Acta Biologica Hungarica 69(4), pp. 505–509 (2018)
DOI: 10.1556/018.69.2018.4.11

DIURNAL CHANGES IN TOMATO GLUTATHIONE TRANSFERASE ACTIVITY AND EXPRESSION

SHORT COMMUNICATION

Ágnes Gallé,[1,*] Zalán Czékus,[1] Krisztina Bela,[1] Edit Horváth,[2] Jolán Csiszár[1] and Péter Poór[1]

[1]Department of Plant Biology, Faculty of Science and Informatics, University of Szeged, Közép fasor 52, H-6726 Szeged, Hungary
[2]Institute of Plant Biology, Biological Research Centre, H-6726 Szeged, Hungary

(Received: June 28, 2018; accepted: August 30, 2018)

Although the participation of glutathione transferases (GSTs) in light-dependent pathways and the circadian changes in the whole detoxification system have been studied, there are fewer results regarding the exact daily fluctuation of GSTs. In the present study, it was demonstrated that light up-regulated, while dark period decreased the plant GST activity and the expression of the selected tau group GST genes in tomato. These findings provide additional information on our current knowledge on the circadian rhythm of GSTs in plants and could help in further defining detoxification processes.

Keywords: Circadian – cis-acting elements – darkness – glutathione transferase – light regulation

Through the effective detoxification system of plants, xenobiotics can undergo several modification and degradation steps, among them several are mediated by glutathione transferases (GSTs). GSTs can be divided into several groups, and in the last decade the number of plant GST clades reached fourteen [7, 8]. The two largest plant specific groups, tau and phi classes, participate mainly in conjugating reactions and possess high affinity towards a broad spectrum of harmful compounds such as herbicides [3, 4, 8]. However, plant defence reactions can be regulated by light and circadian rhythm [10].

Light not only provides an energy source for plant photosynthesis, but also acts as an important signal to regulate gene expression and various aspects of plant development [6]. In *Arabidopsis* the light dependent regulations of two GST genes were determined. Chen et al. [2] identified that AtGSTU20 is interacting with a protein coded by *FIN219* gene, hereby is a part of the phyA mediated, far-red (FR) induced signaling network. Furthermore Jiang et al. [5] found in dark-light transition experiments that AtGSTU17 protein is not only influenced by phyA dependent pathway but also mediates the signaling and has a strong impact on GSH/GSSG ratio thus on redox status of the cells.

*Corresponding author; e-mail address: gallea@bio.u-szeged.hu

Although the participation of GSTs in light dependent pathways and the circadian changes in the whole detoxification system have been extensively studied, there are fewer results regarding the exact daily fluctuation of plant GSTs. The aim of this study was to define the light dependent changes in the total GST activity and in the gene expression of selected GST genes in tomato plants.

Solanum lycopersicum L. cvar. Ailsa Craig was cultivated in hydroponic culture for 6 weeks in 12/12 h light/dark cycle as described by Poór et al. [9]. The plants were grown in a controlled environment under 300 μmol m^{-2} s^{-1} light intensity (F36W/GRO lamps, Sylvania, Germany) for six weeks, with 12-h light/12-h dark period, a day/night temperatures of 24/22 °C and relative humidity of 55–60%. The light period started at 6 o'clock and ended at 18 o'clock. Samples were taken from the second, fully expanded young leaves in three replicates in every 3 hours. Glutathione transferase (GST, EC 2.5.1.18) activity was determined as described in Benyó et al. [1]. RNA was extracted from leaf samples using a Quick RNA miniprep (Zymo Research Corp., Irvine, California, USA) and first-strand cDNA was synthesized by using 1 μg RNA and M-MuLV reverse transcriptase (Thermo Fisher Scientific, Waltham, Massachusetts, USA) according to manufacturer's instructions. The expression rate of the selected genes was monitored by quantitative real-time PCR (qRT-PCR, Analytik Jena AG, Jena, Germany) using SYBR Green PCR Master Mix (Thermo Scientific, Waltham, Massachusetts, USA) as described in Csiszár et al. [3]. Data were normalized to the transcript levels of first control sample. Differences between means were determined by Duncan's multiple range test (Sigma Plot 12.0 software, SPSS, Erkrath, Germany).

The conjugating activity towards CDNB multiplied during the day, between 15 and 18 o'clock it increased to a five times higher value. This elevated activity was detectable at 18 and 21 o'clock, and then decreased during the dark period (Fig. 1A).

