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Gene expression of a putative glutathione S-transferase is responsive to abiotic stress in embryogenic cell cultures of *Cyclamen persicum*

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Abstract

Background: *Cyclamen persicum* is an economically important ornamental crop that is propagated exclusively through seeds as vegetative propagation using cuttings is not possible. Therefore a micropropagation method through somatic embryogenesis is of high interest; however the method suffers from low reliability concerning quality and quantity of the produced plantlets. A crucial step of the protocol is the removal of plant growth regulators (PGRs) that triggers embryo development. In order to get a better insight in this crucial step of the propagation process, a gene expression analysis has been set up using five different genes of glutathione S-transferases (GST) as these are known to be auxin responsive as well as stress reactive. **Results:** One out of the five genes of glutathione S-transferases (CpGST1) displayed a clear down-regulation 72 hrs after removal of PGRs compared to 4 hrs after, implying auxin responsiveness. However, a more detailed analysis including the time points 0, 4 and 72 hrs revealed an initial strong up-regulation after 4 hrs before it was down-regulated after 72 hrs. In comparison fold-changes of the additional four GST-genes were marginal. Comparing cultures on semisolid medium to that in suspension, transcript abundances of CpGST1 were clearly decreased in suspension culture. **Conclusions:** Against the initial hypothesis CpGST was not auxin responsive but stress reactive, probably especially indicating drought stress imposed on the cells upon transfer from submerged suspension culture to semisolid medium. Mechanical stress caused by shaking of suspensions cultures seemed to be less important.

Keywords: auxin, callus, micropropagation, semisolid medium, suspension culture

INTRODUCTION

Cyclamen persicum is an ornamental potted plant with international economic importance. Because of the plant habits and the regeneration characteristics conventional vegetative propagation (through cuttings) is not possible. Therefore, *Cyclamen* are traditionally propagated through seeds although this is associated with problems due to variability (in case of population cultivars), inbreeding depression (in case of generation of F1-parent lines), and high costs (in case of production of F1-seed) (Ruffoni et al. 2000). Accordingly, the establishment of a vegetative propagation system in vitro is highly desirable. In *Cyclamen* one of the most efficient micropropagation systems is a protocol for somatic embryogenesis starting from unfertilised ovules (Schwenkel and Winkelmann, 1998). From these explants callus develops on medium containing 2,4-D and 2iP as plant growth regulators (PGRs). Callus can be propagated by regular sub-culturing on identical medium. Besides, Winkelmann et al. (1998) described suspension cultures in liquid medium of the same composition (lacking Gelrite). The differentiation of somatic embryos is initiated by transferring the callus to PGR-free medium. In order to investigate the developmental processes of this propagation system a microarray analysis has been performed

analyzing the expression of 1,216 genes in various stages and tissues (Rensing et al. 2005; Hoenemann et al. 2010).

In this analysis a group of five putative genes of glutathione S-transferases (GSTs) displayed differential expression during important steps of the micropropagation protocol. GSTs are enzymes that catalyse the conjugation of the tripeptide glutathione to a wide variety of hydrophobic, electrophilic, and cytotoxic substrates. Many GSTs also act as glutathione-dependent peroxidases by catalysing the reduction of organic hydroperoxide to the less toxic monohydroxy alcohols (Marrs, 1996). GSTs were found to be present at every stage of plant development from early embryogenesis to senescence and in every tissue type examined (Sari-Gorla et al. 1993; McGonigle et al. 2000; Jain et al. 2010). However, the function and temporal-spatial appearance of the different GST isoenzymes is highly specific (Sari-Gorla et al. 1993; Moons, 2003). GSTs have been subdivided into eight distinct classes: seven classes (phi, tau, zeta, theta, lambda, dehydroascorbate reductase (DHAR) and tetrachlorohydroquinone dehalogenase (TCHQD)) are soluble (cytoplasmatic), whereas the eighth is microsomal (Basantani and Srivastava, 2007). Plant GSTs have been intensively studied for their ability to detoxify herbicides (Reade et al. 2004). Besides, a major focus of research on plant GSTs is their function in phytohormone physiology, especially auxin metabolism (Jones 1994; Bilang and Sturm, 1995). Moreover, they are involved in the response to biotic and abiotic stresses (*e.g.* Marrs, 1996). Due to their key role in the cellular physiology they are also important for many developmental processes. GSTs are known for their relevance during somatic embryogenesis of plants as described by Pan et al. (2009) for *Citrus sinensis*.

Our study aims to analyse the transcript abundances of different *GST* homologues in the process of somatic embryogenesis of *C. persicum* during the crucial step of transfer to PGR-free medium which triggers the realisation of embryo development. By this we want to learn more about the role of different in vitro manipulation steps, auxin removal and mechanical stress.

