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ZmGT1 Transports Glutathione Conjugates and Its Expression Is Induced by Herbicide Atrazine

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Abstract Reduced glutathione (GSH) plays an important role in plant resistance to biotic and abiotic stresses. A putative glutathione transporter gene, *ZmGT1*, was cloned from maize (*Zea mays*) using RACE-PCR. The deduced *ZmGT1* protein is highly homologous to glutathione transporters from other plants. A yeast mutant (*hgt1*Δ) deficient in glutathione transport was used to characterize the physiological functions of this gene. Complementation by *ZmGT1* restored growth of the *hgt1*Δ mutant strain on a medium containing GSH as the sole sulfur source and also mediated the uptake of a model glutathione conjugate, GS-N-ethylmaleimide (GS-NEM). *ZmGT1* was expressed in all organs of maize seedlings, with a higher level of transcript being found in leaves. The expression of *ZmGT1* was strongly induced by atrazine with a 4～5 fold increase in transcript level being detected in leaves after 96 h treatment. The strong up-regulation of *ZmGT1* by atrazine suggests that this glutathione transporter may be involved in the detoxification of xenobiotics.

Key words detoxification, glutathione conjugation, herbicides, atrazine
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The widespread use of pesticides is causing serious environmental problems, including water and soil contamination. Phytoremediation has been studied as a potential method to restore the polluted environment. This technique relies on the use of plants that are able to accumulate and compartmentalize high concentrations of organic xenobiotics [1–2]. Although plants possess highly efficient root systems for the removal of xenobiotics from soil and groundwater, detoxification and degradation of these compounds within plant cells is a prerequisite for the success of this technique.

Metabolism and detoxification of xenobiotics in plants can generally be divided into three phases. In phase Ⅰ, the xenobiotic may be oxidized, reduced or hydrolysed to introduce or reveal a functional group. In phase Ⅱ, the xenobiotic or activated xenobiotic is conjugated to glutathione, glucose or malonate by the respective transferases to form a water-soluble conjugate. In phase Ⅲ, conjugates of xenobiotics are removed from the cytosol and transferred to the large central vacuole for further degradation[3–5].

Glutathione is a tripeptide (γ-glutamyl-cysteinyl Gly) synthesized in both the cytosol and chloroplasts of plant cells. Reduced glutathione (GSH) is the most abundant source of non-protein thiols in plant cells[6]. It plays numerous roles in plants including detoxification of endogenously and exogenously derived toxins, storage and transport of reduced sulfur, control of sulfur assimilation, control of redox status and protection against biotic and abiotic stresses[7–9].

Glutathione conjugation reactions play a major role in plant resistance to xenobiotics, such as heavy metals [9–10], and herbicides [11]. Conjugation reactions between electrophilic compounds and glutathione detoxify a broad spectrum of compounds used in agriculture and industry including triazine herbicides [12], nitroaromatic compounds [13], chloroacetanilide herbicides [14], sulfonylurea herbicides [15],

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diphenyl ether herbicides[16-17], polyaromatic hydrones[18], pyrrolizidine alkaloids[19].

Glutathione reactions with activated xenobiotic compounds are catalyzed by glutathione S-transferase enzymes to form various glutathione conjugates (GS conjugates). Increases in glutathione, glutathione reductase, and glutathione S-transferase levels have been linked to plant resistance and adaptation to a variety of physical and chemical stresses encountered in the environment[20]. However, accumulation of the resulting metabolites in cells can lead to a decrease in the detoxification activity of the phase Ⅱ system. Indeed, several GS conjugates have been found to inhibit both glutathione S-transferases and glutathione reductase activities[3]. It is generally believed that the various GS conjugates are transferred into the vacuole for storage or further degradation. Although the biochemical and molecular basis of GS conjugate transport into the vacuole has been extensively described [21], relatively little is known about the possible carrier of GS conjugates located in the plasma membrane, which mediates the transport of such compounds from apoplast to symplast and between different tissues and cells.

