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Drought response strategies during grain filling in wheat. Glutathione transferase activity and expression pattern in flag leaves

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Summary

Total GST (glutathione S-transferase, EC 2.5.1.18) and glutathione peroxidase activity were measured spectrophotometrically in *Triticum aestivum* cv. MV Emese and cv. Plainsman (drought tolerant) and cv. GK Élet and Cappelle Desprez (drought sensitive) flag leaves under control and drought stress conditions during the grain filling period, in order to reveal possible roles of different GST classes in the senescence of flag leaves. Six wheat *GSTs*, members of 3 GST classes, were selected and their regulation by drought and senescence was investigated. High glutathione peroxidase activity (EC 1.11.1.9) was measured in well-watered controls of the drought tolerant Plainsman cultivar. At the same time *TaGSTUIB* and *TaGSTF6* sequences, investigated by Real-Time PCR showed high expression levels that increased with time indicating that the gene products of these genes may play important roles in monocarpic senescence of wheat. The expression levels of these genes were also induced by drought stress in all of the four investigated cultivars, but extremely high transcript amounts were detected in cv. Plainsman. Our data indicate genotypic variations of wheat GSTs; high expression levels and early induction of two senescence-associated GSTs under drought during grain filling in flag leaves correlated with high yield stability.

Key words

Drought stress, glutathione transferase, grain filling, wheat

Abbreviations

CDNB, 1-chloro-2,4-dinitrobenzene; GPOX, glutathione peroxidase; GST, glutathione S-transferase; AOS, activated oxygen species; RWC, relative water content; DHAR, dehydroascorbate reductase; DPA, days post anthesis

Introduction

Higher plants have developed a wide range of defence systems to survive stress caused by pathogens, constantly changing weather and other environmental conditions (Cushman and Bohnert 2000, Zhu 2002, Wang et al., 2003). One of the most important stress factors is drought, which often leads to an imbalance between antioxidant defence and the amount of activated oxygen species (AOS). AOS are necessary for inter- and intracellular signalling (Breusegem et al., 2001), but at high concentrations can cause damage at various levels of the organization (Asada 1999). To protect against the toxicity of AOS, aerobic organisms are equipped with an array of defence mechanisms, one of them is based on the glutathione transferases (GSTs).

Plant GST genes encode 25 to 29 kDa proteins, which form heterodimers, homodimers or monomers. GSTs play important roles in protection against cytotoxic endogenous and xenobiotic compounds (Marrs 1996, Dixon et al., 1998a, 2002). Besides detoxification by conjugating a glutathione tripeptide to a wide range of xenobiotics, GST isoenzymes have a function in hormone transport and maintaining homeostasis, including the cellular response to auxins (Bilang et al., 1993), cytokinins (Gonneau et al., 1998) and ethylene (Zhou and Goldsbrough 1993). Some GST isoforms have glutathione peroxidase (GPOX) activity, suggesting that their main function could be the reduction of toxic lipid peroxidation products and the maintenance of membrane integrity e.g. under osmotic stress (Dixon et al., 2003). The protective role of GSTs against different kinds of stress has been proven in several plant species (Marrs 1996, Edwards et al., 2000, Basantani and Srivastava 2007), and transgenic tobacco plants overproducing a GST gene with GPOX activity exhibit significant oxidative stress tolerance (Roxas et al., 2000). GSTs catalyze alternative GSH-dependent biotransformation reactions e.g. conversion of maleylacetoacetate to fumarylacetoacetate or reduction of dehydroascorbate (Dixon et al., 2002b), and also have a role in the metabolism of secondary products such as anthocyanins and cinnamic acid (Alfenito et al., 1998). These different functions coincide with the high diversity of the protein and nucleotide sequences. Plant GSTs fall into eight classes, seven soluble: phi, tau, theta, zeta, dehydroascorbate reductase, lambda and tetrachlorohydroquinone dehalogenase and one membrane bound (microsomal) GSTs (GSTF, GSTU, GSTT, GSTZ, GSTDHAR, GSTTCHQD, GSTM, respectively; Edwards and Dixon 2005).