MATERIALS AND METHODS

Tissue culture

The cell line of the genotype “56/2” (kindly provided by Traud Winkelmann, Institute of Floriculture and Woody Plant Science, Leibniz University Hannover) was established as described by Schwenkel and Winkelmann (1998) from unfertilised ovules of a single plant of the cultivar ‘Maxora Light Purple’ (Varinova, Berkel en Rodenrijs, The Netherlands). The cell line was cultivated on MS medium containing the plant growth regulators 2,4-D and 2iP (in the following called “standard medium”) as described by Schwenkel and Winkelmann (1998). Subculturing was performed by transfer to fresh standard medium every four weeks. As described by Winkelmann et al. (1998) suspension cultures were established and maintained by transfer to fresh standard medium every two weeks. Embryo development was induced by transferring the cells to PGR-free standard medium (Schwenkel and Winkelmann, 1998). Subculturing of callus as well as of cell suspension comprised severe mechanical manipulations by disintegration of cell clumps (callus) and sieving through a 500 µm-mesh (cell suspension). Therefore, mechanical manipulations were performed four days before transfer to PGR-free medium (with continuing culture on fresh standard medium for four days) in order to separate mechanical manipulations from medium change. After four days cells were gently transferred to either solid or liquid standard medium lacking PGRs. In case of cell suspensions, cells were collected on a 200 µm-mesh and have subsequently been washed three times in PGR-free medium. Culture density on PGR-free medium was standardised to 500 mg/jar for cultures on semisolid medium and to a packed cell volume of 4% for cell suspensions. A flow chart of the experimental procedure is given in Figure 1.

Cell material was collected 0, 4 and 72 hrs after transfer to PGR-free medium for RNA isolation. These time points have been chosen because of the results obtained in foregoing studies (Rensing et al. 2005; Hoenemann et al. 2010). Each analysed tissue was represented by three independent biological replicates.

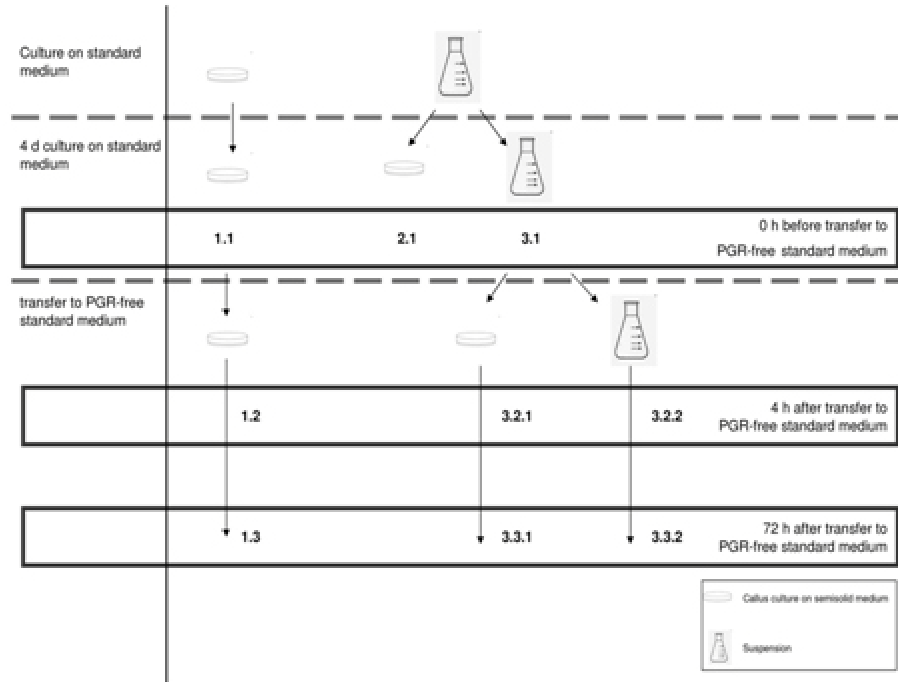

Fig. 1 Flow chart of the experimental procedure. Samples taken for further analysis are encircled by boxes and are specified with tissue IDs.

Isolation of RNA and cDNA synthesis

For RNA isolation the RNeasy Plant Mini Kit (Qiagen) was used according to the manufacturer's instructions except for modifications as described by Dhanaraj et al. (2004). The protocol was altered by supplementing the 'RLT' lysis buffer (Dhanaraj et al. 2004) with 0.5% (v/v) beta-mercaptoethanol, 1.25% (w/v) polyvinylpyrrolidone-3000 (PVP 3000) and 2% (w/v) sodiumsarcosyl. According to the manufacturer's instructions the on-column DNase digestion was applied for eliminating genomic DNA contamination. RNA was quantified using the Quibit fluorimeter (Invitrogen).

The QuantiTect Reverse transcription Kit (Qiagen) was used to synthesise first strand cDNA from up to 1 µg of total RNA.