The first eukaryotic glutathione transporter, named *HGT1*, was cloned from *Saccharomyces cerevisiae*[22]. It exhibited a high affinity for GSH and a GS conjugate, GS-N-ethylmaleimide (GS-NEM). Subsequently, genes for glutathione transporters were isolated and characterized from *Brassica juncea* (*BjGT1*)[23], *Oryza sativa* (*OsGT1*) [24] and *Arabidopsis thaliana* (*AtOPT6*) [25]. When expressed in a yeast mutant deficient in glutathione transport, all of the plant glutathione transporters were able to confer the yeast mutant the ability to take up GSH and GS conjugates from the medium. In the present paper, we report the molecular cloning of a glutathione transporter from a major crop plant, maize (*Zea mays*); functional analysis of this gene using a yeast complementation technique; and expression analysis in different organs of maize plants as well as in response to herbicide treatment. Our findings provide new insights into the possible role of glutathione transporters in the detoxification of xenobiotics.

1 Materials and methods

1.1 Plant material

Maize (*Zea mays*, cv Nongda 108) plants were grown on sand in the glasshouse using the method described by Schafer *et al*[26]. Plants grown to the 3-leaf stage were transferred to a hydroponic culture (three plants per pot). Each pot contained 50 ml of nutrient solution. The nutrient solution comprised of macronutrients: 1 150 mg/L $Ca(NO_3)_2 \cdot 4H_2O$; 200 mg/L KNO_3; 200 mg/L KH_2PO_4; 200 mg/L $MgSO_4 \cdot 7H_2O$; and micronutrients: 30 mg/L EDTA-2NaFe; 2.86 mg/L H_3BO_4; 2.13 mg/L $MnSO_4 \cdot 4H_2O$; 0.22 mg/L $ZnSO_4 \cdot 7H_2O$; 0.08 mg/L $CuSO_4 \cdot 5H_2O$; 0.012 mg/L $Na_2MoO_4 \cdot 2H_2O$. Plants were adapted for 7 d and the nutrient solution was changed every 2 d. After the 7 d adaptation period, atrazine (90% water dispersible granule, Welex SA Holding China Ltd), a herbicide of the triazine family, was added into the nutrient solution to a final concentration of 250 μmol/L and the nutrient solution was changed daily. At the end of the treatment, leaves were harvested separately, immediately frozen in liquid nitrogen, and stored at –80℃.

1.2 RNA isolation

Frozen tissues of maize seedlings were ground into a fine powder in liquid nitrogen. Total RNA was isolated from roots, stems and leaves using TRIzol reagent (Invitrogen, USA), and then digested by DNase Ⅰ (TakaRa, China). First strand cDNA was synthesized with AMV reverse transcriptase and Oligo $(dT)_{15}$, according to the manufacturer's protocol of Reverse Transcription System (Promega, USA).

1.3 Cloning of *ZmGT1* cDNA

According to our previously isolated partial sequence of *ZmGT1* cDNA (AY387493, gi 37362135), a pair of primers (P1 5′ CGT CGA TGC CCG TGA TGA CAT 3′ and P2 5′ TCA AAA CAC CGG GCA GCC CTT 3′) was designed to amplify a 2 125-bp internal fragment of *ZmGT1* using maize leaf cDNA as a template. The amplification product was cloned into the pGEM-T Easy vector (Promega, USA) and sequenced. Based on the sequence of this fragment, which contained 3′ end of the ORF, but lacked the 5′untranslated and 5′ ORF regions, two gene specific primers, P3 5′ CTG CCT GTA CCT CTG CAT CAG CTT GG 3′ and P4 5′ GCT GAG CGT CTG CTT GTA GTA GGC C 3′ (nested primer), were designed to perform 5′ RACE-PCR. The 5′ end of the gene was cloned using the SMART™ RACE cDNA Amplification Kit (Clonetech, USA) and sequenced. The full length cDNA including 5′ untranslated region, ORF and 3′ untranslated region, was then re-amplified, re-sequenced and submitted to GenBank (FJ573212).

