

**Cultivar-dependent physiological and transcriptional changes during drought
stress in wheat**

Ph.D. Theses

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Introduction

Drought is becoming a more and more severe ecological problem, especially for the agriculture and consequently for food supply. Our important cultivated plants, such as wheat, corn, rice, are subjected to a severe water deficit as well resulting a lower yield. Breeders aim to find drought-resistant cultivars for the efficient crop production with the support of physiologists, biochemists and molecular biologists. The reason why the latter ones are involved, the complex plant responses to water-deficit stress that are realized at morphological, physiological, enzymatic, and transcriptional level following drought avoidance, drought escape or drought adaptation strategies. The main coordinator of stress responses is the stress hormone, abscisic acid (ABA) that is responsible for the stomata closure, decrease of transpiration, increase of root growth, development of side roots as well as water uptake of roots, and inhibition of shoot growth. Up- or down-regulation of different genes regulated via ABA-dependent or ABA-independent pathway is the basis of all of the changes. These genes encode proteins with distinct functions, such as enzymes of osmoprotectant biosynthesis, protecting proteins, antioxidant enzymes, aquaporins, proteases, transporters, proteins related to cell wall biogenesis, and regulatory proteins.

During drought stress, limited availability of CO₂ - as a consequence of stomata closure - facilitates electron transfer to O₂, increasing the generation of reactive oxygen species (ROS) such as superoxide anion radicals (O₂^{•-}), hydrogen peroxide (H₂O₂) and hydroxyl radicals (•OH). To prevent oxidative damage by these ROS, plants have evolved a complex antioxidant defense system, including both non-enzymatic and enzymatic constituents. The most important members of the first group are glutathione (GSH) and ascorbic acid (AsA). Key enzymes include superoxide dismutase (SOD), ascorbate peroxidase (APX) and catalase (CAT). AsA is a crucial antioxidant in plant cells which may act either directly as a free radical scavenger or indirectly as an electron donor to APX during the enzymatic reduction of H₂O₂. The product of ascorbate oxidation is a monodehydroascorbate (MDAsA) radical that is reduced back to AsA enzymatically by monodehydroascorbate reductase (MDAR). Two molecules of MDA may also disproportionate spontaneously to AsA and dehydroascorbate (DHAsA). DHAsA is reduced to AsA by a GSH-dependent enzyme, dehydroascorbate reductase (DHAR). Oxidized glutathione

(GSSG) is restored by glutathione reductase (GR) using NAD(P)H as an electron donor. APX, MDAR, DHAR and GR are the enzymes of the ascorbate-glutathione (AsA-GSH) cycle.

Aims of our study

The objects of our work were wheat genotypes with different drought stress tolerance and following their stress responses subjected to long-term, moderate water-deficit stress in the phase of vegetative development.

1. Comparing two drought-tolerant wheat genotypes (Plainsman V, Kobomugi) following distinct adaptation strategies, we aimed to answer the following questions:

- Which genes are up-regulated in the roots of the two genotypes during long-term, moderate water-deficit stress?
- Does the function of proteins encoded by the up-regulated genes reflect the different adaptation strategy of the two genotypes?

2. Comparing two drought-tolerant (Plainsman V, Xiaoyan-54) and two drought-sensitive wheat cultivars (Cappelle Desprez, Jing-411), we aimed to answer the following questions:

- Which physiological parameters are changed by long-term, moderate water-deficit stress?
- Which genes are up-regulated in the roots of the examined genotypes?
- Are there any “tolerance genes” showing different transcript profile in the drought-tolerant and sensitive cultivars subjected to water-deficit stress?

3. Comparing two genotypes with different drought tolerance (Plainsman V, Cappelle Desprez), we aimed to answer questions related to oxidative stress:

- Which isoforms of the ascorbate-glutathione cycle can be found in wheat?
- How does the transcript level of different isoforms change in the leaves of the two genotypes during long-term, moderate water-deficit stress?
- Is there any relation between transcriptional data and ascorbate/dehydroascorbate content measured during the experiment?

