Summary of the Ph.D. Thesis

The effects of abiotic stresses (drought and aluminium stress) on wheat (*Triticum aestivum* L.) glutamine synthetase

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Introduction

Glutamine synthetase (GS, 6.3.1.2) is the key enzyme of primary N assimilation, as well as ammonia reassimilation and detoxification. This enzyme has been found in all kinds of living beings from prokaryotes to eukaryotes. Early studies have showed that GS is widely distributed in the plant and occurs in two major forms, one in the chloroplast (GS2) and one in the cytosol (GS1). GS2 reassimilates the ammonia released as a result of photorespiration and nitrate reduction. The expression of GS1 is enhanced in later stages of flag leaf development, which may facilitate the recovery of nitrogen during senescence.

Wheat (*Triticum aestivum* L.) is one of the main crops consumed by humans and it is cultivated in different environments. In 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 analysis of stress response is important in the selection of plants. In our study we have investigated the drought and aluminium stress responses in wheat (*Triticum aestivum* L.) from the aspect of nitrogen metabolism and glutamine synthetase.

Aims of the study

The quantity and quality of crop yield depends on the stress resistance of the plants. On the one hand adequate agrotechnics, nutrition, minerals, water supply, protection against weeds and causative agents and diseases are needed for the improved yield. On the other hand we need plants that have adapted to a non-optimal environment. Of course the best result is the parallel and aligned methods and plants. The analysis of the stress responses is important in the selection of plants. In our study we investigate the drought and aluminum stress responses in wheat (*Triticum aestivum* L.) from the aspect of the nitrogen metabolism and glutamine synthetase.

Water stress has a considerable impact on the ecosystem and agriculture. During the grain filling period drought reduces photosynthesis, induces early senescence and shortens the grain filling period. Wheat shows a sequential type of leaf senescence. During growth of the whole plant, new young leaves successively form at the top, while lower and older leaves develop gradually toward the phase of senescence. In the course of this process, leaves from the base to the top pass different developmental stages, from maturation up to the last phase of

senescence including cell death. In monocarpic plants, developing grains represent the most important sink for carbon and nitrogen and other nutrients after anthesis.

Early studies have showed that glutamine synthetase is widely distributed in the plant and occurs in two major forms, one in the chloroplast (GS2) and one in the cytosol (GS1). GS plays a central role in nitrogen metabolism and there are multiple regulatory controls at the gene and protein level to modify its activity. We examined protein, Rubisco and GS amounts and activities in leaves during the grain filling period in search for the traits that ultimately determine the sequential senescence and the stay-green stage.

Acidification of soils may release water soluble, toxic aluminium species from clay minerals. Aluminium interferes with a wide range of physical and cellular processes. Aluminium toxicity could result from complex Al interactions with apoplastic, plasma membrane and symplastic targets. Plant GS requires two divalent cation binding sites, where magnesium binds under normal conditions. This makes GS a potential target of metal stress. Thet physiological, genetic and molecular basis of Al resistance is still the focus of investigation. Organic acids play a central role in aluminium tolerance mechanisms. Some plants detoxify aluminium in the rhizosphere by releasing organic acids that chelate aluminium. The other objective of this investigation was to prove that the activation of GS extracted from leaves of in vivo aluminium-treated wheat seedlings is because of aluminium ions bound to the polypeptide structure, potentially occupying the specific metal-binding sites of the GS molecule.

The main goals of this Ph.D. work were the following:

- How do the sink-source relations change under drought stress in sensitive and tolerant wheat genotypes?
- What are the connections between carbon and nitrogen metabolism under drought stress?
- How does the activity, amount and isoenzyme ratio change during drought stress?
- What is the effect of aluminium in wheat seedlings in an acidic environment?
- Does aluminium have a direct influence on glutamine synthetase?
- What is the effect of Al(III)NTA on glutamine synthetase?

Materials and methods

Plant material:

Our drought stress experiments were carried out on two Hungarian and four internationally known wheat genotypes: *Triticum. aestivum* L. cv. MV Emese, a drought-resistant Hungarian cultivar, *T. aestivum* L. cv. GK Élet, a drought-sensitive Hungarian cultivar, *T. aestivum* L. cv. Plainsman V, a drought-resistant North American cultivar, the drought-sensitive French *T. aestivum* L. cv. Cappelle Desprez, and two special landrace breeds. Kobomugi is a facultative spring landrace derived from inner Asia (China, Central Deserts); Kharchia is an Indian tall landrace wheat cultivar. The aluminium treated plants were *T. aestivum* L. cv. Jubilejnaja-50 seedlings.

