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Effect of Water Stress on Protein Content of Some Calvin Cycle Enzymes in Different Wheat Genotypes

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The dynamics of protein content changes of some photosynthetic enzymes under water stress has been studied by immunoblotting method in bread (*Triticum aestivum*) and durum (*Triticum durum*) wheat genotypes differing in their drought tolerance. In all tested genotypes protein content of studied enzymes under weak and mild drought stress was similar to their content in normal watered variants. While protein content of phosphoribulokinase (PRK) and fructose-1,6-bisphosphatase (FBP) remained unchanged, sedoheptulose-1,7-bisphosphatase (SBP), transketolase (TK), NADP-glyceraldehyde-3-P-dehydrogenase (NADP-GAPDH) and Rubisco activase decreased under severe water stress. This decrease was more apparent for TK, SBP and Rubisco activase. However in drought tolerant genotypes Azamatli-95 and Barakatli-95 this decrease was less pronounced compared to genotypes Garagylchyg-2 and Giymatli-2/17, which are sensitive to drought. At the early stage of heading of the genotype Garagylchyg-2, during 2-3 days of water stress protein content of PRK, FBP and NADP-GAPDH was unchanged in flag leaves, while in ear elements protein content of these enzymes decreased significantly.

Keywords: *Calvin cycle, drought, wheat genotypes*

INTRODUCTION

At present in consequence of global climate changes a progressive increase of the average annual temperature of the earth results in the development of some stress factors. This leads to the significant decrease of the productivity of agricultural plants in regions having water deficit. Modern biotechnological methods have been used now to investigate molecular-genetic bases of drought resistance to create cultures tolerant to drought and high temperature (Raines, 2006). Modern investigations of genotypes differing in their drought tolerance within the same species and among the ancestors of crops may serve as a marker in obtaining more productive genotypes. Photosynthetic CO_2 assimilation in C_3-plants is affected by environmental variables including temperature, CO_2 concentration and water availability. Of these variables, water is the main biotic factor limiting plant productivity in many regions of the world (Chaves et al., 2002). Water stress can affect photosynthesis directly by causing changes in plant metabolism or indirectly by limiting the amount of CO_2 available for fixation (Lawlor and Cornic, 2002). Drought resistance of a plant is related to its ability to maintain higher relative water content in the leaves under water stress. Many changes in gene expression occur in plants growing under limited water conditions (Bray, 2002).

The data on water stress induced regulation of the activity of photosynthetic enzymes other than Rubisco are scarce. Thimmanaik et al. (2002) studied the activity of several photosynthetic enzymes under progressive water stress in two different cultivars of *Morus alba*. Unlike Rubisco, which is highly stable and resistant to water stress, the activity of some enzymes involved in the regeneration of ribulose-1,5-bisphosphate (RuBP) are progressively impaired from very early stages of water stress. Thus, these results present the possibility that some enzymes involved in the regeneration of RuBP could play a key regulatory role in photosynthesis under water stress. During water stress induced by polyethilenglycole, Rubisco activity significantly increased in young potato leaves, while decreased in mature leaves (Bussis et al., 1998). But NADP-GAPDH and PRK activities have been decreased and this change became faster in the course of drought. While decreased Rubisco activity may not be the cause of photosynthetic

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reduction during water stress, its down-regulation may still be important because it could preclude a rapid recovery upon rewatering (Ennahli and Earl, 2005). Similarly, some reports have shown strong drought-induced reductions of Rubisco activity per unit leaf area (Maroco et al., 2002) and per mg showed that the decrease of Rubisco activity *in vivo* was not connected with the protein content. It occurs because of CO_2 concentration decrease in the carboxylation center in consequence of the partly closing of stomata (Flexas et al., 2006). But it is known, that enzyme regulation occurs not only in transcription, but also in posttranscriptional level. Activities of the tested enzymes are regulated by light as well as by the concentration of photosynthetic metabolites (Raines, 2006). Reductions of more than 50% in the levels of NADP-GAPDH, FBP, PRK, and plastid aldolase were also needed before photosynthetic capacity was affected (Stitt and Schulze, 1994). Although the level of control exerted by any single enzyme in the cycle varied with environmental conditions and developmental status, the data from the transgenic plants strongly indicated that a number of enzymes in the Calvin cycle are present in excess. The implication from this is that the levels of Rubsico, NADP-GAPDH, FBP, and PRK are not close to limiting carbon fixation through this cycle and therefore would not be useful targets for overexpression to increase photosynthetic capacity.

In contrast, photosynthesis has been shown to be sensitive to small reductions in the levels of the enzymes TK and SBP (Harrison et al., 2001). TK catalyzes three reactions in the regenerative phase of the Calvin cycle. A small decrease (20-30%) in TK resulted in a reduction of photosynthetic carbon fixation, and flux to phenylpropanoid metabolism was also decreased, making this enzyme a potential target for overexpression.

