

Plant Gene Register

Soybean Root Nodule cDNA Encoding Glutathione Reductase¹

Xiaoyan Tang and Mary Alice Webb*

Department of Botany and Plant Pathology, Purdue University, West Lafayette, Indiana 47907

GSH is widely distributed in organisms and has diverse functions in protein synthesis, sulfur storage, and protection against a variety of stresses (Meister and Anderson, 1983). GR (EC 1.6.4.2) is a flavoprotein that catalyzes the reduction of GSSG to reduced GSH using NADPH as the reducing cofactor. It is a key enzyme in the GSH-ascorbate cycle, which provides protection against oxidative stress, particularly in photosynthetic tissues of plants (Foyer and Halliwell, 1976). Tobacco transformed with the *gor* gene, encoding GR in *Escherichia coli*, showed mixed results in resistance to oxidative stress (Aono et al., 1991; Foyer et al., 1991). Recently, a cDNA clone encoding GR was isolated from a cDNA library of pea leaves (Creissen et al., 1991). An N-terminal leader sequence encoded by the pea cDNA was consistent with targeting of the gene product to the chloroplast.

In root nodules, enzymes of the GSH-ascorbate cycle have been suggested to function in peroxide scavenging and protection against other oxidative stresses (Dalton et al., 1986). In soybean, GR activity was much higher in nodules than in uninfected roots and was enhanced in nodules exposed to higher than normal pO₂ (Dalton et al., 1991). GR activity also was about 4-fold higher in effective nodules than in ineffective nodules, with a range of factors controlling effectiveness (Dalton et al., 1993). Soybean (*Glycine max*) nodules contain both homo-GSH as well as GSH, and the combined concentration of thiol tripeptides in nodules was calculated to be about 1 mM (Dalton et al., 1993). Recent studies examining the role of GSH in relation to pathogen defense responses (Wingate et al., 1988; Edwards et al., 1991) have implications for GSH function during nodule development as well.

A full-length cDNA encoding soybean nodule GR (Table I) was isolated serendipitously in screening a soybean nodule cDNA library with antibodies against nodule allantoinase. The deduced amino acid sequence of the cDNA was found to share a high degree of homology to sequences for GR in other organisms and was 90% similar and 83% identical to pea GR (Creissen et al., 1991). The soybean GR sequence also had an N-terminal leader sequence characteristic of a chloroplast transit peptide, which would target the gene product to nonphotosynthetic plastids in root nodules. South-

Table I. Characteristics of the cDNA clone encoding GR from soybean nodules

Organism:	<i>Glycine max</i> (L.) Merr. cv Williams 82.
Location in Genome:	Nuclear genome.
Gene Product and Function:	Glutathione reductase (EC 1.6.4.2); reduction of GSSG to GSH.
Source:	A cDNA expression library was constructed from poly(A) ⁺ RNA isolated from soybean root nodules 24 d after inoculation of seeds.
Method of Identification:	Similarity of deduced amino acid sequence to GR in pea (90%) and other organisms.
Features of cDNA:	2099 bp in length; translational start site at nucleotide 59 and stop site at nucleotide 1691; an 18-nucleotide poly(A) tail at the 3' end.
Structural Features of the Deduced Protein:	The 1632-bp open reading frame encodes a deduced polypeptide of 544 amino acids, including an N-terminal leader sequence of 50 to 60 residues characteristic of a plastid targeting sequence. Cys residues forming redox-active disulfide bridge and Arg residues required for binding NADPH are conserved.
Expression:	mRNA of about 2.1 kb was present in poly(A) ⁺ RNA isolated from soybean leaves and root nodules. Transcript was detected in total RNA isolated from root nodules between 8 and 24 d after inoculation. Expression in infected region of nodules was shown by in situ hybridization.

ern analysis showed multiple hybridizing bands after digestion of genomic DNA with several restriction enzymes, suggesting the presence of a multigene family. The GR cDNA was used to rescreen the nodule cDNA library, and three additional clones were isolated. Partial sequencing of these clones has shown that they have similar coding regions but different 3' untranslated regions.

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Abbreviation: GR, glutathione reductase.

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* Corresponding author; fax 1-317-494-5896.

LITERATURE CITED

- Aono M, Kubo A, Saji H, Natori T, Tanaka K, Kondo N** (1991) Resistance to active oxygen toxicity of transgenic *Nicotiana tabacum* that expresses the gene for glutathione reductase from *Escherichia coli*. *Plant Cell Physiol* **32**: 691–697
- Creissen G, Edwards EA, Enard C, Wellbern A, Mullineaux P** (1991) Molecular characterization of glutathione reductase cDNAs from pea (*Pisum sativum* L.). *Plant J* **2**: 129–131
- Dalton DA, Langeberg L, Treneman NC** (1993) Correlations between the ascorbate-glutathione pathway and effectiveness in legume root nodules. *Physiol Plant* **87**: 365–370
- Dalton DA, Post CJ, Langeberg L** (1991) Effects of ambient oxygen and of fixed nitrogen on concentrations of glutathione, ascorbate, and associated enzymes in soybean root nodules. *Plant Physiol* **96**: 812–818
- Dalton DA, Russell SA, Hanus FJ, Pascoe GA, Evans HJ** (1986) Enzymatic reactions of ascorbate and glutathione that prevent peroxide damage in soybean root nodules. *Proc Natl Acad Sci USA* **83**: 3811–3815
- Edwards R, Blount JW, Dixon RA** (1991) Glutathione and elicitation of the phytoalexin response in legume cell cultures. *Planta* **184**: 403–409
- Foyer CH, Halliwell B** (1976) Presence of glutathione and glutathione reductase in chloroplasts: a proposed role in ascorbic acid metabolism. *Planta* **133**: 21–25
- Foyer C, Lelandais M, Galap C, Kunert KJ** (1991) Effects of elevated cytosolic glutathione reductase activity on the cellular glutathione pool and photosynthesis in leaves under normal and stress conditions. *Plant Physiol* **97**: 863–872
- Meister A, Anderson MA** (1983) Glutathione. *Annu Rev Biochem* **52**: 711–760
- Wingate VPM, Lawton MA, Lamb CJ** (1988) Glutathione causes a massive and selective induction of plant defense genes. *Plant Physiol* **87**: 206–210