1.4 Yeast strain and growth medium

The yeast (*Saccharomyces cerevisiae*) strain ABC822 bearing a deletion in *HGT1* (Matα ura3-52 leu2-Δ1 lys2-801 his3-Δ200 trp1-Δ63 ade2-101 hgt1Δ:: LEU2)[22] is deficient in glutathione uptake and was used for complementation studies. The yeast expression (shuttle) vector used was pDR196[27]. Both the yeast strain ABC822 and the vector pDR196 were kindly provided by Andrée Bourbouloux (University of Poitiers, France).

Synthetic dextrose minus sulfur (SD-S) medium was prepared according to DIFCO's Bacto yeast nitrogen base without amino acids and ammonium sulfate (DIFCO Laboratories, Detroit). The only modification was that all sulfur-containing reagents in macroelements, microelements, and vitamins were substituted with equal amounts of the corresponding chloride salts [24]. The non-transformed yeast strain ABC822 and the same strain transformed with empty vector pDR196 (ABC822/pDR196) were grown in DIFCO's SD medium with ammonium sulfate, or in synthetic complete (SC) medium [28], which contained ammonium sulfate and sulfur amino acids.

1.5 Heterologous expression of *ZmGT1* in a yeast *hgt1*Δ mutant

Primers (P9 5′ CGA CTA GTT GGA GGG AGG GTG GGT AG 3′; P10 5′ TAG AAT TCG ACA AAG CAC TGC ATC GGT T 3′) and nested primers (P11 5′ CCA CTA GTA TGG CGT CGC GGA AGT C 3′; P12 5′ TAG AAT TCT CAA AAC ACC GGG CAG CC 3′) were designed to amplify the entire *ZmGT1* ORF and also introduce a *Spe* Ⅰ site upstream of the translation initiation codon ATG and an *Eco*R Ⅰ site downstream of the stop codon, TGA. Maize leaf cDNA was again used as a template. The amplified fragment was digested by *Spe* Ⅰ and *Eco*R Ⅰ, and directionally cloned into the yeast shuttle vector pDR196.

The yeast strain ABC822 was transformed with either the empty vector pDR196 or pDR196+*ZmGT1* plasmid by the LiOAc/polyethylene glycol method[29]. The transformants growing on a selection medium (SC) lacking uracil were further selected for growth on an SD-S medium containing 100 μmol/L GSH[24].

1.6 Yeast growth assay

For yeast growth assays in liquid and on solid media, the ABC822 strain transformed with either the empty vector pDR196 or pDR196 +*ZmGT1* plasmid was grown in SD medium to A_{600} = 0.6, washed 3 times in cold sterile water, and diluted to A_{600} = 0.001 with liquid SD-S medium containing 100 μmol/L GSH or inoculated on the solid SD-S medium containing 100 μmol/L GSH.

1.7 Transport experiments

Strains of ABC822/pDR196 and ABC822/pDR196+ *ZmGT1* were grown overnight to an A_{600} = 0.6 in liquid SD and SD-S medium, respectively. A [^{14}C]-labeled model GS-conjugated compound, [^{14}C]GS-NEM was prepared using GSH and [^{14}C]-NEM (specific activity: 1.23×10^3 GBq/mol, PerkinElmer, USA) following the method reported by Martinoia *et al*[30]. Uptake of [^{14}C]GS-NEM by the ABC822 yeast mutant strain was measured as described by Zhang *et al*[24]. Briefly, after a 5-min incubation of the yeast cells at 28℃ in the transport medium (pH 5.0) containing 20 mmol/L MES/KOH, 0.5 mmol/L $CaCl_2$, 0.25 mmol/L $MgCl_2$ and 2% glucose, [^{14}C]GS-NEM was added to the buffered transport medium to a final concentration of 100 μmol/L GS-NEM (radioactivity: 18.5×10^3 kBq/L). At selected time intervals, uptake was terminated by diluting the medium with a 20-fold volume of water (4℃), and cells were collected by vacuum filtration using glass fiber filters (GF/C; Whatman) and washed 3 times with the same volume of cold water. The filter was dried, and the radioactive GS-NEM retained was measured by liquid scintillation counting.

