

Original article:

**A NEW MEMBER OF TAU-CLASS GLUTATHIONE S-TRANSFERASE
FROM BARLEY LEAVES**

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ABSTRACT

Glutathione S-transferase is a family of multifunctional detoxification enzymes which are mainly cytosolic that detoxify natural and exogenous toxic compounds by conjugation with glutathione. Glutathione, an endogenous tripeptide, is important as either a reducing agent or a nucleophilic scavenger. This molecule alleviates the chemical toxicity in plants by reaction of glutathione S-transferase, and its conjugates can be transported to vacuole or apoplast. The plant soluble glutathione S-transferases grouped today into seven distinct Phi, Tau, Zeta, Theta, lambda, dehydroascorbate reductase, and tetrachlorohydroquinone dehalogenase classes. In this study, bioinformatics analysis of glutathione S-transferase gene in barley was carried out using Tau-class of barley glutathione S-transferase sequences in NCBI GenBank and isolated sequence. DNA extraction, primer design, PCR, electrophoresis, column purification, DNA sequencing and analysis by some software led to identify new sequences of Tau-class of glutathione S-transferase from barley, which is similar to Tau GST of the diploid wheat. Comparison of the deduced amino acid sequences of the three barley GST genes showed that they have 99 % identity with each other but only 45 % identity with the new GST. This sequence was submitted to NCBI GenBank with F1131240 accession number.

Keywords: glutathione S-transferase, Tau-class, glutathione, barley, detoxification

INTRODUCTION

Glutathione S-transferases (GSTs) belong to multigene families common to all plants (McGonigle et al., 2000; Edwards & Dixon, 2005). They are well known for their responses to numerous endogenous and xenobiotic stresses for example their roles in herbicide detoxification (Cho & Kong, 2007). GSTs are mostly soluble cytoplasmic enzymes, but microsomal isoforms also known in both plants and animals (Board et al., 2000). The plant GST soluble enzymes according to their sequence relatedness, immunological cross-reactivities, kinetic properties and genome organizations are grouped today into seven distinct Phi, Tau, Zeta, Theta, Lambda, dehydroascorbate reductase (DHAR), and tetrachlorohydroquinone dehalogenase

(TCHQD) classes. In plants, GSTs can compose up to 2 % of soluble proteins (Reinemer et al., 1996; Oztetik, 2008). Genomics projects and gene analysis indicate that plants have more than 40 genes coding for GSTs and that the proteins share as little as 10 % amino acid identity (Thom et al., 2002).

Each soluble GST is a dimer of approximately 26 kDa subunits, typically forming a hydrophobic 50-kDa protein with an isoelectric point in the pH range 4-5. Each subunit contains two distinct domains (the N-terminal domain and C-terminal domain), and has an active site consisting of a GSH-binding site (the G-site) and a hydrophobic substrate binding site (the H-site). In the case of Phi and Tau GSTs, only subunits from the same class will dimerize. Within a class, however, the subunits can

dimerize even if they are quite different in amino-acid sequence (Dixon et al., 2003). GST enzymatic activity could involve direct glutathione conjugation to toxic electrophilic molecules, or glutathione-dependent peroxidase activity, using glutathione as reductant for the detoxification of toxic oxygen species, oxygen radicals, and lipid peroxides formed during or after plant stress (Dixon et al., 1998; Kampranis et al., 2000; Mueller et al., 2000; Kilili et al., 2004; Edwards & Dixon, 2005).

In both *Arabidopsis* and maize 28 Tau GST genes have been identified (McGonigle et al., 2000; Wagner et al., 2002), and 20 and 40 in soybean and rice, respectively (McGonigle et al., 2000; Soranzo et al., 2004).

