Abstract of Ph.D. thesis

Acclimatisation of wheat cultivars during osmotic and drought stress, the role of glutathione transferases in the stress responces

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1. Introduction

Higher plants are in permanent relationship with their environment and strongly dependent on its effects. Different environmental factors influence their development, growth and determine their yield. When these factors fall out of an optimal range that is regarded to a certain species (such as too low or too high temperatures, unbalanced nutrient ratios, lack of light exposure or drought) the plant will be subjected to a varying level of stress. There are stress sensitive and resistant plants based on the effectiveness of the protective responces caused by stress. The most important concept of agricultural plant breedings is to gradually form stress resistant crops from stress sensitive cultivars. To obtain these tasks, it is highly important to shed light on the complexity of such stress responces. Firstly, those physiological parameteres are important to identify, which basically determines not only the survival of the plant but also guarantees to avoid the decrease of crop yield. Such effective physiological stress responces are usually characterized by the activation of certain genes, or groups of genes, which are important targets of research to help the breeding process.

Wheat is one of the most valuable and most widely cultivated crop in the world. Yield of the wheat is usually determined by two factors, the process of fertilization and grain filling. During grain filling transport of nutrients is initiated from the flag leaf into the developing grains, which starts to undergo the well-regulated process of senescence. Drought stress can influence the senescence of flag leaf, causing drop in crop yield. Both drought stress and senescence can result in the accumulation of toxic metabolites in plants, which are mainly eliminated by different types of glutathione transferases. There are numerous, multifunctional members in the isoenzyme family of glutathione transferases, which has eight groups (phi, zeta, tau, theta, dehydroascorbate reductase, lambda, tetrachlorohydrokinin dehalogenase and the microsomal GST). One of their well-known functions is the catalysis of detoxification of toxic metabolites with GSH conjugation. Increase of detoxification triggered by glutathione transferase (GST) enzymes after decrease of water potential in the soil can be an important factor in stress protection strategies. Therefore, one of the aims of our experiments was to reveal the relation between drought stress induced physiological responses and GST activity and to identify such GST transcripts, which can play an important role in the protection of senescence and/or drought stress.

In our study, we compared different drought resistant and sensitive wheat cultivars in two experimental designs. One series of experiments were performed on greenhouse-grown flowering plants. However, these experiments are time-consuming, that is why these experiments were also repeated on wheat seedlings grown in hydoponic culture. Osmotic stress was induced by increasing the osmolarity of the nutrient solution. After the comparison of the two different sets of experiments, we concluded that some gene transcripts were common in both of the stress responses, while drought-induced gene expression changes were higher in the samples of the flowering plants. The above mentioned results were also examined in the context of abscisic acid level and biosynthesis. In the field of stress physiology our novel findings can provide new ideas to wheat breeders for developing new drought resistant cultivars.

2. Aims

Wheat is one of the most sensitive cultivated plants to the deficiency of water. Drought stress-caused induction of GST activity and expressions are well-known in wheat, however, it is not clear which genes take part in this increase, what role it plays in drought tolerance of cultivars, and which GST group is more active in the different developmental stages. In case of two cultivars (Kobomugi and GK Öthalom) we studied to reveal the relationship between ABA content and GST expression profiles.

In my thesis I present the results which intend to answer the following questions:

1. Are there detectable signs of flag leaf senescence during the early grain filling period in any of the used cultivars? Is there correspondence between the initiation of senescence, GST activity and expression?

2. What does drought stress effects on GST and glutathione peroxidase (GPOX) activity in the resistant and sensitive cultivars? Which GST groups are induced by stress in those cultivars, which showed the highest yield stability?

3. What changes can be measured in water relation parameters due to osmotic stress? Is there any connection between osmotic stress responce and drought tolerance of the cultivars?

4. What changes are triggered in the GST activity and in the expression of the selected phi, tau, zeta GST genes due to osmotic stress?

5. Does the ABA content show differences during the acclimatisation process of isohydric and desiccation postponement plants? What roles does ABA play in the regulation of GST expressions in those cultivars, which showed the highest GST inductions due to stress?