The study of gene expression and polypeptide content changes of some Calvin cycle enzymes under water stress will probably promote to evaluate the decrease of CO_2 photosynthetic assimilation.

MATERIALS AND METHODS

Plant material and growth conditions. The seeds of two bread wheat (*Triticum aestivum* L.) genotypes (Giymatli-2/17, Azamatli-95) and two durum wheat genotypes (Garagylchyg-2, Barakatli-95) were supplied by Research Institute of Crop Husbandry (Baku, Azerbaijan). The germinated seeds were planted in 12.5 cm pots filled with peat- and loam-based compost and grown in a temperature-controlled greenhouse at day/night temperature of 24/18°C. The plants were watered regularly with Hoagland's solution. Recently fully expanded 4th or 5th leaf and flag leaf and ear elements were used in experiments.

Leaf Relative Water Content (RWC) determination. RWC was measured simultaneously on two leaves from both stressed and well-watered plants. After weighing (fresh weight, FW), leaves were cut into parts and placed in water in a closed Petri dish. After 24 h at 4°C, leaf pieces were weighed (turgid weight, TW). Dry weight (DW) was measured after 48 h at 60°C. RWC was calculated as (100 x (FW-DW)/(TW-DW)).

Protein Extraction and Western-Blot Analysis. Leaf discs (two leaf discs 0.75 cm^2) were isolated from the leaves and frozen in liquid nitrogen. The frozen leaf discs were ground to a fine powder in liquid nitrogen using a mortar and pestle in extraction buffer (50 mM Hepes, pH 8.2; 5 mM $MgCl_2$; 1 mM EDTA; 1 mM TGTA; 10% glycerol; 0.1 % Triton X-100; 2 mM benzamidine; 2 mM amino capronic acid; 0.5 mM phenylmethylsulfonylfluoride (PMFS); 10 mM dithiothreitol (DTT)), transferred to a prechilled tube and spun in a microcentrifuge for 1 min at 4°C. The supernatant was removed for protein estimation and Western blotting. Protein was determined by the method of Bradford (1976) with bovine serum albumin as a standard. An equal volume of SDS-loading buffer (150 mM Tris-HCl, pH 6.8, 4% (w/v) SDS, and 10% (v/v) 2-mercaptoethanol) was added to the supernatant for Western blot. The homogenates were boiled for 5 min and centrifuged at 10 000 g for 10 min. Samples were loaded on an equal protein basis (5 mg), separated using 12% (w/v) SDS-PAGE gel according to the method of Laemmli (1970). Then blotted onto nitrocellulose in transfer buffer (50 mol m^{-3} Tris base, 380 mol m^{-3} Gly, 0.1% [w/v] SDS, and 20% [v/v] methanol) at approximately 4°C overnight at 50 V. Prior to incubation with antibody the membranes were washed in phosphate-buffered saline (PBS) containing 0.05 (v/v) Tween 20 (PBS-T) and then blocked in PBS-T containing 6% milk powder (w/v). The nitrocellulose membranes were incubated with the appropriate primary polyclonal antibodies for 1.5 h after blocking for 2 h at room temperature with 6% (w/v) skimmed milk in phosphate-buffered saline (PBS) containing 0.0005% (v/v) Tween 20. After six washes with PBS-T, blots were incubated for 2 h at room temperature with a 1:5.000 dilution in PBS-T of sheep anti-rabbit secondary antibody conjugated to horseradish peroxidase. After six PBS-T washes, the secondary antibodies were detected using enhanced chemiluminescences according to the manufacturer's directions. Prior to re-probing of the membranes, antibodies were removed following ECL detection by immersing in buffer containing 100 mM 2-

mercaptoethanol, 2% SDS, 62.5 mM Tris-HCL, pH 6.7, for 30 min at 50°C.

RESULTS AND DISCUSSION

Wheat is one of the most important food crops and its productivity is markedly influenced by soil water availability. Two durum (Barakatli-95, Garagylchyg-2) and two bread (Azamatli-95, Giymatli-2/17) wheat genotypes were used as the investigation objects. Results showed that depending on the duration and severity of drought protein content of some Calvin cycle enzymes changed differently. Effect of drought on protein content of some Calvin cycle enzymes (NADP-GAPDH, Rubisco activase, TK, PRK, SBP and FBP) was tested by immunoblotting method. When RWC reached 85-80%, protein content of tested enzymes remained unchanged. Protein content of almost all the tested enzymes under progressive stress was similar to their content in normal watered variants (Figure 1).