All uptake experiments were conducted with 3 replicates and the results expressed as nmol of GS-NEM absorbed per mg of protein. Protein content was measured by the method of Bradford [31] using bovine serum albumin as a standard.

1.8 Quantitative real-time RT-PCR

A primer pair (P5 5′ CGT GGG ATT CGT CAT GTT C 3′ and P6 5′ GCT TCC CGT AGC TGT CGT AG 3′) was designed from the ORF region of the *ZmGT1* sequence to amplify a single 191-bp fragment. Another pair of primers (P7 5′ CTA CGA CTG CTG AGC GAG AA 3′ and P8 5′ GGA TGG CTG GAA TAG AAC CT 3′) was used to amplify a 194-bp fragment from maize actin gene (*ZmACT1*, accession number DQ492681.1), chosen as an internal reference. Quantitative real-time RT-PCR was performed with four technical replicates on a DNA Engine Option™ 2 (MJ Research Co., USA) following the manufacturer's recommendations. The real-time RT-PCR reactions were performed in a final volume of 25 μl containing 2.5 μl 10×PCR buffer (Mg^{2+} Plus), 2 μl dNTP mixture (10 mmol/L), 1 μl of each primer (10 μmol/L), 1 μl

cDNA (equivalent to 0.5 ng DNA), 0.8 μl SYBR Green Ⅰ (*v*/*v*, 1∶1 000), 0.125 μl TaKaRa Taq HS (5 U/μl) and 16.575 μl ddH_2O. The PCR program consisted of 95℃ for 5 min, followed by 40 cycles of 95℃ for 30 s, 56℃ for 30 s, 72℃ for 30 s, and 1 s at 81℃ for plate reading. After the cycling protocol, the final step was applied to all reactions by continuously monitoring fluorescence while increasing the temperature at a rate of 0.1℃/s to generate a melting curve.

Relative quantification method using C_T values and efficiency correction was adopted to estimate the gene expression levels [32]. The final results were expressed as a fold change relative to control. Due to the large variation of real-time PCR results, all experiments were conducted three times using different RNA preparations.

1.9 Statistic analysis

Data were analyzed by SAS (version 6.12).

2 Results

2.1 Functional complementation of *ZmGT1* in the yeast mutant

As the *HGT1* gene encoding the glutathione transporter is disrupted, the ABC822 strain of *S. cerevisiae* is unable to grow on a medium containing GSH as the sole source of sulfur [22]. Heterologous expression of *ZmGT1* restored the growth of ABC822 cells in a liquid medium (Figure 1a), as well as on a solid medium (Figure 1b) each containing 100 μmol/L GSH as the sole sulfur source. This indicates that *ZmGT1* is functional in yeast and confers the yeast mutant strain the ability to take up GSH from the media, and to use it as a source of reduced sulfur in its metabolism. As expected, this same yeast strain transformed with the empty pDR196 vector was not able to grow in the presence of GSH as the unique sulfur source (Figure 1a, 1b).

2.2 *ZmGT1*-mediated uptake of GS-NEM by the yeast mutant

The above growth assays indirectly showed that the *ZmGT1* protein can transport GSH across the yeast plasma membrane. Further evidence that this transporter is able to mediate transport of GS conjugates was sought by studying the uptake of radiolabeled GS-NEM. When the ABC822 strain carried the pDR196+*ZmGT1* plasmid, [^{14}C]GS-NEM uptake into yeast cells was much faster compared to the same strain transformed with the empty vector (Figure 2).