Quantitative realtime PCR

Partial sequences of five putative *GST* genes have been obtained by EST sequencing (Rensing et al. 2005; http://www.cyclamen-est.de: CYC01T7_E12, CYC32T7_B11, CYC29T7_E07, CYC16T7_B04, CYC33T7_F07). In the following the putative *GST* genes are named *CpGST1-5* (Table 1). Specific primers for all genes have been designed using Primer3 (Rozen and Skaletsky 2000, available from the internet: http://biotools.umassmed.edu/bioapps/primer3_www.cgi).

PCR amplification was performed in a Stratagene Mx3000P realtime PCR System (Stratagene, La Jolla, CA, USA) using SYBR Green as described by Hoenemann and Hohe (2011). Primer concentrations for optimal amplification results have been determined for each gene (Table 1). The reactions were performed in triplicate for each of three independent biological samples. Evaluation of primer efficiency was carried out by calculation of standard curves with the Mx3000P Software (MxPro) and all primers passed successfully.

Table 1. Primer sequences, primer concentrations, product sizes and annealing temperatures for amplification of putative *Cyclamen persicum GST* genes.

Abbreviated gene designation	Putative gene product	GenBank	No. Cyclamen EST databased	Forward Primer (F) Reverse Primer (R) 5' → 3'	Product size [bp]	Primer concentration [nM]
CpGST1	Probable glutathione S-transferase parA	AJ886042	CYC01T7_E12	F: CATCCTGGGAGAACAATGTG R: ACCCCCAAAGTAGGGTTTGT	118	200
CpGST2	Probable glutathione S-transferase	AJ886910	CYC32T7_B11	F: GCTCGGGATTTTGCTAGAAG R: TTCCCTGATGACAGAGCAAT	109	200
CpGST3	Glutathione S-transferase GST 20	AJ887722	CYC29T7_E07	F: GTTGGGACCGATCGAAGTA R: CAAGTGGAAGCTCGAGGAA	101	200
CpGST4	Glutathione-S-transferase/glutaredoxin	AJ887143	CYC16T7_B04	F: AAGGGCATGAGGTGGATTT R: CTCATCAGCCTCGCTATGG	98	100
CpGST5	Putative glutathione S-transferase	AJ886941	CYC33T7_F07	F: TGTGAAGCTGCTCGATGAA R: TGGGATCGCATTTTTCACT	91	100
Ef-Tu	Elongation factor tu	AJ886626	CYC16T7_A05	F: TATCCAGAGGGGGATGGTT R: TGCCTACCTCCCTCTTCCT	102	200

The experimental data were normalized to the mean value of the reference gene (a putative *Ef-Tu*) as described by Hoenemann et al. (2010). The relative quantity was calculated according to Hoenemann et al. (2010) by the ΔΔCt-method (Livak and Schmittgen, 2001). The calculated relative quantity for one tissue is given as the ratio (fold change) to the tissue to which it was compared. If this value was less than one, the (negative) reciprocal is given. Every reported fold change represents the arithmetic mean of three independent experiments and three biological replicates. Differential gene expression was statistically calculated using a two-samplet-test ($p \leq 0.05$).

RESULTS AND DISCUSSION

In a broad microarray experiment comparing the expression of 1,216 transcripts in various stages of somatic embryogenesis in *Cyclamen persicum* genes of five putative GSTs were found to be differentially expressed in at least one of the experiments that are specified with tissue IDs in Figure 1. Regarding the crucial step of transfer to PGR-free medium (which triggers the development of somatic embryos) a previous study showed that two out of the five *GST*-homologues displayed a reduced transcript abundance in cells 72 hrs after transfer to PGR-free medium compared to cells right before the transfer (Hoenemann et al. 2010). This might be interpreted as a hint on auxin-regulated gene expression which should be validated by additional experiments.

In the microarray experiments gene expression in cells right before the transfer to PGR-free medium was compared to that of cells 4 hrs and 72 hrs after transfer. Here, it had been concluded that major changes in transcript abundances did only occur between 4 hrs and 72 hrs after transfer to PGR-free medium (Hoenemann et al. 2010). Therefore, in a first additional experiment the gene expression levels of the five different *GST*-homologues have been compared to each other 4 hrs (tissue 3.2.1) and 72 hrs after transfer (tissue 3.3.1) to PGR-free standard medium. Figure 2 shows that especially the transcript abundance of the *CpGST1* was extensively decreased after transfer to the PGR-free medium (fold-change: 99). This result supports the initial hypothesis that transcription of this gene was induced by auxin and repressed upon auxin-removal. The GST enzyme group is quite diverse and some GSTs are considered to be auxin induced (Flury et al. 1995; Kop et al. 1996; Domoki et al. 2006; Singla et al. 2007). Moreover, this hypothesis has been supported by a sequence comparison to *GST*-genes of other plants showing high similarities of the here presented *CpGST1* to *GST*s from *Malva pusilla* (NCBI Acc. No. AAO61854), *Ricinus communis* (NCBI Acc. No. XP_002532823 and XP_002515772), *Populus trichocarpa* (NCBI Acc. No. ADB11373 and ADB11374) and *Glycine max* (NCBI Acc. No. AAG34800). According to the data given by NCBI all these GSTs are related to the tau-class, which is reported to be auxin induced (Marrs, 1996). Singla et al. (2007) also described a strong repression of the expression of *GST*-homologues upon auxin removal in tissue cultures of *Triticum aestivum*; however they do not relate the GSTs encoded by these genes to one of the different GST classes as described by Basantani and Srivastava (2007).

