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Plant Gene Register PGR 98–071

Nucleotide Sequence of a cDNA Encoding the Dihydrolipoyltransferase (E2) Subunit of the Branched-Chain Alpha-Keto Acid Dehydrogenase Complex from *Arabidopsis thaliana* (Accession No. AF038505).

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Plant Gene Register PGR 98–072

Characterization of a Genomic Clone Encoding a Novel Storage Protein, Gea8 (Accession No. U62395) from Carrot.

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Plant Gene Register PGR 98–073

Isolation and Characterization of AW13 (Accession No. AF047898) from Water-Deficit-Stressed *Sorghum bicolor*.

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Plant Gene Register PGR 98–074

Isolation and Characterization of a cDNA Encoding a Glyoxalase-I (Accession No. AJ224520) from *Cicer arietinum* L. Epicotyls Up-Regulated by Stress.

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Plant Gene Register PGR 98–075

Cloning of a cDNA Encoding a Putative Glutathione Peroxidase Protein from *Arabidopsis thaliana* (Accession No. AF030132).

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Plant Gene Register PGR 98–076

Cloning of a cDNA Encoding a Two-Fingered C2H2 Zinc-Finger Protein from *Arabidopsis thaliana* (Accession No. AF030304).

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Plant Gene Register PGR98-074

Silvia Romo, Emilia Labrador and Berta Dopico (1998) Isolation and Characterization of a cDNA Encoding a Glyoxalase-I (Accession No. AJ224520) from *Cicer arietinum* L. Epicotyls Upregulated by Stress (PGR98-074) Plant Physiol. 117: 331

Isolation and Characterization of a cDNA Encoding a Glyoxalase-I (Accession No. AJ224520) from *Cicer arietinum* L. Epicotyls Upregulated by Stress

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The glyoxalase system comprises the enzymes glyoxalase-I (EC 4.4.1.5) and glyoxalase-II (EC 3.1.2.6). Glyoxalase-I catalyzes the formation of S-D-lactoylglutathione (S-LG) from methylglyoxal (MG) and glutathione (GSH). S-LG is further metabolized to D-lactate and GSH by glyoxalase-II (Thornalley, 1990). The glyoxalase pathway might have evolved to remove the MG that is unavoidably formed as a by-product of triosephosphate metabolism (Richard, 1993).

Rapidly proliferating cell lines have enhanced levels of glyoxalase enzyme activity. Thus, human colon carcinoma cells showed a 12-fold increase in glyoxalase-I transcript level over normal colon cells (Ranganathan *et al.* 1993). In plants, increased glyoxalase-I activity has been found in dividing cells, like those from cell suspension, calli, seedlings and root tips (Deswall *et al.* 1993, Paulus *et al.* 1993). In soybean cell suspension glyoxalase-I was induced by auxin, with a peak of maximum activity that preceded cell division (Paulus *et al.* 1993). Thus, the glyoxalase system has been also regarded as a marker for cell growth and division, although the causal relationship between glyoxalase activity and rapid growth has not been established.

The knowledge of the biological function of the glyoxalase system may come from the molecular cloning of their respective genes. Until now, only two cDNAs encoding a glyoxalase-I has been described in higher plants, the one of *Lycopersicon esculentum* (Z48183) and of *Brassica juncea* (Y13239). Here, we describe the cloning of a cDNA encoding a glyoxalase-I (CapGLX-I) from *Cicer arietinum* epicotyls. The chickpea cDNA was isolated as a clone whose expression increased when epicotyls were grown under osmotic stress conditions using PEG. The 805 bp insert contained an open reading frame of 558 bp. The protein is 186 amino acids in length, and showed extensive sequence similarity to glyoxalase-I from *L. esculentum* and *B. juncea* (81.1% homology to both). Also, the chickpea glyoxalase-I shares 53.3 % homology with the human glyoxalase-I. The increase in the expression of chickpea CapGLX-I under osmotic stress conditions induced by PEG is consistent with the upregulation by stress conditions (salt stress, water deficit, ABA treatment) described for tomato GLX1 (Espartero *et al.* 1995). As in tomato, it could be suggested that the increased expression of glyoxalase-I may be linked to a higher demand for ATP generation and to enhanced glycolysis in stressed plants.

Glyoxalase-I has been described as a metalloprotein containing one Zn atom that is essential for activity (Mannervik and Ridderstrom, 1993). Zinc forms complexes with three amino acid, the first two ligands are separated by 1-3 aminoacids, and are followed by a third ligand 20-120 amino acids downstream (Vallee and Auld, 1990). Usually His and Glu participate in zinc binding. Thus, as referred in tomato glyoxalase-I (Espartero *et al.* 1995), the zinc binding domain of chickpea glyoxalase-I might be formed by residues Glu-97, His-100 and His-125 (numbering referred to the *Cicer* sequence), since they are in a highly conserved regions and have the appropriate spacing.

Acknowledgements

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Table I. Characteristics of CapGLX1 from *Cicer arietinum*

Organism:

Cicer arietinum L cv. castellana

Clone Type, Designation:

cDNA, full length, CapGLX-I

Source:

cDNA library in lambda-ZAP constructed from poly A+ RNA from *Cicer arietinum* 4-day-old epicotyls treated during the last 24 h with PEG.

Method of isolation:

Differential screening. Replicas of the library were hybridized to labelled cDNA prepared from 4-day-old chickpea epicotyls growing in water or 3-day-old chickpea epicotyls followed by 24 h of polyethylenglycol (PEG) treatment.

Gene identification:

Nucleotide and amino acid sequence comparisons to published sequences in GenBank and EMBL data bases and Swiss-Prot and Blitz data bases respectively.

Feature of the cDNA

The clone is 795 bp in length, including a complete ORF of 558 bp, 57-bp 5' untranslated sequence and 180-bp 3' untranslated sequence.

Features of deduced protein:

The ORF encodes a 186 amino acid polypeptide. The encoded protein has a predicted molecular mass of 21 kD, and an isoelectric point of 5.21. Residues Glu-97, His-100 and His-125 might form the zinc-binding domain.

Gene product:

A glyoxalase-I; lactoyl glutathione lyase.

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