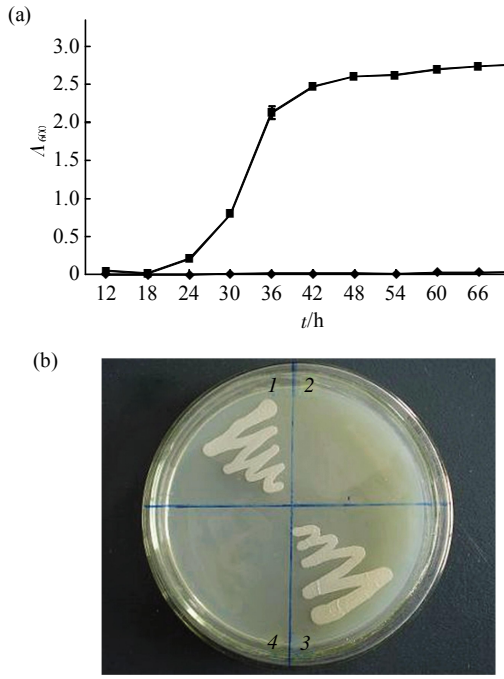

Fig. 1　Complementation of the yeast strain ABC822 (*hgt1*Δ) by *ZmGT1*

(a) ABC822 strain transformed with either the empty vector pDR196 or the pDR196+*ZmGT1* plasmid was grown in synthetic dextrose (SD) medium to A_{600} = 0.6, washed three times in cold sterile water, and diluted to A_{600} = 0.001 in synthetic dextrose minus sulfur (SD-S) medium containing 100 μmol/L GSH. ♦—♦: pDR; ■—■: pDR196+*ZmGT1*. (b) Growth of ABC822 strain after transformation with the empty vector pDR196 (*2* and *4*) or pDR196+*ZmGT1* plasmid (*1* and *3*) on a medium containing 100 μmol/L GSH as the sole sulfur source.

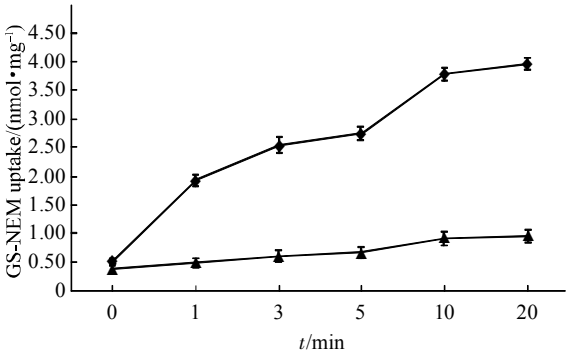

Fig. 2　Uptake of [^{14}C]GS-NEM (100 μmol/L) by the ABC822 strain transformed with either the empty vector pDR196 or the pDR196+*ZmGT1* plasmid

The uptake data are the $\bar{x} \pm s$ of three replicates. ▲—▲: pDR; ♦—♦: pDR196+*ZmGT1*.

[^{14}C]GS-NEM uptake mediated by *ZmGT1* in the ABC822 strain varied with the pH of the incubation medium. The uptake rate peaked at pH 5.0, and decreased at either a higher or lower pH (Figure 3).

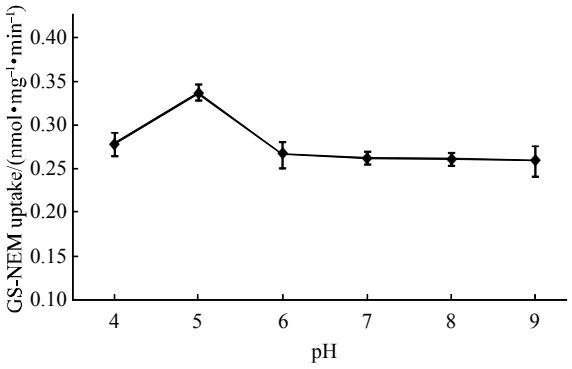

Fig. 3 Influence of medium pH on the uptake of [^{14}C]GS-NEM by the ABC822 strain transformed with the pDR196+*ZmGT1* plasmid

The buffered transport medium contains 20 mmol/L MES/KOH, 0.5 mmol/L $CaCl_2$, 0.25 mmol/L $MgCl_2$ and 2% glucose. Uptake was measured after 5 min incubation. Each data point represents the $\bar{x} \pm s$ of three replicates.