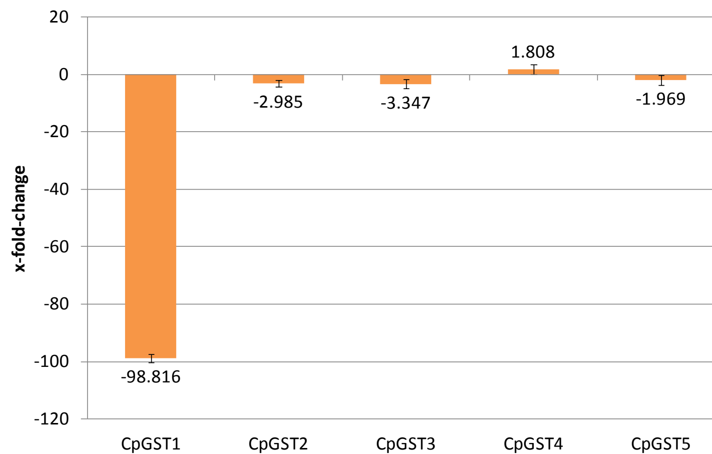

Fig. 2 Transcript abundances of *GST* homologues in samples 72 hrs after transfer to PGR-free standard medium (tissue 3.3.1) compared to 4 hrs (tissue 3.2.1) presented as x-fold-change. Positive values describe up-regulation while negative values describe down-regulation.

In order to validate the putative auxin-responsive gene expression of *CpGST1* it was necessary to check changes of the transcript abundance over time. This has been done in an additional experiment comparing gene expression of the *GST*-homologues in cells 4 hrs and 72 hrs after transfer to PGR-free medium to that right before transfer. The results are presented in Figure 3. Again, transcript abundances of *CpGST*1 displayed the most distinct results while changes in the transcription level of other putative *GST*-transcripts were much less pronounced. Indeed, the expression of all putative GST-transcripts was repressed in the period between 4 hrs and 72 hrs after auxin removal (Figure 3b), which is highly comparable to the preceding experiment (Figure 2). However, comparing samples 0 hrs and 4 hrs after transfer to PGR-free medium transcript abundance of *CpGST*1 was manifold increased in the samples 4 hrs after the medium exchange regardless whether cells were transferred to liquid or to solidified medium. This observation clearly contradicts our initial hypothesis about the auxin-responsiveness of the expression of *CpGST*1, because in this case transcript abundance was expected to be reduced upon auxin removal. Thus - in order to avoid misinterpretations - in this context it seems to be very important to analyse a time period (Figure 3) rather than randomly chosen time points (Figure 2), especially since transcript abundances of the *GST*-homologues change rather fast. This is in line with results of Zhu et al. (2008) who analysed *GST* expression during initial cellular dedifferentiation in cotton seedlings. They described high *GST* expression levels only within a time period from 6 hrs to 24 hrs after induction by PGR treatment. These results of Zhu et al. (2008) imply that also in our experiments the high expression levels of *CpGST1* are probably not caused by stored auxins or a late reaction to the exchange of PGR-containing medium 4 days before transfer to PGR-free medium. Therefore, we expect other factors than auxin to be responsible for the pronounced changes in transcript abundance of *CpGST1* upon transfer to the PGR-free medium.