Four tau class GST genes (*SlGSTU6*, *SlGSTU23*, *SlGSTU24*, *SlGSTU26*) were selected, according to their catalytic activity and their inductions to various environmental stresses based on our previous results [3]. The transcript levels showed time dependent inductions (Fig. 1B) similarly to the GST activity during the investigated light period. The transcript amounts of *SlGSTU23, SlGSTU24, SlGSTU26* increased and reached a peak at 18 o'clock. The expression of *SlGSTU6* was halved between the first two sampling, but before the end of the light period it also elevated. From the beginning of the dark period the transcript amounts decreased.

In our earlier publication the 5' *cis*-regulating elements (CRE) of tomato GSTs were collected and categorised [3]. Several light dependent elements were identified. To reveal the differences among the elements in the promoter of the four genes and to determine which element could be involved in the elevated activity and expression, the light dependent CRE were compared (Table 1). Variable number and types of light regulatory elements were found in the four promoter regions: *SlGSTU6* contained 9, *SlGSTU24* contained 13, while *SlGSTU23* and *SlGSTU26* both have 17. There was one common element: Box 4, which is a part of a conserved DNA module involved in light responsiveness. The highly conserved G-box and Box I also presented in 3–3 promoters.

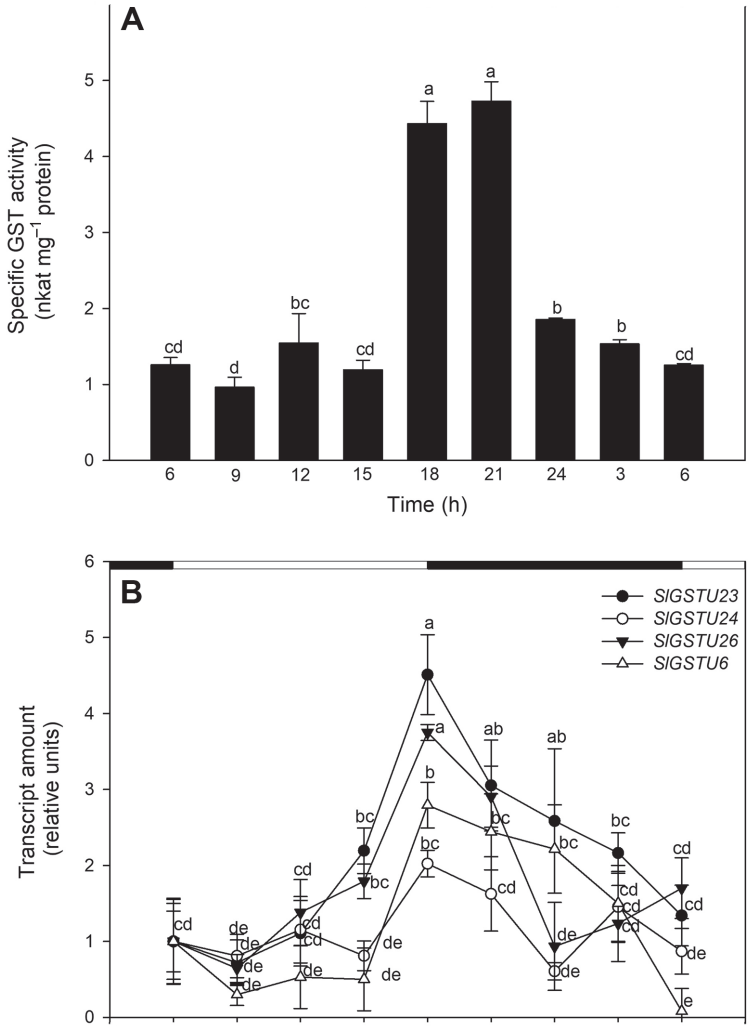

Fig. 1. Circadian changes in the GST expression and activity in tomato plants: A – Specific GST activity towards artificial substrate CDNB. Data consist of means±SD obtained from at least 3 measurements. Means denoted by different letters are significantly different at $P \leq 0.05$ as determined by Duncan's test. B – Light caused changes in the expression of *SlGSTU6, SlGSTU23, SlGSTU24, SlGSTU26* genes. The transcript amounts of the first sampling points (6 a.m.) was taken as arbitrary unit

Our findings underline the circadian fluctuation of the GST activity with a maximum late in the light period. Darkness also highly affected the expression of the selected GST genes. These findings could complete our knowledge on the circadian rhythm of GSTs in plants and could help in further defining their detoxification system.