GSTs comprise approximately 2% of the soluble protein in wheat seedlings (Pascal and Scalla 1999). High GST activity is a common characteristic of the most widespread *Triticum* cultivars, and several studies have revealed a high correlation between their GST activity and stress tolerance (Bartoli et al., 1999). Recently, the results of several transcriptome analyses revealed that GSTs may play an important role in senescence (Buchanan-Wollaston et al., 2005, Gregersen and Holm 2007), and Kunieda et al. (2005) identified a special, senescence-induced GST in barley leaves.

Senescence has attracted attention, particularly in monocarpic plants, such as cereals, especially in the grain filling period. The duration and rate of grain filling determine the final grain weight, a key component of the total yield. Water stress during grain filling usually induces early senescence and shortens the grain filling period but increases remobilization of assimilates from the leaves to the grains (Plaut et al., 2004). Using wheat cultivars with different drought susceptibility, pre- and post-anthesis water deficit induced early senescence in both the resistant and the sensitive wheat lines. According to parallel experiments in two drought resistant wheat lines, earlier senescence under drought resulted in a faster and more successful remobilization of pre-stored carbon from vegetative tissues, and also a better yield (than in two drought sensitive cultivars (Guóth et al., 2009).

In this study the changes in GST activity and expression patterns in the flag leaves of wheat cultivars with different drought resistance were investigated under drought stress during the grain filling period. Our aim was to define the role of different types of GSTs in defence under drought stress conditions during grain filling.

Materials and methods

Plant material

Our experiments were carried out on four wheat genotypes: *Triticum aestivum* L. cv. MV Emese, a drought resistant Hungarian cultivar, *T. a.* cv. GK Élet a drought sensitive Hungarian cultivar, *T. a.* cv. Plainsman V. a drought resistant American cultivar and the drought sensitive French Cappelle Desprez. The breeding pedigree analysis showed closer genetic relations between the two Hungarian cultivars (GK Élet and MV Emese, three similar ancestors were found), but not data were found regarding the common origin of Plainsman and Cappelle Desprez. Plants were grown in plastic plots (3 plants per pot) containing a mixture of soil (Terra, Hungary) and sand (1:1, v/v) under 300 $\mu\text{mol m}^{-2} \text{s}^{-1}$ light intensity provided by OSRAM HQL

400 W/R lamps, 12/12 hour day/night illumination, at 25/20 °C day/night temperature, at 55-60% air humidity. Drought stress was induced by reducing the water supply 4 days before the booting stage (7-8 days before anthesis). The plants were irrigated every 2nd day to 60% total soil water capacity for control plants, and 25% for stressed plants. The experiments were carried out in two seasons. Samples were taken at anthesis, and 4, 9, 12 days post anthesis (DPA) from the whole flag leaves of 6-7 plants.

Relative water content (RWC)

To determine the relative water content, penultimate leaves were weighed immediately to obtain fresh weight (FW), then floated on distilled water for 24 h and weighed again for turgid weight (TW). Leaves were then dried at 80 °C for 24 h for dry weight (DW) measurements. The RWC was calculated according to the following formula: $RWC = 100 \times (FW - DW) / (TW - DW)$.

Chlorophyll a, b and carotenoid content

The fully expanded flag leaves were homogenized in ice-cold 100% (v/v %) acetone (1,5 mL for 250 mg sample), and extracted for 24 hours. Samples were centrifuged at 5000 g for 15 minutes at 4 °C. The pellet was extracted again with 80% (v/v %) acetone (1,5 mL for 250 mg sample) for 24 hours. After centrifugation (5000g, 15 min, 4 °C), the supernatants were collected. The pigment composition was measured by a double beam spectrophotometer according to Lichtenthaler and Wellburn (1983). This method implies measurement of absorbed light in plant extract at 470, 646.8 and 663.2 nm.

Malondialdehyde content determination

MDA formation was assayed by using a thiobarbituric acid method (Ederli et al. 1997). 100 mg leaf tissue was homogenized with 1 ml of 0.1% trichloroacetic acid (TCA); to avoid further lipid peroxidation 100 µl of 4% butylhydroxytoluene (BHT) was added to the extract. After centrifugation at 12 000 g for 20 min, 250 µl of supernatant was mixed with 1 ml 0.5% thiobarbituric acid in 20% TCA and the mixture was incubated in boiling water for 30 min. The absorbance was read at 532 nm and adjusted for nonspecific absorbance at 600 nm. MDA concentration was estimated by using an extinction coefficient of $155 \text{ mM}^{-1} \text{ cm}^{-1}$.

