

Plant Gene Register

A cDNA Sequence Encoding Glutamine Synthetase Is Preferentially Expressed in Nodules of *Vigna aconitifolia*

Zhenwu Lin¹, Guo-Hua Miao², and Desh Pal S. Verma*

Department of Molecular Genetics and Biotechnology Center, The Ohio State University, Columbus, Ohio 43210

GS (EC 6.3.1.2) occupies a key position in controlling nitrogen metabolism, a pathway directly affecting plant productivity. It catalyzes the first step in the assimilation of ammonia in higher plants, converting inorganic ammonia produced by different metabolic routes into organic nitrogen. GS is encoded by a small gene family in higher plants, the members of which are expressed in different tissues, cell types, and subcellular compartments under the control of both metabolic status and developmental programs of the plant (Hirel et al., 1993). This temporal and spatial regulation of GS genes meets the requirements of ammonia assimilation under various physiological conditions. In legume-*Rhizobium* symbiosis, the symbiotically reduced ammonia is assimilated by host root nodule-specific and/or root/nodule-specific GS. Both nodule-specific (alfalfa, Dunn et al., 1988; and soybean, Roche et al., 1993) and nodule-enhanced (French bean, Cullimore et al., 1984) GS isoforms have been reported. In pea no nodule-specific GS has been found (Tingey et al., 1987). In addition to the nodule-specific GS, soybean also contains a root/nodule GS, the expression of which is strongly enhanced in nodules. The latter occurs in direct response to the availability of ammonia (Hirel et al., 1987; Miao et al., 1991). We report here the isolation of a full-length cDNA sequence encoding mothbean (*Vigna aconitifolia*) GS, expression of which appears to be nodule specific.

Mothbean is a diploid tropical legume that can be easily transformed by *Agrobacterium*-mediated transformation methods (Lee et al., 1993) and regenerated (our unpublished results). Thus, mothbean is a plant of choice toward understanding the regulation and role of specific GS isoforms in plant nitrogen metabolism. Using a soybean cytosolic GS sequence (GS20; Miao et al., 1991) as a probe, we isolated several GS cDNA sequences from a mothbean nodule cDNA library (Table I). One of these sequence, VGSn-1, contains an open reading frame of 1068 nucleotides and encodes a GS polypeptide of 356 amino acids. Sequence homology comparison showed that VGSn-1 shares more than 85% similarity at the amino acid level

Table I. Characteristics of a GS cDNA from mothbean (*V. aconitifolia*)

Organism:	Mothbean (<i>Vigna aconitifolia</i>).
Gene Function:	Encodes a cytosolic GS preferentially expressed in root nodules.
Techniques:	Screening of nodule cDNA library in pCDNA II (Invitrogen, San Diego, CA); double-strand plasmid sequencing using the dideoxy chain termination method for both strands.
Method of Isolation and Identification:	The cDNA clone was isolated from mothbean nodule cDNA library in pCDNA II vector using a soybean GS cDNA sequence (Miao et al., 1991) as a probe.
Expression and Regulation:	Expressed strongly in root nodule. The transcript was not detected in root, and treatment with ammonia did not induce expression of this gene in roots (cf. Miao et al., 1991).

with GS of other leguminous plants. VGSn-1 is strongly expressed in symbiotically effective mothbean root nodules but not in roots as shown by northern blot analysis using the 3'-end-sequence-specific probe. Treatment with 10 mM of ammonia did not induce the expression of VGS-1 gene. This suggests that expression of VGSn-1 is controlled by nodule developmental program regulated by infection of *Rhizobium*. Genomic Southern blot analysis using full-length or 3'-end-sequence-specific probe of VGSn-1 showed that VGSn-1 is encoded by a small gene family in mothbean genome. These data confirmed the presence of nodule-specific GS genes in legumes. However, a low level of expression of this gene was also observed in leaf. It is not clear whether this is due to the low-level expression of VGSn-1 gene or to the expression of a related sequence. The *gln-γ* gene of *Phaseolus vulgaris* was initially shown to be specifically expressed in root nodules. However, a lower level of expression of this gene has also been detected in other tissues of the plant using more sensitive methods (Forde et al., 1989).

In soybean, two different control mechanisms appear to be involved in controlling expression of GS genes in root nodules. Although expression of one of the soybean GS genes, GS20, is enhanced by symbiotically fixed or externally supplied ammonia (Miao et al., 1991), other GS genes

Abbreviation: GS, glutamine synthetase.

¹ Present address: Coriell Institute for Medical Research, 401 Haddon Avenue, Camden, NJ 08103.

² Present address: Agricultural Products, E. I. du Pont de Nemours & Company, Wilmington, DE 19880-0402.

* Corresponding author; e-mail dverma@magnus.acs.ohio-state.edu; fax 1-614-292-5379.

appear to be specifically induced during nodule development (Roche et al., 1993). Thus, it is becoming apparent that both developmentally controlled nodule-specific or nodule-enhanced GS and metabolic regulated pre-existing root-specific GS genes contribute to the symbiotic nitrogen assimilation in root nodules. Understanding the regulation and role(s) of these genes in nitrogen metabolism may allow further manipulation and improvement of plant nitrogen assimilation.

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LITERATURE CITED

- Cullimore JV, Gebhardt C, Saarelainen R, Mifflin BJ, Idler BK, Barker RF** (1984) Glutamine synthetase of *Phaseolus vulgaris* L.: organ-specific expression of a multigene family. *J Mol Appl Genet* 2: 589-599
- Dunn K, Dickstein R, Feinbaum R, Burnett BK, Peterman TK, Thoidis G, Goodman HM, Ausubel FM** (1988) Developmental regulation of nodule-specific genes in alfalfa root nodules. *Mol Plant-Microbe Interact* 1: 66-74
- Forde BG, Day HM, Turton JF, Shen W-J, Cullimore JV, Oliver JE** (1989) Two glutamine synthetase genes from *Phaseolus vulgaris* L. display contrasting developmental and spatial patterns of expression in transgenic *Lotus corniculatus* plants. *Plant Cell* 1: 391-401
- Hirel B, Bouet C, King B, Layzell D, Jacobs F, Verma DPS** (1987) Glutamine synthetase genes are regulated by ammonia provided externally or by symbiotic nitrogen fixation. *EMBO J* 5: 1167-1171
- Hirel B, Miao G-H, Verma DPS** (1993) Metabolic and developmental control of glutamine synthetase genes in legume and non-legume plants. In DPS Verma, ed, *Control of Plant Gene Expression*. CRC Press, Boca Raton, FL, pp 443-458
- Lee N-G, Stein B, Suzuki H, Verma DPS** (1993) Antisense expression of nodulin-35 RNA in *Vigna aconitifolia* root nodules retards peroxisome development and the availability of nitrogen to the plant. *Plant J* 3: 599-606
- Miao G-H, Hirel B, Marsolier MC, Ridge RW, Verma DPS** (1991) Ammonia-regulated expression of a soybean gene encoding cytosolic glutamine synthetase in transgenic *Lotus corniculatus*. *Plant Cell* 3: 11-22
- Roche D, Temple S, Sengupta-Gopalan C** (1993) Two classes of differentially regulated glutamine synthetase genes are expressed in the soybean nodule: a nodule-specific and a constitutively expressed class. *Plant Mol Biol* 22: 971-983
- Tingey SV, Walker EL, Coruzzi GM** (1987) Glutamine synthetase genes of pea encode distinct polypeptides which are differentially expressed in leaves, roots and nodules. *EMBO J* 6: 1-9