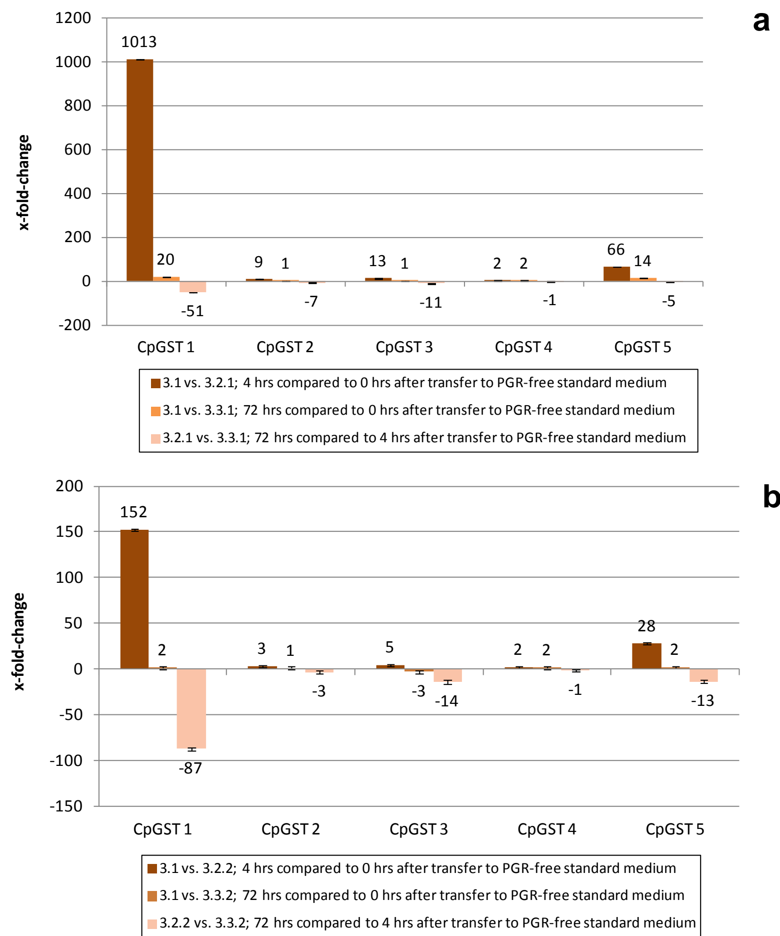

Fig. 3 Gene expression of *GST* homologues 0, 4 and 72 hrs after transfer to PGR-free medium. (a) culture on semisolid medium; (b) suspension culture. Positive values describe up-regulation while negative values describe down-regulation.

Levine et al. (1994) and Tenhaken et al. (1995) mentioned the function of *GST*-homologues in oxidative or mechanical stress response. As described we tried to separate the effects of severe mechanical manipulations and the medium change by a sequential subculture procedure. However, even during the gentle procedure of medium change, mechanical stress cannot be completely avoided, since upon transfer of cell suspensions to the PGR-free medium, the cells have been collected on a 200 µm-mesh and subsequently washed three times in PGR-free medium before they were inoculated into the new flasks. This procedure might impose stress by the mechanical treatment as well as by sudden exposure of the submerged cells to dry clean bench air. Accordingly, a new hypothesis on induction of *CpGST1*-expression has been formulated proposing that it might be induced by mechanical and/or drought stress. Therefore, transcript abundances of the *GST*-homologues have also been compared in cells growing in different culture systems, *i.e.* on semisolid medium in petri dishes and as suspension culture in shake flasks (Figure 4). Except for *CpGST3* all other putative *GST*-transcripts were down regulated in the cells cultured in liquid medium (tissue 3.1) compared to the cells on semisolid medium (tissue 2.1). However, whereas fold-changes were only marginal for *CpGST2-5*, transcript abundance of the *CpGST1* was reduced 60-fold in shake flask compared to petri dish culture. Whereas mechanical stress is only imposed on cells in shake flask culture, cells transferred to petri dish culture have to cope with sudden exposure to dry air. The same comparison has been made 4 hrs after transfer to PGR-free medium (Figure 4b). Again all putative GST-transcripts were repressed in suspension culture compared to the tissue cultivated on semisolid medium. However, the fold-change of *CpGST1* amounts only to factor 7 in this comparison. Since these cells have undergone the washing procedure 4 hrs before the samples were taken, we believe that in this experiment, the large effect of this treatment (Figure 3) has masked a part of the differences induced by the different culture systems. Nevertheless, again the transcript abundance of *CpGST1* was increased in petri dish culture compared to suspension culture.

If expression of *CpGST1* was induced by drought stress, no significant changes in the transcript abundance should occur, if only cells from semisolid media of different culture stages are compared,