Enzyme assays

Tissue homogenization and extraction steps were carried out at 4 °C. Crude protein extracts were prepared by homogenizing 0.2 to 0.5 g of flag leaf tissues in 2 mL of extraction buffer (0.1 M phosphate buffer pH 7.0, containing 1 mmol L⁻¹ phenylmethylsulfonyl fluoride and 1% polyvinyl-polypyrrolidone). The homogenate was then centrifuged at 10000g for 15 min, and supernatant was decanted.

Glutathione transferase (EC 2.5.1.18) activity was determined spectrophotometrically by using an artificial substrate, 1-chloro-2,4-dinitrobenzene according to Habig et al., (1974). Reactions were initiated by the addition of CDNB, and the increase in A₃₄₀ was determined. One unit (U) is the amount of enzyme producing 1 μmol conjugated product in 1 min, $\epsilon_{340} = 9.6 \text{ mmol L}^{-1}\text{cm}^{-1}$. The enzyme activity was expressed in terms of specific activity (U g⁻¹ FW). FW was used for calculation, because in this developmental stage the protein content is greatly affected by the amount of RUBISCO, which is remobilised more rapidly from leaves than the other proteins during senescence (Pécsváradí et al., personal communication). Glutathione peroxidase (GPOX, EC 1.11.1.9) activity was measured by the method of Awasthi et al. (1975), with cumene hydroperoxide as a substrate, as described in Csiszár et al. (2004). The reaction mixture contained 4 mmol L⁻¹ GSH, 0.2 mmol L⁻¹ NADPH, 0.05 U glutathione reductase (GR, Type II from wheat, Sigma), 100 μL enzyme extract, and 0.5 mmol L⁻¹ substrate in phosphate buffer (0.1 mol L⁻¹, pH 7.0) in a total volume of 1 mL. The decrease of NADPH was followed by measuring the absorbance at 340 nm; one U was equalled to converted NADPH μmol min⁻¹. The non-specific absorbance decrease was corrected for by using additional measurements without substrate, $\epsilon_{340} = 6.22 \text{ mmol L}^{-1}\text{cm}^{-1}$.

Screening of databases, phylogenetic analyses

Wheat GST sequences were identified using an *in silico* approach. Screening for wheat GSTs was initially performed on the TIGR (The Institute for Genomic Research) wheat database using published plant GST sequences from DDBJ/EMBL/GenBank sequence database (<http://compbio.dfci.harvard.edu/tgi>). A minimum cut-off E value ($\leq e^{-20}$) was applied to select significant matches. To discriminate between duplicated genes a threshold of at least 95% nucleotide sequence identity was used. Nucleotide sequences of known wheat GST genes and tentative consensus sequences (TCs) were aligned using the CLUSTALW program (Thompson et al., 1994). According to the conserved sequences used for classification of GST proteins (Dixon et al., 2002a), and using genes which were already assigned to GST classes, we could

identify six classes of wheat GSTs (Dixon et al., 2002b). A family tree was composed from approximately 300 amino acid long sequences and drawn with Phylodendron D.G. Gilbert version 0.8d.

RNA purification, expression analyses with Real-Time RT-PCR

RNA was extracted from flag leaf samples harvested at different developmental stages (anthesis, 4, 9, 12 DPA) according to Chomczynski and Sacchi (1987). DNase digestions were applied (Fermentas). First strand cDNA was synthesized using MMLV reverse transcriptase (Fermentas). Primers were designed using Primer express and Primer 3 softwares. Primers were synthesised in the Nucleic acid synthesis laboratory, Biological Research Center (Szeged, Hungary). Primer pairs are shown in Table 1. The expression rate of GST genes was monitored by Quantitative Real-Time PCR (BioRad, MJ Research) using SYBR green probes (Applied Biosystems; Karsai et al., 2002). Each reaction was repeated at least three times. QRT-PCR was initiated with denaturation at 95 °C for 10 min followed by 41 cycles of denaturation at 95 °C for 15s and annealing, extension at 60 °C for 1 min. Data analysis was performed using Opticon monitor software. To determine the specificity of the reaction, a melting curve analysis of the product was performed immediately after the final PCR cycle by increasing the temperature from 55 °C to 90 °C (0.2 °C 0.2 s⁻¹). Data were normalized using the wheat elongation factor α subunit (EF-1) and a gene with unknown function (NP-1) as high and low controls, respectively (Jukanti et al., 2006). These two internal standards showed constant expression levels in senescing leaf tissues during grain filling period (Jukanti et al., 2006).

