Summary of the Ph.D. Thesis

PHYSIOLOGICAL INDICATORS OF DROUGHT TOLERANCE OF WHEAT

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INTRODUCTION

Wheat (*Triticum aestivum* L.) is one of the main crops consumed by humans and it is cultivated in different environments. Under the temperate zone early-summer droughts are increasingly frequent and limit grain yield since they coincide with the grain filling period of most cereals, including wheat. The most important parameter of the wheat for which genotypes are screened during the process of breeding is the grain yield. But determining grain yield is time-consuming as the wheat plants have to be bred until the maturity of the grains. Therefore the selection would be time and energy-saving if a standard test system were worked out based on the correlations of certain physiological parameters and the drought tolerance and grain yield.

To reach this aim several experiments have been carried out on wheat in field and in greenhouses as well. In a greenhoue the drought stress can be stimulated in two different ways: with water deficit if the plants are grown in soil, or with germination and growth of seedlings in solution with high osmotic potential (e.g. in mannitol, polyethylene glycol, PEG).

For working out a test system, which could be used for selecting genotypes for drought toelrance the different conditions (filed, greenhouse) and varieties with different drought tolerance characteristics should be compared.

AIMS OF THE STUDY

Plant physiology is one of the most important areas of plant breeding.
We investigated several uncleared questions which can facilitate the breeding process of wheat. Our aim was to find physiological parameters which correlate with drought resistance. These correlations can be useful for the breeders who could select the varieties according to the drought resistance related beneficial properties in young plants.

Our aim was to compare the changes of the phisiological parameters in the young seedling stage under osmotic stress and in the in the garin filling period during water-defici and to find out whether the changes of these experiments correlate, and the changes obserbed in the seedling stage can charactherise the drought tolerance and final grain yield. We investigated whether the results of the experiment carried out in young seedlings can be used for charatherising the attitude of plants in the generative stage and for early determination of drought resistance or sensitivity of a wheat variety.

The other main goal of our work was to charactherising the drought resistance and sensitivity and to determine which physiological parameter can *unequivocally charactherise* the agronomic traits of drought resistance, namely the yiled parameters.

During our research work we were looking for the answers for the following questions:

a) Are the changes of the phisiological parameters of the two sets of experiments comaparable? Do the changes generated with PEG-6000 in the young seedlindgs correlate with those generated with soil dry in the grain filling period?

b) Is there any correlations between the photosynthetic activity of the flag leaves and the degree of grain filling?

c) How does the water-deficit effect the ABA (abscisic-acid) content of the flag leaves and grains and how do the changes of ABA concentartions influence the grain filling, final number and mass of grains?

d) How does the osmotic stress effect the ABA content of leaves and root in seedling stage? Is there any correlation between these ABA contents and the root and shoot growth, biomass production and stomatal conductance?

e) How does the osmotic stress effect the root growth of the drought tolerant and sensitive varieties? What are the role of NO (nitric oxide), ROS (reactive oxygen species) and ABA molecules in root growth under osmotic stress?

f) How does of the activity of abscisic aldehyde oxidase chnage in root under osmotic stress?

Materials and methods

Plant material:

Two drought-tolerant (*Triticum aestivum* L. cv. Plainsman V and MV Emese) and two drought-sensitive (cv. GK Élet and cv. Cappelle Desprez) winter wheat cultivars were investigated

Apllied treatments:

Osmotic stress was induced from day 7 after germination by adding increasing amounts of polyethylene glycol (PEG 6000) to the culture medium, on day 7 to reach 100, on day 9 to reach 200 and on day 11 to reach the final value of 400 mOsm. In the grain filling period the dought stress was generated by withholding irrigation. Plants were irrigated every 2 days 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:

The water potential was measured by pressure chamber, the osmotic potential by digital osmometer, and the realtive water content by the floatation method.

Pigment analysis:

The pigment composition of the extract made from plant tissue was measured with a double-beam spectrophotometer using the method of Lichtenthaler and Wellburn (1983)

Measurement of Chlorophyll Fluorescence and Photosynthesis:

The Chl a fluorescence induction parameters, the CO2 assimilation and the stomatal conductance were measured using a portable photosynthesis system (LI-6400, LI-COR, Inc., Lincoln, NE, USA). To determine the fuorescence induction parameters the methode of quenching analysis was used.