The GSTs of Phi and Tau classes, which are plant-specific and the most abundant, are chiefly involved in xenobiotic metabolism for example catalyzing the detoxification of herbicides in crops and weeds and play important roles in plant development, stress tolerance and secondary metabolism (Neuefeind et al., 1997; Dixon et al., 2003; Edwards & Dixon, 2005; Cho & Kong, 2007). Members of Tau GSTs overlap in function, differ in their substrate specificity, thus providing the plant a broad range of protection, although the molecular mechanisms for various functions are largely unrevealed. It has shown that GST function cannot infer from their high sequence similarity to other members of known function (McGonigle et al., 2000). Thus, to understand the evolution of GSTs in structure and function, each member should be characterized individually by biochemical and genetic analysis. In this study, we isolate and characterize a new member of Tau-class GST from barley leaves.

MATERIALS AND METHODS

Plant material

The experiment performed using Karoun cultivar of an Iranian barley (*Hordeum vulgare* L.). Seedlings were grown from seeds which had been surface-

sterilised by shaking in 20 % sodium hypochlorite for 10 min followed by repeated washes in sterile water. Seeds were germinated at 22 °C in the dark in sterile tissue culture pots containing two layers of filter paper moistened with 6 ml of sterile water.

DNA extraction and PCR amplification

Seedling leaves (200 mg) were harvested from 7-d-old plants and ground to a fine powder in liquid nitrogen. DNA was extracted using modified CTAB method (Sumer et al., 2003). The primers 5'-AAGGGCCTGAGCTACGAG-3' as forward primer and 5'-TGCTGGCGGCTCACTTG-3' as reversed primer were designed to amplify Tau-class of GST DNA sequence from barley leaves. The PCRs were carried out in 20 µL solution comprising 10 ng of samples cDNA, 1 x PCR buffer, 1.5 mM MgCl₂, 0.25 mM each dNTP, 2 µM of each primer, and 1 U *Taq* DNA polymerase. The PCR profiles were an initial denaturation at 94 °C for 5 min followed by 35 amplification cycles (94 °C for 1 min, 52 °C for 1 min, and 72 °C for 1 min) and final extension at 72 °C for 10 min. The PCR amplification products were separated in 1 % (w/v) agarose gel.

Sequence analysis

PCR products were purified using High Pure PCR Product Purification Kit (Roche), and then sequenced (MWG, Germany). Phylogenetic relationships among the GSTs were evaluated using the neighbour-joining method (Saitou & Nei, 1987). Some software such as Prodom, Block, Pfam, SMART, CDART and Prints were used for bioinformatics analysis. The DNA sequence was translated to protein by Transeq software. N- and C-terminal of deduced amino acid sequence analysis was carried out by PROSITE database. Alignment of sequences were carried out using ClustalW algorithm in BioEdit software (Thompson et al., 1994; Hall, 1999).

RESULTS

The 484-bp sequence was obtained from sequencing report and submitted to NCBI GenBank with FI131234 accession number. Protein BLAST and secondary protein databases search showed that this barley sequence is partial of Tau-class glutathione S-transferase. N- and C-terminal analysis of deduced amino acids sequence by bioinformatics tools showed that the sequence is Tau-class of GST. Figure 1 shows alignment of deduced GST protein sequence from Karoun cultivar with three-barley GST protein of Tau-class in NCBI GenBank. Phylogenetic tree of the 4-Tau GST protein sequences of barley contains Karoun cultivar sequence and one

Karoun cultivar sequence and one Tau-class GST protein sequence from *Aegilops tauschii* seen in Figure 2. According to BLAST search, Karoun cultivar GST sequence has 90 % identity with 72 % coverage and E value $1e-123$ to *Aegilops tauschii* Tau-class GST gene. Comparison of the deduced amino acid sequences of the three barley GST genes showed that they have 99 % identity with each other but only 45 % identity with the Karoun GST. Domains of GST protein were confirmed by ProDom database. Bioinformatics analysis showed that the protein sequence has two β -sheets, eight α -helix and some intermediate loops in its secondary structure.

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      10      20      30      40      50
Karoun  -----DLLLGSNPVHK
ECGST  MAGEKGLVLLDFWVSPFGQVRVIALAEKGLPYEYVEEDLIAGKS.R.R.....
SIGST  MAGEKGLVLLDFWVSPFGQVRVIALAEKGLPYEYVEEDLIAGKS.R.R.....
IPGST  MAGEKGLVLLDFWVSPFGQVRVIALAEKGLPYEYVEEDLIAGKS.R.FR.....

