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Isolation and characterization of Phi class glutathione transferase partial gene of Iranian barley

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A B S T R A C T

Glutathione transferases are multifunctional proteins involved in several diverse intracellular events such as primary and secondary metabolisms, signaling and stress metabolism. These enzymes have been subdivided into eight classes in plants. The Phi class, being plant specific, is the most represented. In the present study, based on the sequences available at GenBank, different primers were designed for amplifying the Phi class of glutathione transferase gene in the genome and transcriptome of Iranian barley, Karoun cultivar. After extraction of DNA and total RNA, Phi class was amplified and sequenced. Bioinformatics analysis predicted that the deduced protein sequence has two ß-sheets, eight α-helices and some intermediate loops in its secondary structure. Consequently, the sequences were submitted to NCBI GenBank with GS262333 and GW342614 accession numbers. Phylogenic relationships of the sequences were compared with existing sequences in GenBank.

Key words: *Hordeum vulgare*, Glutathione S-transferase, Stress, Xenobiotics

INTRODUCTION

Glutathione S-transferases (GSTs; EC 2.5.1.18) are an ancient and diverse protein family, existing as multi-gene families [1-3]. GST activities have been identified in most eukaryotes and prokaryotes analyzed to date [3, 4]. A vast diversity of GSTs allows the enzyme superfamily to perform various enzymatic and non-enzymatic functions. As an example, GSTs comprise a number of various isoforms that recognize at least 100 different xenobiotic chemicals [4, 5]. The GST superfamily in plants is subdivided into eight classes, seven of which (Phi, Tau, Zeta, Theta, Lambda, DeHydro Ascorbate Reductase (DHAR), and TetraChloro Hydro Quinone Dehalogenase (TCHQD) are soluble and one microsomal. In plants, GSTs can compose up to 2%

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of the soluble proteins [6, 7]. Gene analysis and genomic projects indicate that plants have more than 40 genes coding for GSTs and that the proteins share as little as 10% amino acid identity [3, 8]. Since their identification in plants in 1970, these enzymes have been well established as phase II detoxification enzymes that perform several other essential functions in plant growth and development [9]. The GST of Phi class, which is plant-specific and the most abundant, is chiefly involved in xenobiotic metabolism.

GSTs catalyze the transfer of the tripeptide glutathione (γ-glutamyl-cysteinyl-glycine; GSH) or natural homologues, such as homoglutathione (γ-glutamyl-cysteinyl-β-alanine) and hydroxymethyl glutathione (γ-glutamyl-cysteinyl-serine), which are commonly found in legumes and grasses respectively, to a co-substrate (R-X) containing a reactive electrophilic center to form a polar S-glutathionylated reaction product (R-SG) [1, 2, 10].

Plant GSTs, induced by factors that cause oxidative membrane damage such as wounding, pathogen attack, senescence, and certain xenobiotics, strongly suggest the function of plant GST isoenzymes against oxidative stress and/or in plant-pathogen interactions [6]. The diverse physiological functions performed by GSTs have direct cytoprotective activities and might be essential to preserve plants during environmental stress and disease, as well as supporting their normal development. Because of the extreme divergence among GSTs, sequence analysis alone cannot reveal their function. Manipulation of the GSTs' expression in plants will allow future investigations to test these models of the GST function in vivo. Phi- and Tau-class GSTs, which are specific to plants, are the most abundant, conjugate a diverse array of xenobiotics, and influence the effects (e.g., selectivity) of herbicides on crops and weeds. This study is the first research regarding the isolation and characterization of Iranian Phi class of GST in barley. The aim is to isolate and characterize Phi class glutathione transferase gene of Iranian barley in order to improve the quality and quantity of the crop via gene transfer.

MATERIALS AND METHODS

Plant material: The experiment was performed using the Karoun cultivar of 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 sterile water.

DNA and total RNA extraction and PCR: 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 [11]. The primers 5´-ATGGCGCCGGTGAAGGTGTAC-3´ (as forward primer) and 5´-ACCAGGCCTTCACGTGCGG-3´ (as reversed primer) were designed to amplify Phi-class of GST DNA sequence from barley leaves. The PCRs were carried out in a 20-µL solution comprising 10 ng samples of 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, 45 °C for 1 min, and 72 °C for1 min) and a final extension at 72 °C for 10 min. The PCR amplification products were separated in 1% (*w*/*v*) agarose gel. Extraction of total RNA was carried out after herbicide treatment (100 µM alachlor). Total RNA was extracted using Aurum ™ Total RNA Mini Kit (Bio-RAD). A single strand of cDNA was synthesized from extracted RNA and by a cDNA

Synthesis system Kit (Roche). The cDNA template was then amplified using a gene-specific primer and the anchor primer with oligo(dT). The housekeeping gene, actin, was used as the internal standard for reverse transcriptase polymerase chain reaction (RT-PCR).

Sequence analysis: PCR products were purified using High Pure PCR Product Purification Kit (Roche), and were sequenced subsequently (MWG, Germany). Phylogenetic relationships among the GSTs were evaluated using the neighbor-joining method [12]. Software such as Prodom, Pfam and CDART were used for bioinformatic analysis. The DNA sequence was translated to protein by Transeq software. Alignment of sequences was carried out by ClustalW algorithem in BioEdit software [13, 14].