The *ZmGT1*-mediated GS-NEM uptake was also regulated by the available sulfur. When the ABC822 strain expressing *ZmGT1* was grown in liquid SD medium, which contained many sulfur compounds, the uptake rate of [^{14}C]GS-NEM by the yeast cells was very slow and only marginally faster than that observed for the non-complemented strain (Figure 4). By contrast, the uptake was strongly enhanced when the yeast strain carrying the *ZmGT1* gene was grown in SD-S medium where the only source of sulfur was glutathione.

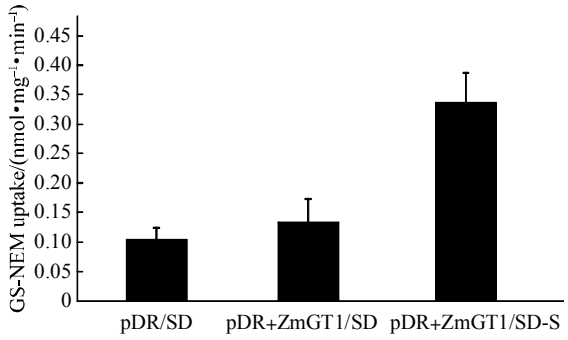

Fig. 4 Effects of sulfur compounds in the growth media on [^{14}C] GS-NEM uptake by the ABC822 strain transformed with either the empty vector pDR196 or the pDR196+*ZmGT1* plasmid

The strains were grown in SD or SD-S media, all containing 100 μmol/L GSH. Uptake was measured after 5 min incubation. Each column represents the $\bar{x} \pm s$ of three replicates.

2.3 Expression of *ZmGT1* in maize

Quantitative real time RT-PCR was used to evaluate *ZmGT1* expression in different organs of hydroponically grown maize seedlings. *ZmGT1* expression was detected in roots, stems and leaves of 3-leaf stage seedlings. The level of transcription in leaves was higher than that in roots or stems(Figure 5). Therefore, in the following experiments, *ZmGT1* expression in leaves of maize seedlings was further studied after herbicide application.

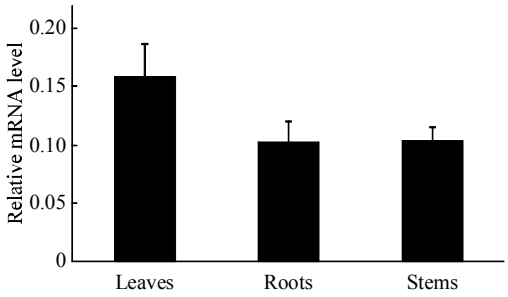

Fig. 5 Quantitative real-time RT-PCR analysis of *ZmGT1* expression in different organs of maize seedlings

Maize leaves, stems and roots were collected separately from hydroponically-grown plants at 3-leaf stage. *ZmGT1* transcript level in leaves, roots and stems was evaluated using real-time RT-PCR and normalized to the level of the maize actin gene (reference gene). Data are $\bar{x} \pm s$ of three experiments.

A time course study was designed to compare the gene expression in maize leaves at 48, 72, 96, 120 and 144 h after treatment by atrazine (250 μmol/L). Atrazine treatment did not significantly affect *ZmGT1* expression in maize leaves up to 72 h after application, but induced a 4～5 fold increase in transcript levels after 96 h, which was followed by a decline in expression (Figure 6).