Analysis and statistics

Enzyme assays and RWC

For activity measurements, the means \pm SD were calculated from the data of at least three separate samples. Differences between treatment means were determined by Duncan's multiple range test. Columns denoted by the same letters did not differ significantly at a probability level of $P < 0.05$. RWC was determined using 3-5 parallel samples. Differences between treatment means were determined by Student's t-test.

Real-Time RT-PCR

Data were calculated using $2^{-(\Delta\Delta Ct)}$ formula (Livak and Schmittgen 2001): the additive effect of concentration, gene and replicate was minimized by subtracting the Ct number of the target gene from that of the average of the two reference genes, which yielded ΔCt . This value was subtracted from all other ΔCt values, which yield the $\Delta\Delta Ct$ (Yuan and Stewart 2005). There were differences between expression levels of GSTs of the

control samples on 0 DPA (initial control) of different cultivars. To demonstrate these differences, the lowest initial control sample's transcript amounts of all cultivars were taken as arbitrary unit.

Results

Changes in RWC, chlorophyll and carotenoid contents, GST and GPOX activity

One thousand grain **mass** of the investigated genotypes under drought stress is a good parameter of the drought stress tolerance of wheat cultivars. Data presented in Fig. 1 show that yield of cv. Cappelle Desprez decreased to a higher extent under drought stress, while Plainsman V has very high yield stability. Drought stress, to which the plants were exposed from the booting stage till the 12th day post anthesis, had a different impact on the water content of the leaves of different wheat cultivars. There were no significant differences in the leaves of tolerant Plainsman either in the control or in the water-stressed plants during this period, while the leaves of **drought sensitive** GK Élet showed some decrease in RWC even in controls, **and the drought stress significantly decreased RWC values even at anthesis in the two sensitive cultivars (Table 2).** In the drought resistant MV Emese, the carotenoid content showed no significant changes, while the chlorophyll *a+b* content decreased both in control and stressed plants on the last sampling days. The MDA measurements resulted higher values even from 4 DPA both in control and stressed plants. The other resistant cultivar, Plainsman did not show changes in pigment content till 12 DPA, however the MDA contents were increased, especially in the drought stressed plants at 12 DPA. In Cappelle Desprez cultivar, the chlorophyll *a+b* decreased on 4 and 9 DPA even control circumstances, but it decreased due to stress even from 0 DPA. The levels of carotenoids decreased similarly at these sampling days, but the MDA content elevated only in drought stressed plants at anthesis and 4 DPA. The other sensitive cultivar, GK Élet showed less decrease in chlorophyll *a+b* content, no changes in the amount of carotenoids and the MDA level enhanced significantly only at 12 DPA in the drought stressed plants.

The highest levels of extractable GST (Fig. 2) and GPOX (Fig. 3) activity were found in the drought resistant Plainsman cultivar. In control plants, the activity of both transferase and peroxidase was enhanced after anthesis. In resistant cultivars, the induction of GST activity following drought stress was detected earlier than in sensitive cultivars. In the sensitive Cappelle Desprez no significant changes were detectable, while in GK Élet, GST activity increased only on 12 DPA in the drought stressed plants (Fig. 2).

In control conditions the extractable GPOX activity increased significantly in every investigated cultivar. In MV Emese, Plainsman and Cappelle Desprez, drought stress further increased glutathione peroxidase activity at the first sampling day, at anthesis. The GPOX activity of the sensitive GK Élet cultivar showed significant decline due to stress (Fig. 3).

These results indicate the involvement of the increased GST and GPOX activity in maintaining normal metabolism during natural senescence and successful acclimatization under stress.

Clustering of GST coding sequences

24 members of the GST gene family were identified from GenBank/DDBJ/EMBL databases (Table 3). 18 wheat *GST* genes (700-1500 bp cDNAs) were selected for homology searching of tentative consensus sequences (TC) in the TIGR database with high homology to known *GSTs*. This screening led to the identification of 98 putative GST sequences. A homology based tree was created after selecting ca. 900 nucleic acid long sequence regions with the highest similarity (Fig. 4). Based on conserved sequences used for classification of GST proteins and the genes already assigned to GST classes, we could identify six classes of wheat *GSTs* (Dixon et al., 2002 a, b).