3. Materials and methods

Plant material:

Six wheat cultivars were used in our experiments:

Triticum aestivum cv. Plainsman – drought resistant

GK Öthalom – medium drought resistant

GK Élet – drought sensitive

Mv Emese - drought resistant

Cappelle Desprez - drought sensitive

Kobomugi – drought resistant, adapted to semi-desert conditions

Applied treatments:

Osmotic stress was induced from day 7 after germination by increasing the osmolarity of the nutrient solution with polyethylene glycol (PEG 6000), on day 7^{th} to reach 100, on day 9^{th} to reach 200 and on day 11^{th} to reach the final value of 400 mOsm. In the grain filling period drought stress was generated by withholding irrigation. Plants were irrigated on every other day to achieve 60% (control) or 25% (stressed) of the total soil water-holding capacity for the control and the water-stressed plants, respectively.

Plant water relation and stomatal conductance parameters:

Water potential was measured by pressure chamber, relative water content by floatation method and the stomatal conductance by steady-state porometer.

Pigment and malondialdehyde content:

Chlorophyll, carotenoid and malondialdehyde content were determined by spectrophotometer analysis.

Abscisic acid content and aldehyde oxidase enzyme activity measurements:

Abscisic acid content was measured by competitive ELISA method, the enzyme activity of abscisic aldehyde oxidase was determined in native activity gel.

GST and GPOX activity measurements:

We determined the GST and GPOX activity by spectrophotometer using CDNB (1-chloro-2,4-dinitrobenzene) and cumene hydroperoxide substrates.

GST sequence searching and analysis:

We performed the GST sequence searching using *in silico* (in DDBJ/EMBL/GenBank and DFCI-TGI databases), and the sequences were aligned by ClustalW program.

Relative transcript amount measurements:

The relative transcript amount of the selected genes was determined by quantitative Real-Time PCR.

4. Results and discussion

Effect of drought stress on flag leaf senescence, physiological parameters and GST (glutathione transferase) expression of wheat cultivars with different drought susceptibility

In our experiments flag leaves of six wheat cultivars (Mv Emese, GK Élet, Plainsman, Cappelle Desprez, Kobomugi and GK Öthalom) were sampled at four time 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 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, which is a key component of the total yield.

1. During the premilk and medium milk periods changes were detectable in the senescence parameters of three wheat cultivars: Mv Emese, Plainsman and Cappelle Desprez. In contrast to data in literature, time-dependent increase of the total extractable GST activity was not detectable in those cultivars, where changes appeared in the senescence parameters. Senescence presumably causes inductions either of other GST isoenzymes, or in a later phase of aging.

2. Higher rate of yield stability indicates the water stress resistance of Plainsman and Kobomugi. GST and GPOX activity and expression of the well-watered flag leaves were the highest in Plainsman cultivar and showed significant induction due to stress. GST activity increased in every sampling day during water deficit in Kobomugi. Drought stress caused the highest elevations in the transcript amounts of the chosen GST genes in Plainsman and Kobomugi.

Drought stress caused high elevations in both phi and tau group GST expressions. The highest increases were detectable in the transcript amount of *TaGSTF6* and *TaGSTU1B/C* in most of the wheat cultivars. These data refer to the important roles of TaGSTF6 and TaGSTU1B/C gene products in the acclimatisation process. There were differences between the stress reactions of Plainsman and Kobomugi (two cultivars with the highest yield stability), among the GST groups different genes were induced due to stress (in case of Kobomugi *TaGSTU1C* and *TaGSTU19E50*, in Plainsman *TaGSTU1B* and *GSTF6*).

Effects of osmotic stress on the physiological parameters and GST expression of wheat cultivars

3. Drought- and osmotic stress resistance mechanisms of plants are divided into several types: some of them are able to function while dehydrated (desiccation postponement), others maintain tissue hydration (isohydric plants). The third category, drought escape, comprises plants that complete their life cycle during the wet season, before the onset of the drought. According to the water relation parameters Cappelle Desprez, GK Öthalom and Plainsman cultivars follow the desiccation postponement strategy, whereas Kobomugi proved to be isohydric. Plainsman and Kobomugi – the two cultivars with the highest yield stability – showed the least changes in the water relation parameters.