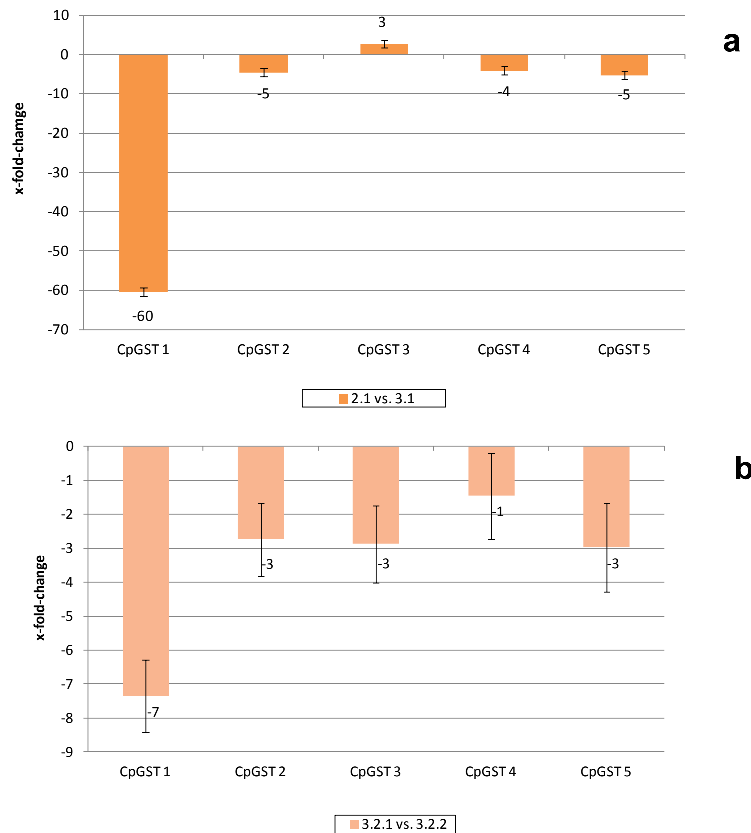

Fig. 4 Gene expression of *GST* homologues in cells growing in suspension culture compared to cells on semisolid medium presented as x-fold-change (a: before transfer to PGR-free medium; b: 4hrs after transfer to PGR-free medium). Positive values describe up-regulation while negative values describe down-regulation.

because these cells do not undergo environmental humidity changes when transferred to different media. Therefore transcript abundances in cells that have only been cultured on semisolid medium have been compared right before the transfer to PGR-free medium as well as 4 hrs and 72 hrs later (Figure 5). Here, only marginal changes in transcript abundance have been detected for all *GST*-homologues. Moreover, by the large standard deviations it becomes apparent how inhomogeneous the callus cultures were compared to cells in suspension culture.

Responsiveness of *GST* gene expression to abiotic stresses has been demonstrated in other plant systems. In *Arabidopsis thaliana* seedlings a drought-associated oxidative stress induced accumulation of a *GST* transcript that is a homologue of an extremely conserved subgroup of tau GSTs (Bianchi et al. 2002). This is of interest as also in our study *CpGST1* shows homologies to *GST* genes of other plants that belong the tau-class (see above). Diao et al. (2010) demonstrated that the expression *LbGST1* was differentially regulated by various abiotic stresses in *Limonium bicolor*. Transcript levels of a tau class *GST* from *Oryza sativa* were observed to vary significantly in response to chlorodinitrobenzene, hydrogen peroxide and atrazine treatments, indicating diverse regulation mechanisms in response to abiotic stresses (Yang et al. 2009). In the study of Jain et al. (2010) the overlapping response of *GST* genes to various stimuli (hormones, abiotic and biotic stresses) and developmental processes were analyzed providing evidence for the role of GSTs in mediating crosstalk between various stresses and hormone response.

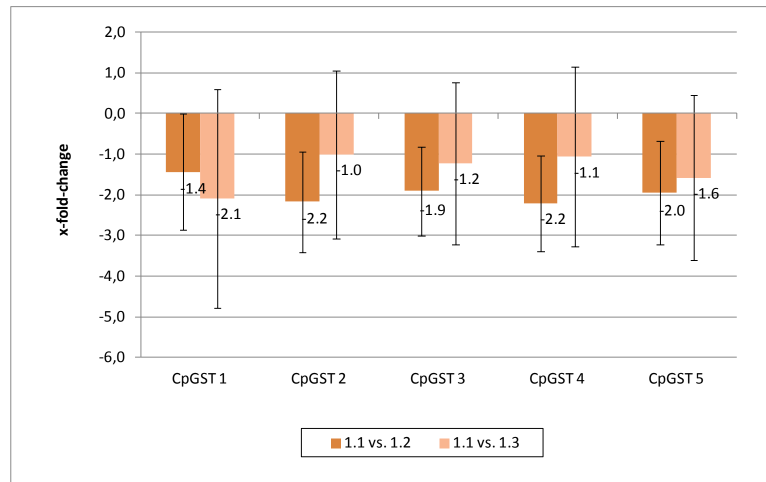

Fig. 5 Gene expression of *GST* homologues in callus cultured for 4 hrs (dark brown bars, tissue 1.2) or for 72 hrs (light brown bars, tissue 1.3) compared to cells before transfer to PGR-free medium (tissue 1.1) presented as x-fold-change. Positive values describe up-regulation while negative values describe down-regulation.

Therefore, we assume that our results demonstrate responsiveness of *CpGST1* to abiotic stress, especially drought stress *in vitro*. It has long been discussed that suspension culture of plant cells might impose strong mechanical stress, because plant cells are comparatively large and possess rigid cell walls (Dunlop et al. 1994; Namdev and Dunlop, 1995), whereas other authors demonstrated that the mechanical sensitivity of plant cells was less pronounced than expected (Scragg, 1995). These inconsistent conclusions might be attributed to the use of different experimental systems but also to effects of unconsidered factors. Our results might be interpreted as a hint that other abiotic stresses, possibly drought stress upon sub culturing of cell suspensions and transfer to semisolid culture, are more important with regard to the comparison of different culture systems in plant tissue culture than has been expected.