Materials and Methods

Examined wheat genotypes:

- *Triticum aestivum* L. cv. Plainsman V – drought-tolerant winter wheat cultivar from North America.
- *T. aestivum* L. cv. Cappelle Desprez – drought-sensitive winter wheat cultivar from France.
- *T. aestivum* L. cv. Jing-411 – drought-sensitive winter wheat cultivar from China.
- *T. aestivum* L. cv. Xiaoyan-54 – drought-tolerant winter wheat cultivar from China.
- *T. aestivum* L. Kobomugi – facultative spring landrace from Inner Asia.

Experimental systems:

- Plants grown in perlite

The treatment was started two weeks after germination by reducing quantity of irrigation solution, while the control plants were irrigated optimally. Samples were harvested every week for four weeks.

- Plants grown in the mixture of sand and perlite

The treatment was started two weeks after germination by decreasing relative field capacity to 30-40% by the end of the second week (2-week-old samples) followed by maintained water content at this FC% for another two weeks (4-week-old samples), while control plants were grown in the mixture with 70-80% FC.

Molecular analyses

- cDNA-microarray - hybridization of wheat root samples, grown in perlite, to a microarray containing barley cDNAs.
- Oligonucleotide-microarray - hybridization of wheat root samples, grown in the mixture of sand and perlite, to a microarray containing 50-60mer wheat oligos.

- real-time quantitative reverse transcription PCR (RT-qPCR) - validation of macro- and microarray data as well as determining transcript levels of the isoforms of ascorbate-glutathione cycle.
- Comparing amino acid sequences - composition of similarity trees of ascorbate-glutathione cycle enzymes in wheat based on multiple alignments of deduced amino acid sequences.

Physiological analyses

- Gas exchange - determining net CO₂ assimilation (*A*), transpiration rate (*E*), stomatal conductance (*g_s*), and water-use efficiency (WUE) in the leaves of plants grown in the mixture of sand and perlite by using IRGA.
- ABA content - in the root of plants grown in the mixture of sand and perlite by using ELISA.
- Relative proline content - in the leaves of plants grown in the mixture of sand and perlite by using spectrophotometry.
- Chlorophyll and carotenoid content - in the leaves of plants grown in the mixture of sand and perlite by using spectrophotometry.
- Relative water content - in the leaves of plants grown in the mixture of sand and perlite by measuring weights.

Results

1. Comparison of two drought-tolerant wheat genotypes subjected to long-term moderate water-deficit stress:

- Comparing the two drought-tolerant wheat genotypes, Plainsman V and Kobomugi, among the up-regulated genes oxidoreductases, peroxidases and cell wall-related genes were significantly induced only in Plainsman V while induction of stress- and defense-related genes was more pronounced in Kobomugi. In addition, the mRNA levels of GSTs used for macroarray validation were increased mainly in landrace Kobomugi. During the four-week-long moderate water-deficit in young plantlets, we identified a set of up-regulated genes displaying transiently increased expression, mostly on the second week in roots of the Kobomugi genotype while their transcript levels remained constantly high in roots of Plainsman V plants. These genes encode proteins with various functions, such as transport, protein and carbohydrate metabolism, osmoprotectant biosynthesis, and detoxification as well as regulatory proteins.

- The genotype Plainsman V follows an avoidance strategy - maintains root growth during water deficit that is in accordance with the induced genes encoding cell wall-related proteins. Furthermore, it decreases CO₂ fixation of leaves followed by growing numbers of ROS that are eliminated by peroxidases, for which mRNA level is increased by water deficit. The result of these changes together with enlarged WUE is a barely declined grain yield that is of high agronomic importance. On the contrary, the landrace Kobomugi closes stomata earlier than Plainsman V during drought stress, which results oxidative stress causing damages in macromolecules (indicated by increased MDA level) followed by switch on of the detoxifying system (GSTs). The RWC is maintained by stomata closure together with osmoprotectant biosynthesis (proline, sugars) however, the consequence of reserving water by decreasing stomatal conductance is a strongly decreased grain yield.