The phi (GSTF) and tau (GSTU) class *GSTs* are the most heterologous classes, containing 38 and 26 sequences. The zeta (GSTZ), theta (GSTT), lambda (GSTL) and DHAR (GSTDHAR) *GSTs* are represented by 8, 11, 7 and 8 TCs, respectively.

For analysis of *GST* expression during the early grain filling period in the four wheat cultivars, 6 *GST* coding sequences probably associated to senescence and stress tolerance were selected. Kunieda et al. (2005) had previously identified and characterized a Senescence Induced GST (SIGST) in the flag leaves of barley. They suggested that the sequence of this type of GST contains highly conservative sequences in higher plants and they found that this sequence was similar to the three alleles of *TaGSTU1* gene. Thom et al. (2002) reported that in the investigated wheat tau group *GSTs* *TaGSTU1* showed the highest activity during treatment with a model stress metabolite, crotonaldehyde. In an earlier independent experiment, *TaGSTU1B*, *TaGSTU1C*, *TaGST19E50* and *TaGSTZ* showed up-regulation, among several *GST* coding sequences, due to osmotic and/or drought stress in two drought stress tolerant wheat cultivars (Secenji et al., unpublished). These sequences are grouped into three classes: *GSTU1B* and *GSTU1C* belong to the tau, *GSTZ* to the zeta and *GST19E50* belongs to the phi class. The phi class *GSTs* have broad substrate specificity; they are also

involved in the response to phytohormones, to oxidative stress caused by salt or temperature stress, phytopathogens or herbicides (Roxas et al., 2000, Cummins et al., 1997). We have chosen two other phi class sequences: *TaGSTF6*, whose encoded protein was characterized by Cummins et al. (2003) which possessed high conjugating activity against stress metabolite analogues, and *TaGSTA2*, which showed a highly significant similarity to a pathogen induced GST (Dudler et al., 1991). *TaGSTA2*, a genomic DNA sequence shows complete homology to TC248571.

Expression patterns of selected wheat GST sequences

Among the control samples, the highest transcript amounts were detected in the drought resistant Plainsman (Fig. 5). *TaGSTU1B* and *TaGSTF6* showed time-dependent induction in the controls of GK Élet and Plainsman cultivars. Among tau class GSTs *TaGSTU1B* showed higher induction under stress than *TaGSTU1C* in all cultivars except the sensitive Cappelle Desprez. Among phi class sequences, the transcript amounts of *TaGSTF6* and *TaGST19E50* were increased by stress in Plainsman, MV Emese and Cappelle Desprez, and to a lesser extent in GK Élet. The transcript level of *TaGST19E50* was induced by stress in all of four cultivars, but the elevation in the drought sensitive Cappelle Desprez was higher. The supposedly pathogen-inducible *TaGSTA2* was down-regulated due to aging on 4, 9 and 12 DPA compared to the day of anthesis in three cultivars (GK Emese, Plainsman and Cappelle Desprez). *TaGSTZ* was the least affected by stress and was mostly down-regulated on the last two sampling days (Fig. 5).

Discussion

The timing of flag leaf senescence is an important factor in grain filling and yield both under stress and optimal conditions. In our experiments, wheat flag leaves were sampled at four points starting from anthesis until the 12th day post anthesis (12 DPA), which period covers the premilk stage and the beginning of the medium milk stage. Senescence involves dynamic intracellular changes and a great number of gene products playing important roles in the regulation of this process. During senescence, maintaining protection against oxidative stress is important to protect the cells from premature death, and delayed senescence mutants have been found to have increased tolerance to oxidative stress (Woo et al., 2004).

Several parameters are able to indicate the leaf senescence, like increase of MDA, decrease of chlorophyll content (Yang et al., 2001b), and changes in P_N , Φ_{PSII} , qP, and NPQ can also be explained by aging (Yang and Zhang 2006). Regarding the chlorophyll and carotenoid contents, some time dependent changes were

detectable in MV Emese and GK Élet cultivars until the 12th day, but more significant symptoms of senescence appeared only after 12 DPA (Guoth et al 2009).

Vagy:

Some affects of the senescence was detectable on the control samples, but according to parallel investigation on these cultivars (Guoth et al, 2009) the chlorophyll degradation becomes more relevant later, on 21st sampling day. These data leads to the conclusion that slight effect could have been detectable of senescence on the expression on the 9th and 12th day, but drought stress caused much higher inductions.