But at 70% of RWC, protein content of TK, FBP, NADP-GAPDH, and SBP began to decrease. This decrease was more pronounced in genotypes sensitive to drought. Protein content of SBP, TK, NADP-GAPDH, Rubisco activase decreased significantly in contrast with PRK (Figure 2) which content remained unchanged under severe water stress. This decrease was also different for each tested genotype. Thus in a drought-tolerant sort Barakatli-95 protein content of the tested enzymes was close to their content in normal watered variants.

In drought-tolerant Azamatli-95 (durum) and Barakatli-95 (bread) varieties this decrease was less apparent, though in drought sensitive Garagylchyg-2 and Giymatli-2/17 protein content of TK and SBP enzymes significantly decreased under severe water stress (Figure 3).

Protein content of PRK was unchanged independently of the severity of drought. But protein content of Rubisco activase was unchanged in drought-tolerant variety Barakatli-95 (Figure 4).

In contrast to flag leaves, in initial ear elements protein content of some photosynthetic enzymes showed different changes from the beginning of

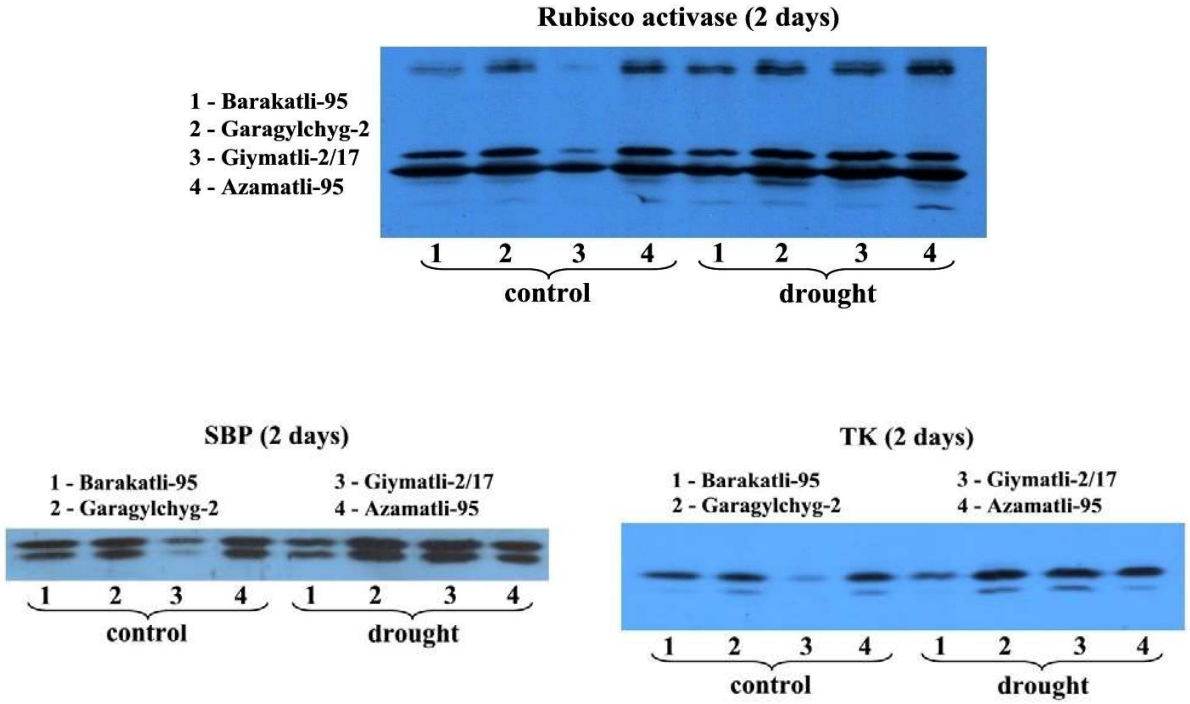


Figure 1. Effects of progressive drought stress on the protein content of Rubisco activase, SBP and TK in different wheat genotypes.

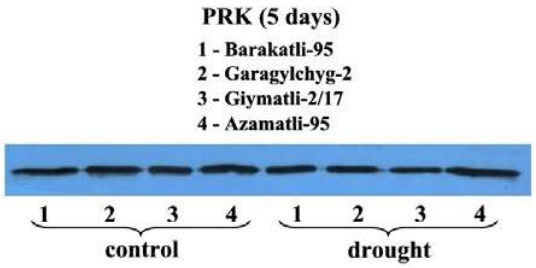
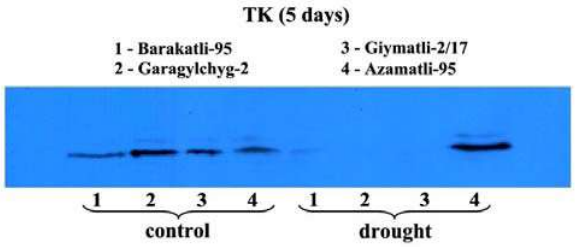


Figure 2. Effects of severe drought stress on the protein content of PRK in different wheat genotypes.