Determination of abscisic-acid and *trans*-zeatin riboside content:

We investigated the abscisic acid and *trans*-zeatin riboside content by competitive ELISA method, the enzyme activity of abscisic aldehyde oxidase was determined in native activity gel.

Detection of ROS and NO:

For visualization of ROS and NO an in situ dyeing method was applied. 4,5-diaminofluorescein-diacetate (DAF-2DA, 10μM) was used for the detection of NO and 2,7-dichlorofluoresceine diacetate (DC-FDA) was used for detection of ROS molecules. Samples were analysed by Zeiss Axiowert 200 M-type fluorescent microscope.

Determination of storage proteins:

The quantitative analysis of gliadins and glutenins was performed by reversed-phase high-performance liquid chromatography (RP-HPLC) after extraction.

RESULTS

Comparing the effects of osmotic stress in seedling stage with dought stress in the grain filling period and the charactherisation of drought resistance and sensitivity of wheat genotypes by physiological parameters are important objects of wheat breeding.
According to ur results we can make the following conclusions:

I. Comparing the osmotic stress in young seedlings with the drought stress in the garin filling period:

- According to the **yiled parameters** (number of grains, mass of grains) it is obvious that the GK Élet and the Cappelle Desprez are **drought sensitive** varieties, while the Mv Emese and the Plainsman are **drought tolerant** varieties. These data of our experiments carried out in greenhouse were similar to the results observed in field.
- **The water status parameters** of the drought sensitive genotypes, GK Élet and and the Cappelle D. decreased earlier and more significantly under water-deficit as well as under osmotic stress. The water status parameters of the leaves of the tolerant varieties did not change significantly until the final, senescence period. These varieties (Mv Emese and Plainsman) belong to the near isohydric plant genotypes. The water status parameters **had the same change tendencies the two sets of experiments**.
- **The changes of the fluorescence induction parameters were different in the two experiments**, the data measured during osmotic stress **did not correlate** with the data measured during drought stress. The tendecies were different, because significant differences between the control and the stressed plants were observed only on the last day of the experiment in the grain filling period, while the osmotic caused significant changes right after 1-2 days. We did not found any fluorescnet induction parameter which changed in the same way in the seedling stgae and in the grain filling period under stress. The CO2 assimilation neraly terminated under osmotic stress in the GK Élet while we observed the same in the Mv Emese in the grain filling period.
- The changes of the **photosynthetic activity of the flag leaves** under drought stress was not in close correlation with the drought tolerance of the varieties.

  According to our results **neither fluorescence induction parameter charactherised obviously the drought tolerance or sensitivity** of the genotypes and the fluorescent induction parameters did not provide definite information on tolerance or sensitivity.

- The results were similar in both experiments as the drought stress as well as the osmotic stress resulted in **higher ABA levels in the flag leaves and in the first and second leaves** in case of the tolerant genotypes. Although the ABA levels in the flag leaves changed significantly in the function of time therefore did not characterise obviously the tolerance.

II. Correlation between the degree of grain filling and the photosynthetic parameters, respectively the plant hormone levels

- The drought stress (and the osmotic stress) did not cause specific changes in the photocemical process of the PSII and the CO2 assimilation of the leaves charactherising the tolerant or the sensitive genotypes. The changes of the fluorescent induction parameters did not charactherise the drought tolerance strategies but the varieties.
- In our experiment obvious differences between the sensitive and tolerant genotypes were found in the **changes of ABA content of grains during drought stress**. The ABA level in the grains was increased significantly in all genotypes exposed to water stress at 9 DPA. In drought-tolerant cultivars Emese and Plainsman, the highest levels were measured at 9 DPA during the experimental period; the hormone levels then decreased rapidly until full maturity.In the sensitive GK Élet and Cappelle D., the ABA content was increased at 9 DPA too, but it **remained high throughout the following phases of grain filling**, allowing it to influence the accumulation of food reserves in the endosperm, hasten grain dormancy and programmed death of the starchy endosperm cells. The early and high ABA levels in the grains of the tolerant varieties can be beneficial for the regulation of the expression of the enzymes having role in the accumulation of the storage proteins.
- According to our results the **sensitivity of the reproductive organs**, the less effective fertilization process resulting lower numer of grains per eras and the continously high ABA content of the grains can be responsible for the decreased yield parameters. While the high ABA content of the grains in an eraly phase and the the senescence of the flag leaves can result in better yiled parameters.