The zeta class GST was shown to accumulate in carnation petals during senescence (Itzhaki et al., 1994). The protein of *Arabidopsis* GSTZ1 plays a significant role in phenylalanine and tyrosine degradation (Dixon et al., 2000). The maleylacetoacetate isomerase (MAAI) function suggests that GSTZ proteins are involved in the re-utilization and translocation of nitrogen metabolites. In our experiments, expression of the zeta class GSTZ1 was a little higher in the drought resistant Plainsman, GK Élet and MV Emese cultivars, but the transcript amount did not change in most of the genotypes. (ezt kihagyni!) Although there were some time-dependent differences in GSTZ1 expression levels in flag leaves in different cultivars, the level of this transcript was rather constant during senescence both in control and stress conditions. These results suggest that zeta class GSTs are less involved in drought stress response mechanisms.

Besides their roles in senescence, GSTs are known as one of the most important gene families involved in oxidative stress response (Marrs 1996, Basantani and Srivastava 2007). Transcriptome analysis of flag leaves of wheat during senescence revealed a cytosolic glutathione peroxidase enzyme and 5 up-regulated genes encoding GSTs (Gregersen and Holm 2007). One of these genes belongs to the zeta class (TC248700), two of them (TC248404 and TC234681 also known as *TaGST19E50*) to the phi class, and TC265396 shows highly significant similarity to *TaGSTU1 A/B/C*. Kunieda et al. (2005) found that GST enzyme activity increased along with progressing senescence, and that GSTs transcriptional regulation is controlled by signal transduction linked to oxidative stress. The participation of tau class GSTs in broad network of catalytic and regulatory functions involved in oxidative stress response has been proven in tomato (Kilili et al., 2004), parsley (Loyall et al., 2000) and rice (Soranzo et al., 2004). Xu et al. (2002) found that *TtGSTU1* and *TtGSTU2* of *Triticum tauschii* were up-regulated by 100 μ M ABA treatment in both shoot and root tissues,

and it was confirmed that the promoter region of *TtGSTUs* contained ABA-, ethylene-, and auxin responsive elements. Both of these findings indicate a potential role in stress responses.

In our experiments, estimation of *GST* expression patterns showed differences in expressed genes in the flag leaves of the investigated cultivars during the early grain filling period. The expression patterns of *GSTU1B* and *IC* differed in the four cultivars under control conditions, and the remarkable increase of *GSTU1B* transcript level was found only in cv. Plainsman and the drought sensitive GK Élet, which produces high yield under well-watered conditions, exhibited a significant induction, but lower transcript levels. The expression level of phi type *TaGSTF6* was similar to that *GSTU1B* in control GK Élet and Plainsman plants. The transcript amounts of *TaGSTU1B* and *TaGSTF6* were up-regulated outstandingly by drought stress on the day of anthesis and on most of the sampling days in the drought tolerant Plainsman cultivar, less increase was found in the expression rate of *GSTU1B* in MV Emese and GK Élet. In the drought sensitive Cappelle Desprez wheat genotype different genes showed some inductions due to aging (Fig. 5). According to our results, the expression level of *TaGSTU1B* and *TaGSTF6* has been elevated in Plainsman and in Élet before the aging showed detectable symptoms /sign on these cultivars which indicate that these genes play a role in the regular progression of senescence. Interestingly, these sequences were extremely induced further under drought stress especially in the drought resistant cultivar. The products of these genes presumably involved in a strong detoxification and/or they can promote the mobilisation or prevent the degradation of macromolecules which facilitate the grain filling under drought stress. Under drought stress, the phi class *TaGST19E50* and tau class *TaGSTU1C* expressions increased in all four cultivars and the highest induction was detectable in Cappelle Desprez.

