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Role of the glutamine synthetase isoenzymes in abiotic stresses

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In plants, glutamine synthetase (GS, EC 6.3.1.2) is the key enzyme of primary nitrogen assimilation, as well as ammonia reassimilation and detoxification. GS catalyses an ATP-dependent conversion of glutamate to glutamine using ammonium derived from fertilizer, nitrate reduction, photorespiration in C3 plants, and numerous other sources including the catabolic release of ammonium during senescence. Plants have two different isoenzymes located in the chloroplast (GS2) and the cytosol (GS1). In young leaves, GS2 plays a major role in fixing ammonium into amino acids. In old, senescing leaves it is proposed that glutamine is synthesized by GS1 isoforms.

Soil acidification and drought stress are the most important abiotic factors in agriculture. A decrease in soil pH may release water soluble, toxic aluminium species from clay minerals. Al interferes with a wide range of physical and cellular processes. Plant GS requires two magnesium ions per subunit for activity, which makes GS a potential target of metal stress. The objective of the first investigation was to prove that Al from an organic metal complex is able to activate GS, and Al becomes bound to the polypeptide structure of the GS molecule. Aluminium(III)–nitrilotriacetic acid complex (Al(III)NTA) activated the GS prepared from wheat (*Triticum aestivum* L.) leaves, as Al^{3+} did in vivo, but could not functionally substitute magnesium ions, which were also necessary for the activity in the in vitro GS assay. GS2 was isolated by non-denaturing polyacrylamide gel electrophoresis, and the Al and Mg content of the enzyme was determined by inductively coupled plasma atomic emission (ICP) spectroscopy. The GS octamer remained intact and contained Mg^{2+} bound to its specific sites after the electrophoretic separation. Al was detected in the Al(III)NTA-treated sample bound to the structure of the enzyme protein, potentially occupying one of the specific metal-binding sites of the subunits. Our results indicate that the activator effect of the Al(III)NTA complex is due to the specific binding of aluminium to the polypeptide chain of GS2; however, the presence of magnesium at least on one of the metal-binding sites is essential for the active state of the enzyme.

Drought stress may have a considerable impact on the ecosystem and agriculture. Drought stress induces early leaf senescence. During this process, chloroplasts are degraded and photosynthesis drastically drops. The objective of this investigation was to look into the regulation of nitrogen and carbon metabolism during water deficit stress. GS isoenzymes are good markers of the plastid status (GS2) and the nitrogen metabolism (GS1). Tolerant and sensitive wheat genotypes were tested, which are widely used in agriculture. The amount of Rubisco (ribulose-1,5-bisphosphate carboxylase/oxygenase) and GS isofroms in leaves were measured during the grain filling period, as indicative traits that ultimately determine the onset and stage of senescence. The symptoms of senescence appear first on the oldest and finally on the youngest leaves. The sequentiality of senescence was disrupted in the sensitive varieties during drought stress. In the flag leaves an untimely senescence appeared, earlier than in the older leaf levels. Total protein and Rubisco content decreased and the GS2 isoenzyme disappeared. These physiological parameters did not change in the tolerant varieties under drought as compared to the control, well watered plants, or only the gradient of senescence became steeper, indicating the acceleration of this process.

Our results revealed the indicator role of GS in different abiotic stresses, which can be applied for characterization (classification) of wheat cultivars in terms of abiotic stress tolerance.

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Focusing on the fuction of endogenous galectin-1 produced by activated T-cells

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The immune system is a tightly regulated network that is able to maintain a balance of immune homeostasis under physiological conditions. Normally, when challenged with foreign antigen, specific response is initiated which aims restoring homeostasis. In this regard the final step during immune response is the elimination of the antigen activated specific lymphocytes by apoptosis. Death of the already functionless cells may occur via different mechanisms including galectin-1 (Gal-1), a β-galactoside binding mammalian lectin, which has been implicated in T-cell apoptosis. Recent studies from our and other laboratories showed that Gal-1 induces apoptosis of activated peripherial inflammatory Th1 cells, hence participating in the down-regulation of the T-cell response. This cytotoxic effect of Gal-1 is exerted by secreted, extracellular protein acting on Gal-1 binding structures on activated T-cells. On the other hand Gal-1 is expressed in activated but not resting T-cells. However the cellular localization and function of the *de novo* expressed Gal-1 in activated T-cells has not been revealed yet. Our aim was to determine the role of T-cell derived Gal-1 in the regulation of T-cell viability.

First we established Gal-1 transgenic Jurkat T-cell line, JGal as a model of activated T-cells. Using JGal, we demonstrated that the transgene Gal-1 remained intracellularly and these cells became more sensitive to apoptosis induced by extracellular exogenous Gal-1 delivered either as soluble or HeLa cell-derived protein. Moreover other cytotoxic reagents, such as hydrogen-peroxide and staurosporine induced lower apoptotic response compared to that of the non-transgenic (Gal-$1^{-/-}$) Jurkat cells.