Table 1
Light regulated promoter elements: list and description of the main circadian/light responsive nucleotide motifs discovered in the 5' regulator regions of the investigated genes (*SlGSTU6, SlGSTU23, SlGSTU24, SlGSTU26)* in tomato plants

Motif name	SlGSTU6	SlGSTU23	SlGSTU24	SlGSTU26
3-AF1 binding site		473 (–)		915 (+)
AE-box			1120 (+) 1206 (–)	1117 (+)
ACE		1278 (+)	1307(–)	
AT1-motif				64 (+)
ATCT-motif	322 (+) 342 (+)	290 (+)		
Box 4	578 (+)	850 (+) 1215 (+) 902 (+) 1372 (+)	39 (+)	199 (+) 280 (+) 367 (+) 747 (+)
Box I		97 (+) 1139 (+) 657 (+) 111 (–)	930 (+)	154 (+) 1109 (–)
Box II			1307 (+)	451 (–)
Box II–like sequence				858 (–)
G-box	669 (–) 1017 (–)	700 (+) 700 (–)	952 (+) 1309 (+) 1308 (–) 1307 (–)	
GATA-motif	896 (+) 1082 (+)			
GA-motif		538 (–)		
GAG-motif		1199 (–)		
GT1-motif			553 (+)	451 (–) 455 (–)
I-box	1084 (+)			
MNF1				906 (+)
Sp1		1187 (–)		
Chs-CMA2b		639 (–)		
Chs-CMA1a			977 (–)	1131 (–)
Circadian	110 (+)		1055 (+)	823 (+) 1473 (+)

ACKNOWLEDGEMENTS

This work was supported by the Hungarian National Scientific Research Foundation [grant number NKFI K 125265 and NKFI PD 112855].

REFERENCES

1. Benyó, D., Horváth, E., Németh, E., Leviczky, T., Takács, K., Lehotai, N., Feigl, G., Kolbert, Zs., Ördög, A., Gallé, R., Csiszár, J., Szabados, L., Erdei, L., Gallé, Á. (2016) Physiological and molecular responses to heavy metal stresses suggest different detoxification mechanism of Populus deltoides and P. x canadensis. *J. Plant Physiol. 201*, 62–70.
2. Chen, I. C., Huang, I. C., Liu, M. J., Wang, Z. G., Chung, S. S., Hsieh, H. L. (2007) Glutathione S-transferase interacting with far-red insensitive 219 is involved in phytochrome A-mediated signaling in Arabidopsis. *Plant Physiol. 143*, 1189–1202.
3. Csiszár, J., Horváth, E., Váry, Zs., Gallé, Á., Bela, K., Brunner, Sz., Tari, I. (2014) Glutathione transferase supergene family in tomato: salt stress-regulated expression of representative genes from distinct GST classes in plants primed with salicylic acid. *Plant Physiol. Bioch. 78*, 15–26.
4. Dean, J. D., Goodwin, P. H., Hsiang, T. (2003) Colletotrichum gloeosporioides infection induces differential expression of glutathione S-transferase genes in Malva pusilla. *Funct. Plant Biol. 30*, 821–828.
5. Jiang, H. W., Liu, M. J., Chen, I. C., Huang, C. H., Chao, L. Y., Hsieh, H. L. (2010) A Glutathione STransferase Regulated by Light and Hormones Participates in the Modulation of Arabidopsis Seedling Development. *Plant Physiol. 154*, 1646–1658.
6. Kendrick, R. E., Kronenberg, G. H. M. (1994) *Photomorphogenesis in plants.* 2nd ed. Kluwer Academic Publishers, Dordrecht, The Netherlands.
7. Lallement, P. A., Meux, E., Gualberto, J. M., Prosper, P., Didierjean, C., Saul, F., Haouz, A., Rouhier, N., Hecker, A. (2014) Structural and enzymatic insights into Lambda glutathione transferases from Populus trichocarpa, monomeric enzymes constituting an early divergent class specific to terrestrial plants. *Biochem. J. 462*, 3952.
8. Liu, Y. J., Han, X. M., Ren, L. L., Yang, H. L., Zeng, Q. Y. (2013) Functional divergence of the glutathione S-transferase supergene family in Physcomitrella patens reveals complex patterns of large gene family evolution in land plants. *Plant Physiol. 161*, 773–786.
9. Poór, P., Gémes, K., Horváth, F., Szepesi, A., Simon, M. L., Tari, I. (2011) Salicylic acid treatment via the rooting medium interferes with stomatal response, CO_2 fixation rate and carbohydrate metabolism in tomato, and decreases harmful effects of subsequent salt stress. *Plant Biol. 13,* 105–114.
10. Poór, P., Ördög, A., Czékus, Z., Borbély, P., Takács, Z., Kovács, J., Tari, I. (2018) Regulation of the key antioxidant enzymes by developmental processes and environmental stresses in the dark. *Biol. Plant* doi:10.1007/s10535-018-0782-7.