2. Comparison of four wheat genotypes differing in drought tolerance during water deficit:

- In this experiment, the RWC and pigment content of leaves of Plainsman V was decreased subjected to water deficit while proline content in shoots as well as ABA level in roots were increased. In case of Cappelle Desprez, stomatal conductance together with transpiration was

increased during drought stress while proline content of shoots was decline, which resulted a lower WUE. In the drought-tolerant Xiaoyan-54, photosynthetic capacity and transpiration was decreased via stomata closure while proline content in shoots together with ABA level in roots was increased. In the sensitive Jing-411, beside the increased ABA level in roots, stomatal conductance, photosynthetic capacity, chlorophyll content together with WUE was declined during water-deficit stress.

- Analyzing the transcriptional changes, genes encoding beta-glucosidases, trehalose-6-phosphate-synthases, catalases as well as receptor kinases were up-regulated in the roots of Plainsman V subjected to 2-week-long water-deficit stress. In case of roots of Cappelle Desprez, genes encoding beta-glucosidases, dehydrins, beta-galactosidases, lipoxygenases, GSTs, CYP450 monooxygenases, serine-type carboxypeptidases, and the enzymes of jasmonic acid biosynthesis were up-regulated during the stress treatment. In the roots of the tolerant Xiaoyan-54, transcript levels of catalases, hydrolases, asparagine synthases together with dehydrins and glycolitic enzymes were increased significantly compared to control conditions. Genes encoding glycolitic enzymes were up-regulated in the sensitive Jing-411, as well together with Myb-type transcription factors, dehydrins, Hsps, hydrolases, non-specific lipid transfer proteins, and glutamine synthetases. Summarizing the expression data, genes encoding proteins involved in signal transduction, nitrogen metabolism, cell elongation, and defense against oxidative stress were up-regulated significantly although each genotype induced different genes to enhance one of the above mentioned biological processes.

- We found several “tolerance genes” showing different transcript profile in the drought tolerant and sensitive cultivars subjected to water-deficit stress, such as GSTs, Hsps, aquaporins, various transporters, non-specific lipid transfer proteins, catalases, and histidine kinases.

3. Transcriptional changes of the genes involved in ascorbate-glutathione cycle in leaves of the drought-tolerant Plainsman V and the drought-sensitive Cappelle Desprez:

- Various isoforms of ascorbate-glutathione cycle were found in wheat according to the similarity trees based on the multiple alignments of deduced amino acid sequences. Their localization was predicted to cytosol (cAPX I, II; cMDAR I, II; cDHAR, and cGR), to stroma of chloroplast

(*sAPX I, II*; *chlMDAR*; *chlDHAR*, and *chlGR*) or bound to the thylakoid membrane (*tAPX*), and anchored to the membrane of peroxisome (*mAPX* and *mMDAR*).

- Among *APX* coding mRNAs, expression levels *cAPX I* and *cAPX II* as well as *tAPX* variants increased significantly in Plainsman V while *cAPX I* and *sAPX II* coding transcripts were found to be higher in Cappelle Desprez after a four-week-long water-deficit stress. Examining the *MDARs*, *cMDAR I* and *cMDAR II* displayed significant up-regulation of mRNA levels in the sensitive genotype, whereas only *cMDAR II* did in the tolerant cultivar. We found an up-regulated *chlDHAR* mRNA only in the sensitive Cappelle Desprez. However, increased expression levels of *cGR* and *chlGR* were detected only in the tolerant Plainsman V.

- After four weeks of reduced irrigation, a significantly lower AsA/DHAsA ratio was detected in leaves of the sensitive Cappelle Desprez than in the tolerant Plainsman V. Comparing the expression data with the ascorbate redox status, mRNA levels of *sAPX II* increased in the sensitive Cappelle Desprez which seems to be in accordance with a shifted AsA/DHAsA ratio in the absence of the up-regulated transcript amount of *chlMDAR*. On the other hand, the tolerant Plainsman V generally possesses a high amount of transcripts of each isoform coupled with a parallel up-regulation of mRNAs of cytosol localized APX (*cAPX I*, *cAPX II*) and MDAR (*cMDAR*).

Publications Related to the Theses

Sečenji M., Hideg É., Bebes A., Györgyey J. (2010a) Transcriptional differences in gene families of the ascorbate–glutathione cycle in wheat during mild water deficit. *Plant Cell Reports*, **29**, 37-50.

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Other publications

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