The *GST* sequences were chosen on the basis of their possible role in the stress response and senescence. It is primarily the plant specific phi and tau class *GSTs* that mediate glutathione transferase activity towards a diverse range of xenobiotics (Edwards and Dixon 2005). Diversity in the substrate specificity and *GST* and *GPOX* activity of some members of phi and tau class *GSTs* in wheat were investigated particularly by Thom et al. (2002) and Cummins et al. (2003). In our experiments, some similarities were found in the tendencies between the extractable *GST* activity and the expression levels of the chosen genes. The drought resistant Plainsman showed the highest *GST* and *GPOX* activity in both drought stressed and well-watered samples. The highly significant increase on the day of anthesis, in correlation with changes in expression patterns

confirms the fast reaction of the Plainsman to water deficit. Our results suggest that the detoxifying activity of antioxidant enzymes including GSTs are able to facilitate/maintain the successful grain filling under water stress up to 12 DPA. In the sensitive cultivar GK Élet, the decreased GPOX activity and the almost unaffected GST activity up to the last sampling day both show that the activation of the defence mechanisms was not sufficient for maximising grain filling under water stress. The GST activity data and expression levels indicate that the faster reaction and the more intense defence against the toxic stress metabolites had an important role in the successful mobilisation of storage reserve material in the Plainsman cultivar. MV Emese and GK Élet both showed a moderate rise in the expression of GSTs, but the effect of drought on the extractable GST and GPOX activity were different between the two cultivars, the GPOX activity remained very low in GK Élet cultivar.

In summary, our results on the wheat GST transcript measurements in flag leaves indicate the major roles of some GST isoenzymes both in monocarpic senescence and drought stress responses during the grain filling period. Differences were found between the genotypes, and the early GST activation detected in drought tolerant wheat lines suggests the involvement of GSTs in the protection of optimal cell metabolism. *TaGSTU1B* and *TaGSTF6* can be important for regular progression of flag leaf senescence. In correlation with the enzyme activity, these gene products presumably stood for a strong detoxification, which facilitated the grain filling under drought stress. The higher expression levels or fast induction of GST coding sequences belonging to tau and phi classes observed in the drought tolerant lines during drought stress conditions may account for highly yield of tolerant cultivars under soil drought.

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Legends of tables and figures:

Table 1. Gene-specific primers used for QRT-PCR. The first two primer pairs were utilised to amplify low- and high-expression standards, while primer pairs 3-8 amplify wheat GST genes (Table 3). F: forward primer; R: reverse primer.

Table 2. The effects of soil drought on the relative water content (RWC %); chlorophyll **a+b**, carotenoid **and MDA** content of wheat plants during grain filling period. (DPA: days post anthesis.) **Means denoted by different letters indicate a significant difference (P<0.05, Duncan test).**

Table 3. Wheat GST genes with their GenBank accession numbers.

Fig. 1. One thousand grain mass of MV Emese, GK Élet, Plainsman, Cappelle Desprez cultivars during the early grain filling period (□ well-watered conditions, ■ drought stress). **R, S are for resistant and sensitive genotypes, respectively.** Statistical differences compared to controls are indicated by * P≤0.05, ** P≤0.01, *** P≤0.001, Student test.

Fig. 2. Specific GST activity (U g⁻¹ FW) in MV Emese, GK Élet, Plainsman, Cappelle Desprez cultivars during the early grain filling period (□ well-watered conditions, ■ drought stress). **R, S are for resistant and sensitive genotypes, respectively.** Means denoted by the same letters were not significantly different (P<0.05, Duncan test).

Fig. 3. Specific GPOX activity (U g⁻¹ FW) in MV Emese, GK Élet cultivar, Plainsman, Cappelle Desprez during the early grain filling period (□ well-watered conditions, ■ drought stress). **R, S are for resistant and sensitive genotypes, respectively.** Means denoted by different letters indicate a significant difference (P<0.05, Duncan test).

Fig. 4. Phylogenetic tree and classes of GSTs, based on the coding sequences of wheat GSTs. Tentative consensus sequences and the corresponding GST genes with highly significant similarity are shown.

Fig. 5. Transcript levels of phi, tau and zeta class *TaGSTs* in GK Élet, MV Emese, Plainsman and Cappelle Desprez cultivars during the early grain filling period (□ anthesis, ■ 4 DPA; ■ 9 DPA, ■ 12 DPA). R, S are for resistant and sensitive genotypes, respectively. Vertical bars represent standard deviations. The lowest relative transcript level in the control samples on the day of anthesis (initial control) was equalled to one for each gene. Data were normalized using the wheat elongation factor α subunit (EF-1) and a gene with unknown function (NP-1) as high and low controls, respectively (Jukanti et al. 2006). Statistical differences compared to controls are indicated by * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$.