Treatments:

The drought stress experiments were carried out in the grain filling period. The plants were grown in plastic pots containing a mixture of soil and sand. The control group of plants received sufficient irrigation, while the treatment group was subjected to water stress by withholding irrigation. Irrigation was adjusted to reach 60% of total soil water capacity for control plants, and the 25% for stressed plants.

Protein extraction and measurement

The GS containing protein extract was prepared from leaves and roots with extraction buffer in a 1:3 and 1:2 ratio. The tissues were homogenized in a mortar with sand. Protein concentration was determined according to Bradford (1976).

Measurement of the glutamine synthetase activity

The enzyme activity of GS was determined in vitro with a modified version of 'synthetase reaction' (Rhodes et al. 1975), measuring the γ-glutamyl monohydroxamate (GMH) formation.

Native gelelectrophoresis

Discontinuous non-denaturating polyacrylamide gel electrophoresis was applied for the separation of protein components of the extract (Laemmli 1970).

Western blot

Semi-dry blotting of proteins onto a polivinyliden-difluoride membrane was performed with the blotter unit, wetted with 50 mM borate buffer (pH 9.0) GS proteins were identified immunologically, with polyclonal anti-GS antibodies in western blot. Protein-A alkaline phosphatase conjugate was used as the secondary antibody (Bennett és Cullimore 1989).

Detection of aluminium and magnesium

The Al and Mg content of the gel subsamples was determined by inductively coupled plasma atomic emission spectroscopy (ICP-AES). Prior to the analysis, the gel subsamples (4 ml each) were digested in 3 ml of concentrated nitric acid. The digestion resulted in clear solutions. The analysis was performed at the Al 396.152 nm and Mg 279.553-nm emissionwavelengths using a Jobin-Yvon 24 type all-argon spectrometer equipped with a Teflon V-groove nebulizer and a Gilson Minipuls III peristaltic pump. Two determinations from each solutions were carried out using two-point background correction, four-point direct calibration and Gaussian spectral drift-compensation. Matrix-matched standard solutions for calibration were prepared from 1 g l21 commercial stock solutions through dilution by Millipore MilliQ quality deionized water and nitric acid addition.

Results

We investigated the effects of drought and aluminium stress on wheat glutamine synthetase.

I. Drought stress

1. The total protein content of the leaves, measured at the 9th day after anthesis, decreased with the age of leaves in well-watered plants.

2. Drought stress changed the protein content gradient in sensitive or less tolerant cultivars (Cappelle Desprez, GK Élet, Kobomugi)**: protein content was lower in the flag leaf compared to the older leaf.** However, the protein contents measured in leaves of tolerant genotype followed the same tendency as well-watered plants.

3. The changes in Rubisco content were in accordance with the changes in total protein content. In the older leaves of well-watered plants the Rubisco content decreased with age in all genotypes. Under drought stress the Rubisco content of the flag leaves of sensitive breeds was lower than that of the older leaves. In the tolerant genotypes high amounts of Rubisco were found in the flag leaf and less in the older leaves.

4. Total glutamine synthetase activity was determined from leaf samples of well-watered and drought stressed wheat cultivars. The changes in total GS activity were similar to the changes in total protein content. **In well-watered plants the enzyme activities in the older leaves of plants was lower than in the younger flag leaf in the sensitive and tolerant breeds as well. Under drought, GS activity was less in the flag leaf of the sensitive breeds than in the leaf below it. However, in tolerant genotypes, the GS activity in the flag leaf remained the highest under drought stress as well.**

5. Our results indicate that chloroplastic GS (GS2) is regulated in a different way than the cytosolic isoform (GS1). The GS2 is good indicator of the status of the chloroplasts**. The increasing ratio of cytoplasmic and chloroplastic GS is the sign of accelerated senescence induced by water deficit.**

6. Under water deficit stress we noticed two different modes of senescence. During drought the order of leaf senescence collapsed in sensitive genotypes (Cappelle Desprez, GK Élet and Kobomugi). In the tolerant genotypes (Plainsman V, Mv Emese and Kharchia),

drought stress did not lead to significant deviation in total protein and Rubisco contents and GS enzyme activities.