4. Osmotic stress caused the highest inductions in GST activity in the root of Kobomugi cultivar. In Plainsman cultivar - which showed also high yield stability - GST activity was almost unaffected by osmotic stress. According to Secenji et al. (2010) activation of the effective ascorbate cycle is the main component of water stress reactions in Plainsman seedlings, which can be the reason for the slight inductions in GST activity and expression during water deficit. In the sensitive Cappelle Desprez, changes in GST activity and expression in consonance with the flag leaf data indicated slow reaction to stress, which led to decrease in the water relation parameters and thousand grain yield. Osmotic stress caused significant increases in GST activity and expression in GK Öthalom. The tau group GSTs were significantly induced due to osmotic stress in all four investigated cultivars. The mRNA content of TaGSTU2 protein was prominently high due to the treatment. According to the literature, this protein has high conjugating activity towards CDNB substrate.

5. The ABA (abscisic acid) content of Kobomugi root and shoot indicated more effective ABA transport to the shoot, than in GK Öthalom. The stomatal conductance of Kobomugi decreased immediately after osmotic stress, which plays an important role in the maintenance of water potential. Induction of AAO2 (abscisic aldehyde oxidase 2) activity in the root might participate in the *de novo* ABA synthesis in both Kobomugi and GK Öthalom cultivars.

Inhibition of the ABA biosynthesis pathway led to the decrease of the *TaGSTU1C* and *TaGSTU2* gene expression in GK Öthalom cultivar in both control and osmotic stress conditions. In case of Kobomugi no significant changes were detecable in the expression of the examined gene, neither in the control, nor in the PEG treated samples due to ABA biosynthesis inhibition.

ABA has less importance in the regulation of the GST isoenzyme expressions in Kobomugi than in GK Öthalom. This enables the effective detoxification of the osmotic stress induced toxic metabolites in Kobomugi, even when the *de novo* synthesized ABA is intensely transported to the shoots.

LIST OF PUBLICATIONS

(* Present thesis is based on articles marked by an asterisk)

*1. **Gallé Á,** Csiszár J, Secenji M, Guóth A, Cseuz L, Tari I, Györgyey J, Erdei L. Glutathione transferase activity and expression patterns during grain filling in flag leaves of wheat genotypes differing in drought tolerance: Response to water deficit J Plant Physiol 2009;166:1878-1891. **IF: 2,456**

2. Guóth A, Tari I, **Gallé Á**, Csiszár J, Pécsváradi A, Cseuz L, Erdei L. Comparison of the drought stress responses of tolerant and sensitive wheat cultivars during grain filling: changes in flag leaf photosynthetic activity, ABA levels and grain yield. J Plant Growth Reg 2009;28:167–176.
IF: 2,109

*3. Sečenji M, Lendvai Á, Miskolczi P, Kocsy G, **Gallé Á**, Szűcs A, Hoffmann B, Sárvári É, Schweizer P, Stein N, Dudits D, Györgyey J. Differences in root functions during long-term drought adaptation: comparison of active gene sets of two wheat genotypes. Plant Biol DOI:10.1111/j.1438-8677.2009.00295.x
IF : 1.944

4. Guóth A, Benyó D, Csiszár J, **Gallé Á,·** Horváth F, Cseuz L, Erdei L,· Tari I. Relationship between osmotic stress-induced abscisic acid accumulation, biomass production and plant growth in drought tolerant and sensitive wheat genotypes. Acta Physiol Plant DOI: 10.1007/s11738-009-0453-6
IF: 0.807

5. Tari I, Kiss Gy, Deér AK, Csiszár J, Erdei L, **Gallé Á,** Gémes K, Horváth F, Poór P, Szepesi Á, Simon LM. Salicylic acid-induced increases in aldose reductase activity and sorbitol accumulation in tomato plants under salt stress. Biol Plantarum. 2010 (accepted)
IF (2008) 1.426

6. Guóth A, Tari I, **Gallé Á,** Csiszár J, Horváth F, Pécsváradi A, Cseuz L, Erdei L. Chlorophyll *a* fluorescence induction parameters of flag leaves characterize genotypes and not the drought tolerance of wheat during grain filling under water deficit. Acta Biol Szeged, 2009;53(1)1-7.