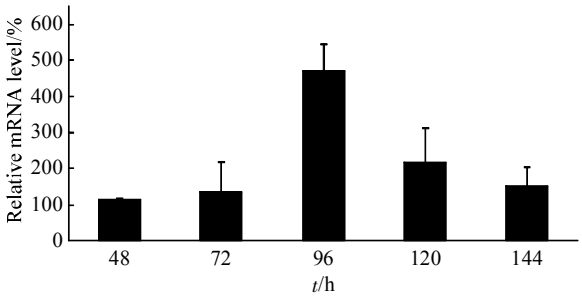

Fig. 6 Quantitative real-time RT-PCR analysis of *ZmGT1* expression in maize leaves at different intervals after the onset of atrazine treatment (250 μmol/L)

Maize leaves were collected from hydroponically-grown plants at 3-leaf stage, which had been treated by atrazine as a root drench. Gene expression in leaves in response to herbicide treatment was quantified using real-time RT-PCR. *ZmGT1* transcript level was normalized to the level of the maize actin gene (reference gene). The normalized transcript levels were then compared between treatment and control samples to obtain the fold change (transcript level in non-treated plants was defined as 100%). Data are $\bar{x} \pm s$ of three experiments.

3 Discussion

The glutathione conjugation reaction is one of the principal metabolic pathways that plants utilize to detoxify herbicides and heavy metals. Ascertaining the transport systems of GS conjugates in plants is important for a better understanding of the mechanisms involved in xenobiotic detoxification. Biochemical results showed that there are specific glutathione transport systems in plants[33], bacteria[34], yeasts [35] and mammalian cells [36]. The first glutathione transporter *HGT1* was successfully identified from yeast relatively recently[22], followed by the isolation of plant homologs from *Brassica juncea* (*BjGT1*) [23] and rice (*OsGT1*) [24]. These transporters all targeted the plasma membrane and mediated the uptake of both GSH and GS conjugates in yeast cells. In the present work, a novel glutathione transporter gene, *ZmGT1*, was cloned from maize. The *hgt1* yeast mutant complemented by *ZmGT1* can grow normally on medium with GSH as the sole source of sulfur. This indicates that *ZmGT1* is functional in yeast and is able to mediate GSH uptake (Figure 1), although direct measurement of GSH uptake was not undertaken in this work.

The ability of the ABC822/pDR196+*ZmGT1* yeast strain to absorb conjugated glutathione was clearly demonstrated through studying the uptake of [^{14}C] GS-NEM, a model GS conjugate. Compared to the yeast mutant strain transformed with the empty vector, the introduction of *ZmGT1* significantly increased the uptake of [^{14}C]GS-NEM by the mutant strain(Figure 2). After a 20-min incubation, the amount of [^{14}C] GS-NEM taken up by the ABC822/pDR196 +*ZmGT1* strain was almost four-fold of that measured for ABC822/pDR196 transformant. This suggests that *ZmGT1* is able to transport GS conjugates in the yeast mutant. A similar property has been reported for other plant glutathione or oligopeptide transporters, *OsGT1*, *BjGT1* and *AtOPT6*[23–25].

ZmGT1-mediated transport of GS conjugates by yeast cells is dependent on the pH of the medium, with the uptake reaching maximal at pH 5, similar to the result obtained for *BjGT1*-mediated GSH uptake [23]. This optimal pH value for *ZmGT1*-mediated uptake activity corresponds to the apoplast pH of plants, suggesting that this membrane carrier would be well suited to transport GS conjugates from the apoplast to cells of plant tissues.

Our results also resemble those obtained with *BjGT1* and *OsGT1* in that the *ZmGT1*-mediated uptake of GS-NEM was observed only when the yeast growth medium was free of any other sulfur source except glutathione [23–24]. When the ABC822 strain expressing ZmGT1 was grown in SD medium containing many sulfur compounds and amino acids, the uptake of [^{14}C] GS-NEM was very low and close to the background uptake value obtained with the ABC822/pDR196 strain (Figure 4). This suggests that the activity of *ZmGT1* in yeast is repressed when other sulfur sources are available.

The fact that the yeast mutant strain carrying ZmGT1 is able to grow normally on a medium containing GSH and take up [^{14}C]GS-NEM indicates that this transporter is able to transport both GSH and GS conjugates. That yeast cells expressing *ZmGT1* are able to take up GSH and GS-NEM from the incubation medium also implies that this protein targets the plasma membrane, distinct from another type of GS conjugate transporters of plants, the ATP-binding cassette (ABC) transporters, which are located in the tonoplast and are active in the transport of GS conjugates from the cytosol to the vacuole[21].