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REFERENCES

BASANTANI, M. and SRIVASTAVA, A. (2007). Plant glutathione transferases-a decade falls short. *Canadian Journal of Botany*, vol. 85, no. 5, p. 443-456. **[CrossRef]**

BIANCHI, M.W.; ROUX, C. and VARTANIAN, N. (2002). Drought regulation of *GST8*, encoding the *Arabidopsis* homologue of ParC/Nt107 glutathione transferase/peroxidase. *Physiologia Plantarum*, vol. 116, no. 1, p. 96-105. **[CrossRef]**

BILANG, J. and STURM, A. (1995). Cloning and characterization of a glutathione *S*-transferase that can be photolabeled with 5-azido-indole-3-acetic acid. *Plant Physiology*, vol. 109, no. 1, p. 253-260. **[CrossRef]**

DHANARAJ, A.L.; SLOVIN, J.P. and ROWLAND, L.J. (2004). Analysis of gene expression associated with cold acclimation in blueberry floral buds using expressed sequence tags. *Plant Science*, vol. 166, no. 4, p. 863-872. **[CrossRef]**

DIAO, G.; WANG, Y. and YANG, C. (2010). Functional characterization of a gluthathione S-transferase gene from *Limonium bicolor* in response to several abiotic stresses. *African Journal of Biotechnology*, vol. 9, no. 32, p. 5060-5065.

DOMOKI, M.; GYÖRGYEY, J.; BÍRÓ, J.; PASTERNAK, T.P.; ZVARA, A.; BOTTKA, S.; PUSKÁS, L.G.; DUDITS, D. and FEHÉR, A. (2006). Identification and characterization of genes associated with the induction of embryogenic competence in leaf-protoplast-derived alfalfa cells. *Biochimica et Biophysica Acta (BBA) - Gene Structure and Expression*, vol. 1759, no. 11-12, p. 543-551. **[CrossRef]**

DUNLOP, E.H.; NAMDEV, P.K. and ROSENBERG, M.Z. (1994). Effect of fluid shear forces on plant cell suspensions. *Chemical Engineering Science*, vol. 49, no. 14, p. 2263-2276. **[CrossRef]**

FLURY, T.; ADAM, D. and KREUZ, K. (1995). A 2,4-D-inducible glutathione S-transferase from soybean (*Glycine max*). Purification, characterisation and induction. *Physiologia Plantarum*, vol. 94, no. 2, p. 312-318. **[CrossRef]**

HOENEMANN, C.; RICHARDT, S.; KRÜGER, K.; ZIMMER, A.D.; HOHE, A. and RENSING, S.A. (2010). Large impact of the apoplast on somatic embryogenesis in *Cyclamen persicum* offers possibilities for improved developmental control *in vitro*. *BMC Plant Biology*, vol. 10, no. 77. **[CrossRef]**

HOENEMANN, C. and HOHE, A. (2011). Selection of reference genes for normalization of quantitative real-time PCR in cell cultures of *Cyclamen persicum*. *Electronic Journal of Biotechnology*, vol. 14, no. 1. **[CrossRef]**

JAIN, M.; GHANASHYAM, C. and BHATTACHARJEE, A. (2010). Comprehensive expression analysis suggests overlapping and specific roles of rice glutathione S-transferase genes during development and stress responses. *BMC Genomics*, vol. 11, no. 73. **[CrossRef]**

JONES, A.M. (1994). Auxin-binding proteins. *Annual Review of Plant Physiology and Plant Molecular Biology*, vol. 45, no. 1, p. 393-420. **[CrossRef]**

KOP, D.A.M.; SCHUYER, M.; SCHERES, B.; ZAAL, B.J. and HOOYKAAS, P.J. (1996). Isolation and characterization of an auxin-inducible glutathione *S*-transferase gene of *Arabidopsis thaliana*. *Plant Molecular Biology*, vol. 30, no. 4, p. 839-844. **[CrossRef]**

LEVINE, A.; TENHAKEN, R.; DIXON, R. and LAMB, C. (1994). H_2O_2 from the oxidative burst orchestrates the plant hypersensitive disease resistance response. *Cell*, vol. 79, no. 4, p. 583-593. **[CrossRef]**

LIVAK, K.J. and SCHMITTGEN, T.D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. *Methods*, vol. 25, no. 4, p. 402-408. **[CrossRef]**

MARRS, K.A. (1996). The functions and regulation of glutathione S-transferases in plants. *Annual Review of Plant Physiology and Plant Molecular Biology*, vol. 47, no. 1, p. 127-158. **[CrossRef]**