III. Correlation between the root growth, biomass production durig osmotic stress and the ABA, NO and ROS leveles and AAO activity

- In seedling stage the **ABA content** of roots of the sensitive GK Élet increased earlier and to a higher extent after PEG exposure which plays an important role in the regulation of the **process of acclimatization**.
- The increase of the **ABA levels of the roots correlated with the increased activity of the AAO 2-3 isoenzymes** during the applied osmotic stress. Our results suggest that that the isoenzymes AAO1 and AAO2+3 could have important role in regulating ABA synthesis under osmotic stress.
- According to our results regarding the ABS, ROS and NO content of apical root segments the higher **ABA content is important in maintaining primary root elongation** under osmotic stress. The effects of ABA **could be modified by the levels ROS and NO molecules in the root elongation zone**. The combined effects of ROS and NO in the lack of the phisiologically important ABA led to a higher reduction of root growth in case of the tolerant Mv Emese.

We can conclude that the test system based on the investigation of young seedlings under osmotic stress conditions did not give exact infromation on the drought tolerance of the different wheat varieties. The drought tolerance of wheat is a complex process in the respects of plant physiology, in order to charactherise the drought tolerance several parameters have to be taken into consideration together, including the fertilazation and the grain filling process.

LIST OF PUBLICATONS

(* Present thesis is based is based on artcles marked by na asterisk)

***Guóth A.**, Tari I., Gallé Á., Csiszár J., Pécsváradi A., Cseuz L., Erdei L. **(2008)**: Comparison of the drought stress responses of tolerant and sensitive wheat cultivars during grain filling: Changes in flag leafe photosynthetic activity, ABA levels and grain yield. *Journal of Plant Growth Regulation* 28 (2): 167-176.
IF: 2,109

***Guóth A**., Benyó D., Csiszar J., Gallé Á., Horváth F., Cseuz L., Erdei L. and Tari I. **(2009)** Relationship between osmotic stress-induced abscisic acid accumulation, biomass production and plant growth in drought tolerant and sensitive wheat genotypes. *Acta Phisiologiae Plantarum* – accepted
IF: 0,807

Gallé Á., Csiszár J., Secenji M., **Guóth A.,** Cseuz L., Tari I., Györgyey J., Erdei L. **(2009)** Drought response strategies during grain filling in wheat. Glutathione transferase activity and expression pattern in flag leaves. *Jornal of Plant Physiology* DOI:10.1016/j.jplph.2009.05016.
IF: 2,456

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IF: 2,32

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

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

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

Király I., Baticz O., Larroque O.R., Juhász A., Tömösközi S., Békés F., **Guóth A.**, Abonyi T., Bedő Z. **(2004)** Relationship between functional properties of wheat dough and the relative proportion of the polymeric fraction
In: Lafiandra D, Masci S, D\'Ovidio R (eds.) *The gluten proteins*. RSC, Oxford, 323-326 (2004) (könyvfejezet)

Király I., Tömösközi S., Baticz O., Abonyi T., **Guóth A.**, Gergely Sz., Lásztity R. **(2007)** Study of the in vivo formation of gluten. 1. Synthesis of gliadins during kernel development. *Növénytermelés* 56 (3): 131-138.

Abonyi T., **Guóth A.**, Király I., Tömösközi S., Baticz O., Gergely Sz., Lásztity R. **(2007)** Study of the in vivo formation of gluten. 2. Synthesis and polymerisation of gluten subunits. *Növénytermelés* 56 (3): 139-145.

Conference abstracts published in scientific journals:

Baticz O., Király I., Abonyi T., **Guóth A.**,Gergely Sz., Tömösközi S., Lásztity R. **(2004)**
Study of the in vivo and in vitro polymerisation of polypeptides of gluten complex: Changes in gliadin components during ripening of wheat. In: Cauvain SP, Salmon SS, Young LS (eds.): 2th ICC Cereal and Bread Congress \'Using cereal science and technology for the benefit of the consumers\', Harrogate 2004.

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2nd World Conference of Stress, 23-26. August 2007, Budapest, Hungary, Book of Abbstracts, pp. 214.

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