Although real-time RT-PCR revealed that *ZmGT1* is expressed in all organs examined, attempts to detect *ZmGT1* transcript by Northern blot proved unsuccessful (data not shown), indicating that under normal growth conditions *ZmGT1* is only weakly expressed. However, upon exposure to the herbicide atrazine gene expression was greatly enhanced. It is known that ABC transporters are involved in the detoxification of xenobiotics by transporting GS conjugates into the vacuole and these tonoplast transporters are inducible by certain herbicides [37–38]. Atrazine is a selective herbicide for maize, and maize GSTs play a major role in the metabolism of this herbicide [12]. This work suggests that alongside GSTs and ABC transporters, *ZmGT1* is likely to take part in the whole detoxification process in the event of massive xenobiotic invasion.

The exact mode of action of *ZmGT1* is still to be elucidated. However, a xenobiotic-inducible GST has been observed in the apoplast of soybean hypocotyls[39]. Thus some of the GS conjugates might be formed in the apoplast that require uptake into the cell by a plasma membrane transporter for further processing. It is also possible that certain tissues or cells possess greater capacity for the storage and/or degradation of GS conjugates. *ZmGT1* might be required to facilitate

the intercellular or long distance transport of such compounds, thus avoiding local toxicity to tissues or cells directly exposed to xenobiotics. In this respect, it would be interesting to compare the expression pattern of GSTs, ABC transporters and *ZmGT1* upon treatment by the same herbicide.

Although atrazine is a selective herbicide for maize when applied at a defined dose, the amount of the herbicide reaching the site of action (chloroplasts) in this experiment may have easily exceeded the tolerated dose as the herbicide was added directly to the hydroponic medium. As a consequence, some physiological processes might be affected before the appearance of phytotoxic symptoms. This may explain why *ZmGT1* expression in leaves was enhanced temporarily and was not maintained beyond 96 h after onset of the herbicide treatment.

In summary, a novel glutathione transporter gene, *ZmGT1*, was isolated from maize, a plant in which glutathione conjugation is thought to be responsible for detoxification of several classes of herbicides. We demonstrated that *ZmGT1* is able to mediate the transport of both GSH and GS conjugates in yeast. The sharp increase in *ZmGT1* transcription in response to atrazine treatment suggests that this gene may be involved in the detoxification xenobiotics in plants, and may contribute to the tolerance of maize against triazine herbicides.

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ZmGT1 基因转运谷胱甘肽轭合物功能研究及除草剂阿特拉津诱导表达分析

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摘要　还原型谷胱甘肽在植物抵御生物压和非生物压过程中扮演着重要的角色．利用 RACE-PCR 技术从玉米体内克隆得到一个编码谷胱甘肽转运蛋白的基因 *ZmGT1*．利用缺失谷胱甘肽转运子基因的突变型酵母菌(*hgt1*Δ)研究 *ZmGT1* 基因的生理功能发现，*ZmGT1* 基因能够修复 *hgt1*Δ 突变型酵母菌在谷胱甘肽(GSH)作为唯一硫源的培养基中的生长，并且具有调控谷胱甘肽轭合物 GS-N-ethylmaleimide (GS-NEM)吸收的功能．*ZmGT1* 基因在玉米幼苗的各个不同器官均有表达，其中在叶片中的表达量更高．玉米 *ZmGT1* 基因能够被除草剂阿特拉津强烈地诱导，经过阿特拉津处理 96 h 后，玉米叶片中 *ZmGT1* 基因的表达量提高约 4～5 倍，该结果表明，谷胱甘肽转运蛋白在玉米解毒外源有害物质的过程中可能发挥着作用．

关键词　解毒作用，谷胱甘肽轭合物，除草剂，阿特拉津

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