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Plant Gene Register

Nucleotide Sequence of a cDNA Encoding a Constitutively Expressed Glutathione S-Transferase from Cell Suspension Cultures of *Silene cucubalus*[1]

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Glutathione conjugation as a method of herbicide detoxification in monocotyledonous plants has been known at least since 1970 (11). This conjugation in plants has been attributed to the action of the enzyme, glutathione *S*-transferase (7). An affinity purification procedure using glutathione as ligand (12) has greatly simplified the purification of this enzyme from various sources leading to a wealth of information concerning the biochemistry and mode of action of herbicide resistance (9) and xenobiotic detoxification. Evidence has been given for the occurrence of isoforms of glutathione *S*-transferase in pea, accumulating in either the ER or the cytosol (6). More recently, cDNA clones for a multigene family have been isolated and characterized from maize (8, 10). Glutathione *S*-transferases have been characterized from human (5), rat (1, 2), and bacteria (4). Of very recent interest is the proposal that human glutathione transferase π interacts with mercury, copper, and cadmium, therefore, potentially having a role in heavy metal detoxification (3). In a continuation of our interest in heavy metal detoxification in plants, we report here on the nucleotide sequence and on the heterologous expression of a cDNA encoding glutathione *S*-transferase from cell suspension cultures of *Silene cucubalus* (Fig. 1; Table I). The transcript for the enzyme was constitutively present in the cell culture as judged by northern analysis. The cDNA contained a 653 base pair reading frame encoding a 217 amino acid polypeptide. The translated nucleotide sequence contained four tryptic peptide and the amino terminal amino acid sequences determined from the protein purified from *S. cucubalus* cell cultures. The cDNA was heterologously expressed in three systems, *Escherichia coli*, *Saccharomyces cerevisiae*, and *Spodoptera frugiperda*, producing, in each case, enzymically active glutathione *S*-transferase.

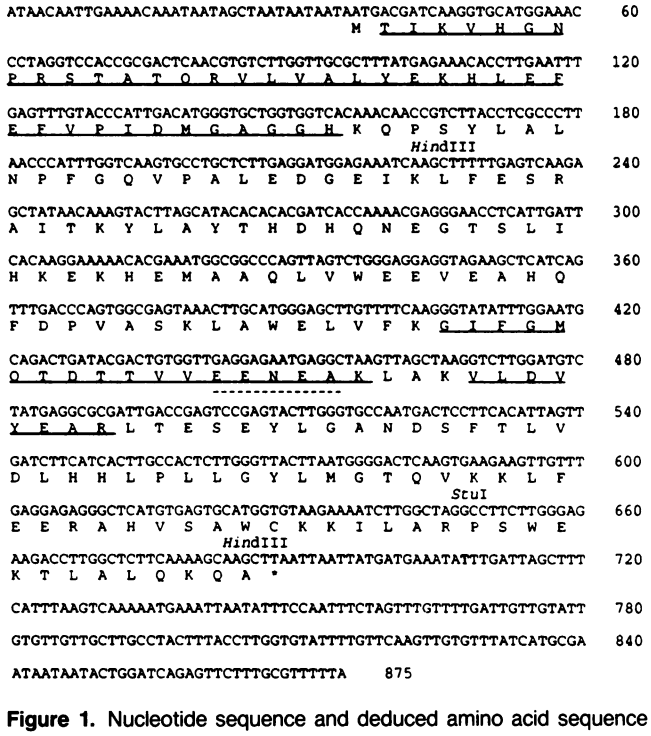

Figure 1. Nucleotide sequence and deduced amino acid sequence of the cDNA for glutathione *S*-transferase from *S. cucubalus*. For an explanation of symbols, see Table I.

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Table I. *Characteristics of a cDNA Encoding Glutathione S-Transferase from Cultured S. cucubalus Cells*

Organism:
Silene cucubalus (Leimkraut), cultured cells.
Location on Chromosome:
Unknown
Gene; Function; Pathway:
GST; encodes glutathione *S*-transferase; transfer of glutathione to certain herbicides resulting in herbicide tolerance.
Clone type:
cDNA, full length.
Sources:
cDNA library in λ gt11, constructed from poly(A)$^+$ RNA of cultured cells.
Sequencing Techniques:
pUC18 supercoil sequencing, unidirectional deletion subcloning and complete dideoxysequencing of both strands.
Method of Identification:
Comparison of translated nucleotide sequence to amino terminal and tryptic peptide amino acid sequences determined by microsequence analysis of homogeneous glutathione *S*-transferase purified from cell-suspension cultures of *S. cucubalus*. These sequences are underlined in Figure 1. The amino acid sequence from which the screening oligonucleotide was designed is additionally underlined with a dashed line. Molecular mass of deduced polypeptide is in agreement with that estimated from SDS-PAGE of glutathione *S*-transferase.
Expression Characteristics:
Transcript of approximately 1000 nucleotides as determined on northern blots. Expressed constitutively throughout the culture period. This clone contains 36n of the 5′ untranslated region (obtained from indirect RNA sequencing) and 185n of the 3′ untranslated region (present on this clone).
Regulation:
Unknown.
Codon Usage:
A general bias against G or C in the wobble position is shown. Codons not used are: CTA (L), AGC (S), CGC (R), GGC (G), TGC (C), CGG (R), CTG (L), TCG (S).
(G + C) Content:
41.0%
Sequence Homologies:
Maize glutathione *S*-transferase III (45.3% identity in 212 amino acid overlap) (8).
Structural Features of Protein:
ORF 217 amino acids; M_r24,571; heterologous protein expression tested and found active in *Escherichia coli*, *Saccharomyces cerevisiae*, *Spodoptera frugiperda*; protein accumulated in cultured *S. cucubalus* cells.
Antibodies:
Not available.
Subcellular Localization:
Cytosolic.
GenBank Accession No.:
M84968