II. Aluminium stress

1. Aluminium chloride **inhibited root and shoot growth** of the wheat seedlings in acidic nutrient solution.

2. The positive effect of organic Al(III) complexes on the wheat glutamine synthetase activity depends on the speciation of the Al(III) complexes. Our study concludes, that the **$AlA(OH)_2$ form of Al(III) complexes is the most effective species in GS assay** in neutral (pH 7.5) conditions *in vitro*.

3. Aluminium(III)-nitrilotriacetic acid complex increased the GS activity in leaf extraction *in vitro*.

4. The aluminium and magnesium content of primary leaves of treated seedlings and isolated GS enzymes was determined with ICP-AES**. Aluminium(III) complex activated the GS, but could not functionally substitute magnesium ions, which were necessary for activity.**

5. Our results indicate that **Al^{3+} taken up by roots** from the acidic nutrient solution can reach the leaf cells and there **it can increase the glutamine synthetase activity *in vivo.***

List of publications

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

* Pécsváradi, A., **Nagy, Z.,** Varga, A., Vashegyi, Á., Labádi, I., Galbács, G. and Zsoldos, F. Chloroplastic glutamine synthetase is activated by direct binding of aluminium. DOI: 10.1111/j.1399-3054.2008.01167.x *Physiologia Plantarum* 135: 43-50, 2009. (IF: 2,708)

Nagy, Z., Németh, E., Gallé, Á., Csiszár, J., Erdei, L. and Pécsváradi, A. Changes in nitrogen metabolism of different wheat cultivars following *Fusarium* infection. HURO/0901/1472.2.2 Szeged – Timişoara axis for the safe food and feed SZETISA1, Book of Final Report, pp. 61-67, Szeged, 2012.

* **Nagy, Z.,** Németh E., Guóth, A., Bona, L., Wodala, B., Pécsváradi, A. Metabolilc indicators of drought stress tolerance in wheat: Glutamine synthetase isoenzymes and Rubisco. DOI: 10.1016/j.plaphy.2013.03.001 *Plant Physiology and Biochemistry* 67, 48-54, 2013. (IF: 2,838)

Posters:

Nagy, Z. and Pécsváradi, A.: Wheat Chloroplastic Glutamine Synthetase Is Activated by Aluminium, 3rd IFSDAA International Seminar on Crop Science for Food security, Bio-energy and Sustainability 1-3 June 2010 in Szeged, Hungary

Nagy, Z., Aleksza, D., Pécsváradi, A.: Aluminium activates the chloroplastic glutamine synthetase in wheat, 11th International Symposium Interdisciplinary Regional Research, 13-15. October 2010, Szeged, Hungary

Nagy, Z., Péter Szabó, K., Németh, E., Pécsváradi, A.: Glutamine synthetase isoenzymes of Nicotiana tabacum callus of leaf origin, A Magyar Növénybiológiai Társaság X. Kongresszusa, 2011. augusztus 31. – szeptember 2., Szeged, Magyarország

Nagy, Z. and Pécsváradi, A.: Role of glutamine synthetase isoenzymes and Rubisco in drought stress, 5th Conference of the Polish Society of Experimental Plant Biology, September 6, 2011 – September 9, 2011, Wroclaw, Poland

Conference abstracts:

Pécsváradi, A., **Nagy, Z.**, Németh, E.: Changes in glutamine synthetase activity and in protein pattern of wheat leaves after *Fusarium* infection. Szeged – Timisoara axis for the safe food and feed (SZETISA1), Hungary – Romania Cross-Border Co-operation Programme 2007-2013, Timisoara, Romania, January 26-27.01.2012

Nagy, Z., Németh, E., Pécsváradi, A.: Separation of protein content of stressed poplar leaves by two-dimensional polyacrylamide gel electrophoresis. Characterization and oxidative stress tolerance in plants: from models to trees (OXIT), Hungary-Serbia IPA Cross-border Co-operation Programme, November 20, 2012, Szeged, Hungary

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