodules showed no or very low reporter activity. To determine the cis-element which enable the high-level expression of *GmPEPC7*, we deleted the upstream region of *GmPEPC7* from the 5' end and examined the promoter activity. A *GmPEPC7* fragment (400 bp in size) which is very similar to the corresponding region of *GmPEPC15*, still showed high activity. Based on these results, we discuss the molecular evolution of the nodule-enhanced PEPC gene in soybeans.

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PROMOTER ANALYSIS OF URICASE II (NODULIN 35) GENES TO IDENTIFY THE ELEMENTS FOR NODULE UNINFECTED CELL - SPECIFIC EXPRESSION.

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Uricase II (a homotetramer of nodulin 35) is a key enzyme to form ureides for transport of fixed nitrogen from nodules to shoot of legumes, such as soybean and cowpea, and is known to localize in peroxisomes in nodule uninfected cells. We have previously isolated two distinct *nod-35* genes, *GmUR9* and *GmUR2*, from soybean plants.

To identify the element(s) responsible to nodule uninfected cell-specific expression (NUSE: Nodule Uninfected cell-Specific Element) of *nod-35* genes, we have examined promoter activities of these *nod-35* genes in transgenic soybean nodules formed on *A. rhizozenes*-mediated hairy roots. Deletion experiments of *GmUR9* promoter indicated that the region from –254 to –148bp (from translation start) is essential for uninfected cell-specific expression. We demonstrated that a motif "GTAATG" contained in this region is one of the NUSE. This motif is present at around 190bp upstream of the translation start of *GmUR9* and *GmUR2* as well as 5'-flanking region of alfalfa uricase II gene, but not in that of non-legume arabidopsis.

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PROPERTIES OF THE *RHODOBACTER CAPSULATUS* RNF PROTEIN COMPLEX THAT IS ESSENTIAL FOR NITROGEN FIXATION.

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Ferredoxin I encoded by the *fdxN* gene and flavodoxin encoded by the *nifF* gene are major electron donors to nitrogenase in *Rhodobacter capsulatus*. In the vicinity of *fdxN*, several genes have been identified to be essential for diazotrophic growth and designated as *rnf* (*rhodobacter nitrogen fixation*) genes. Products of the *rnf* genes are expected to comprise an energy-coupling FdxN reductase complex. We report here 1) membrane topology of RnfD and RnfE, and 2) co-purification of RnfC with hexahistidine-tagged RnfB protein,.

Analysis of translational fusions of *rnfD* to *E. coli* alkaline phosphatase *gene* indicated that RnfD spans membrane 6 times with its central hydrophilic region exposed to periplasm while its N- and C-terminal regions reside in cytoplasm. Similar analysis of RnfE will be reported.

C-terminally $(His)_6$-tagged RnfB can support diazotrophic growth. When membrane proteins were solubilized with dodecylmaltoside at pH 6.5, RnfC was co-adsorbed to Ni-NTA agarose, whereas no RnfC was adsorbed without $(His)_6$-tagged RnfB protein. These indicated that RnfB and RnfC associate together possibly as a subcomplex.