      60      70      80      90     100     110
Karoun  KIPVLIHNGVPVCESMIIVQYIDEAFAGTGPPILPADPHERAVARFWAVYVDDKL
ECGST  .....L.D.R..N..L..LN.L.D..P-DA.AL..S..YA..Q.....D...K.V
SIGST  .....L.D.R..N..L..LN.L.D..P-DA.AL..S..YA..Q.....D...K.V
IPGST  .....L.D.R..N..L..LN.L.D..P-DA.AL..S..YA..Q.....D...K.V

     120     130     140     150     160
Karoun  VSQRTNSRSRLTGEDKATEAMKEMLLAVQTLLEGALRECSGKGFFGDVRVGLVDI
ECGST  YDCG.R-LWKVK..PQ.-Q.RA...EILK..D...GD----P.....KF.F..A
SIGST  YDCG.R-LWKVK..PQ.-Q.RA...EVLK..D...GD----P.....KF.F..A
IPGST  YDCG.R-LWKVK..PQ.-Q.RA...EVLK..D...GD----P.....KF.F..A

     170     180     190     200     210     220
Karoun  ALGSMHSWLMAIEAMSGAKVFDPARTPLLAAWMERFGSLDT-----
ECGST  .FAPFTA.FHSY.RYGEFSLAEV...KI...AK.C.ERESVAKSLYSPEKVYDF
SIGST  .FAPFTA.FHSY.RYGEFSLAEV...KI...AK.C.ERESVAKSLYSPEKVYDF
IPGST  .FAPFTA.FHSY.RYGEFSLAEV...KI...AK.C.ERESVAKSLYSPEKVYDF

      230
Karoun  -----
ECGST  IGLLKKRYGIE
SIGST  IGLLKKRYGIE
IPGST  IGLLKKRYGIE

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Figure 1: Alignment of Karoun cultivar GST protein sequence with three-barley GST protein of Tau-class from NCBI GenBank. Dots show similarity of amino acid and dashes show gaps.

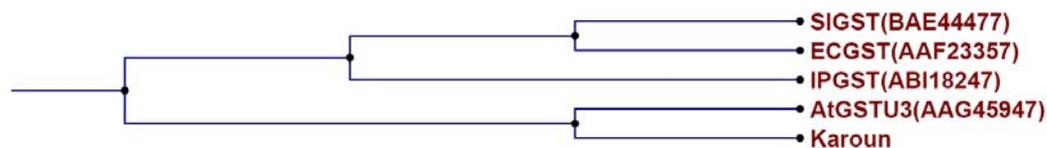


Figure 2: Unrooted neighbour-joining phylogenetic tree obtained for the 4-Tau GST protein sequences of *Hordeum vulgare* contain deduced Karoun cultivar sequence and one Tau GST protein sequence from *Aegilops tauschii*.

DISCUSSION

GSTs comprise a widespread, multi-functional gene superfamily that was thought to have evolved from ancient stress-related proteins (Sheehan et al., 2001), but now the plant GSTs are different in structure and function. The plant GSTs including Tau-class structure, function, genome organization and evolution have been the subject of several reviews (Sheehan et al., 2001; Dixon et al., 2002; Edwards & Dixon, 2005; Oztetik, 2008).

Bioinformatics analysis contains BLAST search, nucleotide and protein sequence alignment, phylogenetic tree, and confirmation by some secondary protein databases proved that Karoun cultivar sequence is a new member of Tau GST protein from barley, which is similar to Tau GST protein of the diploid wheat, *Triticum tauschii* (synonymous with *Aegilops tauschii*). As the figures showed, Karoun cultivar sequence is quite different from three-barley GST protein of Tau-class. Xu and co-workers (2002) reported that the Tau GST nucleotide sequence from *T. tauschii* has no intron. Our Karoun cultivar sequence is also without intron.

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