7. Szepesi Á, Csiszár J, **Gallé Á**, Gémes K, Tari I. Effects of long-term salicylic acid pre-treatment on tomato plants (*Lycopersicon esculentum* Mill. L. cvar. Rio Fuego) under salt stress tolerance: changes in glutathione S-transferase activities and anthocyanin contents. Acta Agron Hung 2008;58:129-138.

8. Csiszár J, Lantos E, Tari I, Madoşă E, Wodala B, Vashegyi Á, Horváth F, Pécsváradi A, Szabó M, Bartha B, **Gallé Á**, Lazăr A, Coradini G, Staicu M, Postelnicu S, Mihacea S, Nedelea G, Erdei L. Antioxidant enzyme activities in *Allium* species and their cultivars under water stress. Plant Soil Environ 2007;53:517-523.

9. Tari I, Csiszár J**,** **Gallé Á**, Bajkán Sz, Szepesi Á, Vashegyi Á. Élettani megközelítések gazdasági növényeink szárazságtűrésének genetikai transzformációval történő javítására. Bot Közlem 2003;90:113-132.

Conference abstracts published in scientific journals:

*1. **Gallé Á**, Csiszár J, Secenji M, Tari I, Guóth A, Györgyey J, Erdei L.: Monitoring the levels of phi and tau group GST genes in wheat cultivars under osmotic stress. Acta Biol Szeged 2008;52:95-96.

2. Guóth A, Tari I, **Gallé Á**, Csiszár J, Cseuz L, Erdei L. Changes in photosynthetic performance and ABA levels under osmotic stress in drought tolerant and sensitive wheat genotypes. Acta Biol Szeged 2008;52:91-92.

*3. **Gallé Á**, Csiszar J, Secenji M, Tari I, Györgyey J, Dudits D, Erdei L. Changes of glutathione S-transferase activities and gene expression in Triticum aestivum during polyethilene-glycol induced osmotic stress. Acta Biol Szeged 2005;49:95-96.

4. Csiszár J, Fehér-Juhász E, Kótai É, Ivankovits-Kiss O, Horváth GV, Mai A, **Gallé Á**, Tari I, Pauk J, Dudits D, Erdei L. Effect of osmotic stress on antioxidant enzyme activities in transgenic wheat calli bearing *MsALR* gene. Acta Biol Szeged 2005;49:49-50.

*5. **Gallé Á**, Csiszár J, Tari I. Erdei L. Changes in water and chlorophyll fluorescence parameters under osmotic stress in wheat cultivars. Acta Biol Szeged 2002;46:85-86.

Conference abstracts published in other journals:

1. Csiszár J, Poór P, **Gallé Á,** Benyó D, Horváth E, Kolbert Zs, Erdei L, Tari I. (2010) Role of H_2O_2, NO and peroxidases in the elongation growth of roots. International Conference on Molecular Aspects of Plant Development, 23-26. February, 2010, Vienna, Austria, Book of Abstracts, pp. 45.

2. **Gallé Á**, Csiszár J, Bartha B, Erdei L. The effect of heavy metal stress on GST activity and transcript amounts of GST and ABC transporter genes in *Brassica juncea.* 16-17 April 2009, COST Action 859 – WG1 & WG2 Workshop and MC Meeting Szeged

3. Csiszár J, **Gallé Á**, Guóth A, Erdei L, Tari I. Different reactions in root growth and peroxidase activities in root segments of wheat genotypes under osmotic stress. International Conference on Plant Abiotic Stress Tolerance, 8-11. February, 2009, Vienna, Austria, Book of Abstracts, pp. 89.