RESULTS

The 456-bp genomic DNA and 689-bp cDNA sequences were obtained from sequencing reports and submitted to NCBI GenBank with GS262333 and GW342614 accession numbers, respectively. A search in Protein BLAST and secondary protein databases showed that this barley sequence is partial Phi-class GST. N- and C-terminal analyses of the sequence of deduced amino acids obtained by bioinformatic tools showed that the sequence is Phi-class of GST. Figure 1 and 2 show the alignment of the deduced GST protein sequence from Karoun cultivar with other proteins of Tau, Zeta and Phi classes in the NCBI GenBank. According to the BLAST search, genomic GST sequence of Karoun cultivar has 100% identity with 92% coverage and an E value of 5e-45 compared to other *H. vulgare* Phi-class GST gene. The domains of the GST protein were confirmed by ProDom database. Bioinformatics analysis predicted that the protein sequence has two ß-sheets, eight α-helices and some intermediate loops in its secondary structure.

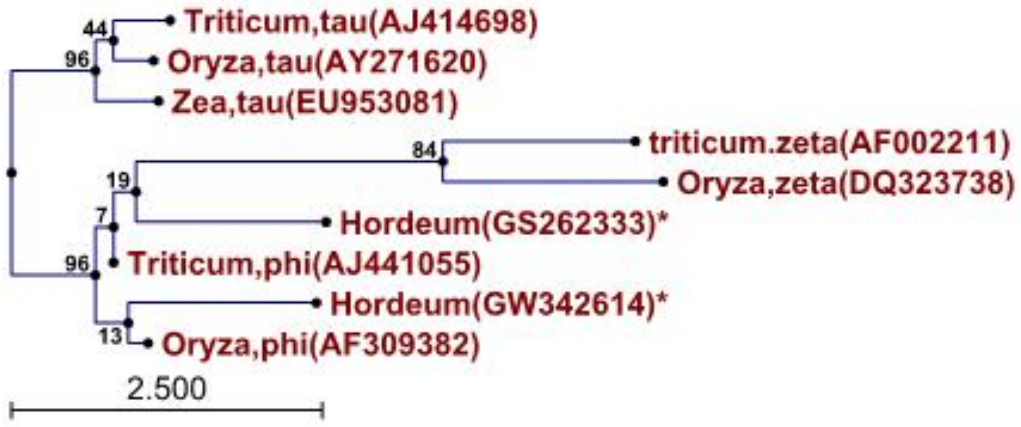

Figure 1: Unrooted neighbour-joining phylogenetic tree obtained for the Tau, Zeta and Phi-class of GST protein sequences of some members of poaceae family in NCBI GenBank contain studied deduced Karoun cultivar sequence marked with asterisk.

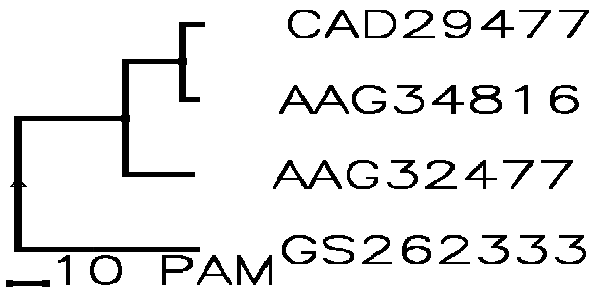

Figure 2: Phylogenetic relationships between GS262333 (*Hordeum vulgare* cv. Karoon deduced protein sequence of GST Phi-class) and some Phi class sequences of plant GSTs in NCBI GenBank including CAD29477 (*Triticum aestivum*), AAG34816 (*Zea mays*) and AAG32477 (*Oryza sativa*). The tree has drowned by Multalin software.

DISCUSSION

GSTs comprise a widespread, multifunctional gene superfamily thought to have evolved from ancient stress related proteins [7, 15]. The plant GSTs including Phi-class structure, function, genome organization and evolution have been the subject of several reviews [1, 8, 15-17]. Phi class includes many first plant GSTs identified for herbicide detoxifying activities. It has been shown that their genes contain two introns in conserved positions [18].

In addition, GST can be a flavonoid-binding protein, having been proposed that it serves as a cytoplasmic flavonoid carrier protein in vivo [19]. GST can also be involved in signaling to chalcone synthase in the cell [20].

The obtained partial Phi class cDNA was composed of a 689-bp open reading frame encoding about 229 amino acids, but different from the DNA sequence found. Two sequences were confirmed to be Phi classes of GST by BLAST search and showed to be polymorphisms of Phi class of GST in barley. Other researchers have also identified Phi class polymorphism. They have found 13, 25, 12 and 16 genes of Phi class in *Arabidopsis*, wheat, maize and rice, respectively [21- 24].

In all analyzed species, a significant subset of GSTs is expressed uniquely or predominantly upon induction by specific factors. Among the first identified are safeners, compounds that enhance the capacity of some crops to detoxify herbicides by increasing the synthesis and activity of metabolizing enzymes, such as GSTs. GST induction by safeners or by the herbicide itself is now well documented in cereals, some weeds and Arabidopsis, and plays a primary role in herbicide tolerance [25- 28].

Gene identification and isolation can help researchers improve the quality and quantitaty of crops via gene transfer. Increasing the gene expression of Phi class of the GST enzyme using gene transfer can help plants, especially cereals, to acclimate to environmental stresses. The full length of the found cDNA is needed for future genetic engineering. The ß-sheets and α-helices' secondary structure is only a prediction by bioinformatics data from the NCBI GenBank, and X-ray crystallography is needed for further certification.

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CONFLICTS OF INTEREST: None

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