MCGONIGLE, B.; KEELER, S.J.; LAU, S.M.; KOEPPE, M.K. and O'KEEFE, D.P. (2000). A genomics approach to the comprehensive analysis of the glutathione *S*-transferase gene family in soybean and maize. *Plant Physiology*, vol. 124, no. 3, p. 1105-1120. **[CrossRef]**

MOONS, A. (2003). *Osgstu*3 and *osgtu*4, encoding tau class glutathione *S*-transferases, are heavy metal- and hypoxic stress-induced and differentially salt stress-responsive in rice roots. *FEBS Letters*, vol. 553, no. 3, p. 427-432. **[CrossRef]**

NAMDEV, P.K. and DUNLOP, E.H. (1995). Shear sensitivity of plant cells in suspensions present and future. *Applied Biochemistry and Biotechnology*, vol. 54, no. 1-3, p. 109-131. **[CrossRef]**

PAN, Z.; GUAN, R.; ZHU, S. and DENG, X. (2009). Proteomic analysis of somatic embryogenesis in Valencia sweet orange (*Citrus sinensis* Osbeck). *Plant Cell Reports*, vol. 28, no. 2, p. 281-289. **[CrossRef]**

READE, J.P.H.; MILNER, L.J. and COBB, A.H. (2004). A role for glutathione *S*-transferases in resistance to herbicides in grasses. *Weed Science*, vol. 52, no. 3, p. 468-474. **[CrossRef]**

RENSING, S.E.; LANG, D.; SCHUMANN, E.; RESKI, R. and HOHE, A. (2005). EST sequencing from embryogenic *Cyclamen persicum* cell cultures identifies a high proportion of transcripts homologous to plant genes involved in somatic embryogenesis. *Journal of Plant Growth Regulation*, vol. 24, no. 2, p. 102-115. **[CrossRef]**

ROZEN, S. and SKALETSKY, H. (2000). Primer3 on the WWW for general users and for biologist programmers. In: KRAWETZ, S. and MISENER, S. eds. *Bioinformatics Methods and Protocols in the series Methods in Molecular Biology*. Humana Press, Totowa, NJ, p. 365-386.

RUFFONI, B.; SEMERIA, L.; PROFUMO, P. and BISIO, A. (2000). *Cyclamen persicum* Mill. somatic embryos developed in suspension cultures: Histological analysis and conversion to plant. *Acta Horticulturae (ISHS)*, vol. 520, p. 83-90.

SARI-GORLA, M.; FERRARIO, S.; ROSSINI, L.; FROVA, C. and VILLA, M. (1993). Developmental expression of glutathione-*S*-transferase in maize and its possible connection with herbicide tolerance. *Euphytica*, vol. 67, no. 3, p. 221-230.**[CrossRef]**

SCHWENKEL, H. and WINKELMANN, T. (1998). Plant regeneration via somatic embryogenesis from ovules of *Cyclamen persicum* Mill. *Plant Tissue Culture and Biotechnology*, vol. 4, p. 28-34.

SCRAGG, A.H. (1995). The problems associated with high biomass levels in plant cell suspensions. *Plant Cell, Tissue and Organ Culture*, vol. 43, no. 2, p. 163-170. **[CrossRef]**

SINGLA, B.; TYAGI, A.K.; KHURANA, J.P. and KHURANA, P. (2007). Analysis of expression profile of selected genes expressed during auxin-induced somatic embryogenesis in leaf base system of wheat (*Triticum aestivum*) and their possible interactions. *Plant Molecular Biology*, vol. 65, no. 5, p. 677-692. **[CrossRef]**

TENHAKEN, R.; LEVINE, A.; BRISSON, L.F.; DIXON, R.A. and LAMB, C. (1995). Function of the oxidative burst in hypersensitive disease resistance. *Proceedings of the National Academy of Sciences of the United States of America*, vol. 92, no. 10, p. 4158-4163. **[CrossRef]**

WINKELMANN, T.; HOHE, A. and SCHWENKEL, H. (1998). Establishing embryogenic suspension cultures in *Cyclamen persicum* 'Purple Flamed'. *Advances in Horticultural Sciences*, vol. 12, p. 25-30.

YANG, X.; SUN, W.; LIU, J.P.; LIU, Y.J. and ZENG, Q.Y. (2009). Biochemical and physiological characterization of a tau class glutathione transferase from rice (*Oryza sativa*). *Plant Physiology and Biochemistry*, vol. 47, no. 11-12, p. 1061-1068. **[CrossRef]**

ZHU, H.; TU, L.; JIN, S.; XU, L.; TAN, J.; DENG, F. and ZHANG, X. (2008). Analysis of genes differentially expressed during initial cellular dedifferentiation in cotton. *Chinese Science Bulletin*, vol. 53, no. 23, p. 3666-3676. **[CrossRef]**

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