4. **Gallé Á**, Csiszár J, Secenji M, Guóth A, Tari I, Györgyey J, Erdei L. GST (glutation S-transzferáz) aktivitás- és génexpressziós vizsgálatok ozmotikus stressznek kitett búza növényeken. IX. Magyar Növénybiológiai Kongresszus, Szeged, 2008. július 7-9

5. Guóth A., Tari I., **Gallé Á.,** Csiszár J., Cseuz L., Erdei L. Fotoszintetikus hatékonyság és ABS tartalom változása ozmotikus stressz hatására szárazságtűrő és szenzitív búza genotípusokban. IX. Magyar Növénybiológiai Kongresszus, Szeged, 2008. július 7-9. 2008

6. Guóth A., Tari I., **Gallé Á.,** Csiszár J., Pécsváradi A, Cseuz L., Erdei L. Drought response strategies under grain filling in wheat. Changes in photosynthesis, ABA levels and grain yield. XVI. Congress of FESPB Federation of European Societies of Plant Biology, 17-22. August, 2008, Tampere, Finland, Abstract book, pp.134 2008

7. **Gallé Á.,** Csiszár J., Secenji M., Tari I., Guóth A., Dudits D., Györgyey J., Erdei L. Studies on glutathione S-transferase activities and gene expression levels in *Triticum aestivum* cultivars during polyethylene glycol-induced osmotic stress. 2nd World Conference of Stress, 23-26 August, Budapest, Hungary, Book of Abstracts, pp. 151. 2007

8. Guóth A., Tari I., **Gallé Á.**, Csiszár J., Cseúz L., Erdei L. Comparison of changes in photosynthesis, chlorophyll fluorescence parameters and abscisic acid levels in wheat cultivars under drought stress during grain filling and in seedlings under osmotic stress. 2nd World Conference of Stress, 23-26 August, Budapest, Hungary, Book of Abstracts, pp. 214. 2007

9. **Gallé Á**., Csiszár J., Secenji M., Tari I., Guóth A., Dudits D., Györgyey J., Erdei, L. Studies on glutathione S-transferase activities and gene expression levels in Triticum aestivum cultivars during polyethylene glycol-induced osmotic stress. 9th International Symposium Interdisciplinary Regional Research ISIRR, Novi Sad, 37 2007

10. **Gallé Á**, Csiszár J**,** Tari I, Erdei L. Induction of senescence during grain filling in wheat cultivars due to water stress. 3th EPSO Conference, Visegrád, Hungary, 2006. 184.

11. Csiszár J, Tari I, **Gallé Á**, Lendvai Á, Miskolczi P, Secenji M, Király É, Györgyey J, Erdei L. The role of glutathione S-transferases in the drought stress tolerance of different wheat genotypes. XVII. International Botanical Congress, Vienna, Austria, 17-23 July 2005

12. Tari I, Csiszár J, **Gallé Á**, Bartha B, Horváth F, Pécsváradi A, Szepesi Á, Zeller D, Erdei L. The role of ABA and NO in the drought stress acclimatisation mechanisms of wheat genotypes. XVII. International Botanical Congress, Vienna, Austria, 17-23 July 2005

13. Csiszár J, Tari I, **Gallé Á,** Király É, Erdei L. Investigation of drought tolerance of wheat varieties: the importance of glutathione S-transferase and peroxidase activities. Acta Physiol Plant 26: S 194 2004

IF: 0,438

Other publications

1**.** Csiszár J, Guóth A, Kolbert Zs, **Gallé Á**, Tari I. Starch content and α-amylase activities in different wheat cultivars. Zárójelentés. 56-65. old. Magyarország-Románia INTERREG IIIA Határon Átnyúló Együttműködési Programkomponens (projekt szám: HURO-06-02/006) 2008

2. **Gallé Á,** Csiszar J, Secenji M, Tari I, Györgyey J, Dudits D, Erdei L. Changes of glutathione S-transferase activities and gene expression in Triticum aestivum during polyethilene-glycol induced osmotic stress. Képzés és innováció a növénybiológiai felsőoktatásban SZTE Növényélettani Tanszék: 151-162 2006

3. Csiszár J**,** Tari I, **Gallé Á**, Király É, Sija É, Pécsváradi A, Erdei L. Antioxidative responces of wheat varieties with different drought stress tolerance under osmotic stress. Képzés és innováció a növénybiológiai felsőoktatásban SZTE Növényélettani Tanszék: 136-150 2006