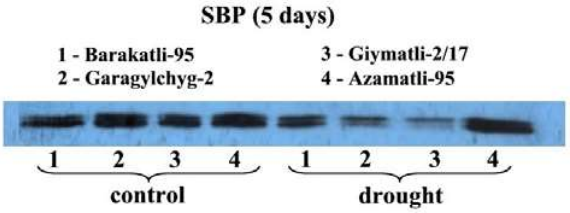


Figure 3. Effects of severe drought stress on the protein content of TK and SBP in different wheat genotypes.

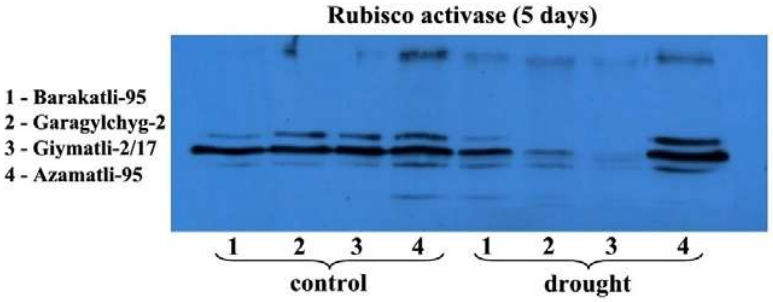


Figure 4. Effects of severe drought stress on the protein content of Rubisco activase in different wheat genotypes.

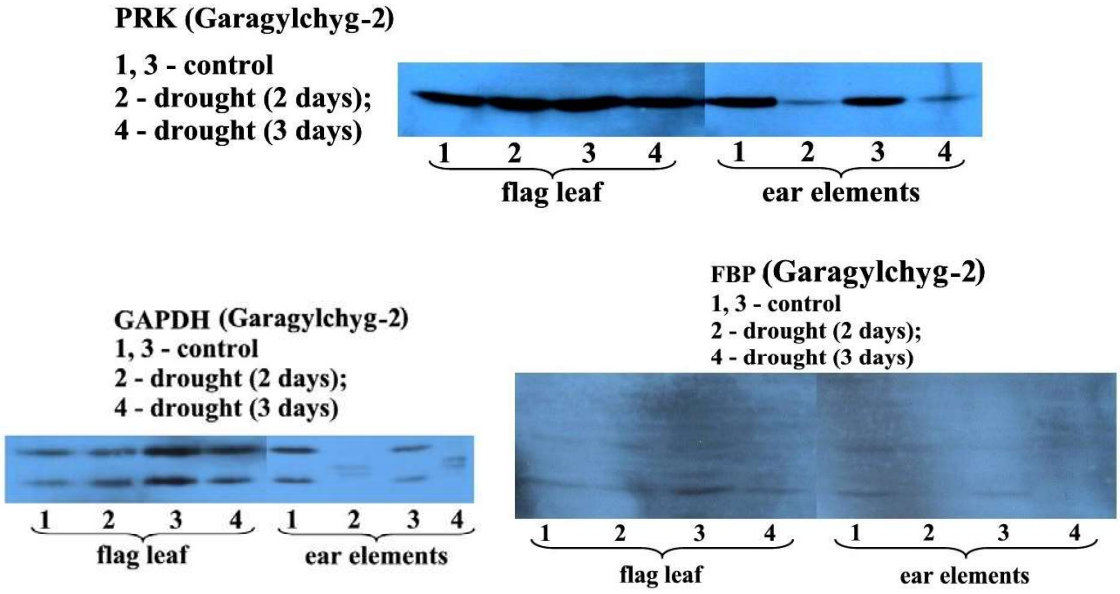


Figure 5. Effects of progressive drought stress on the protein content of some Calvin cycle enzymes in flag leaf and ear elements.

drought stress. During 2-3 days of drought stress protein content of NADP-GAPDH, PRK, FBP was unchanged in flag leaves, while their content sharply decreased in ear elements (Figure 5).

Previous investigations (Guliyev et al., 2008) showed that Rubisco activity was almost unchanged in drought-tolerant genotypes under weak drought stress. But when RWC was less than 80%, the activity decreased faster and this tendency was more pronounced in genotypes sensitive to drought.

In field-grown maize (*Zea mays*), losses in grain yield are maximal when drought occurs during the flowering stage (Andersen et al., 2002). In barley plants depending on the duration and severity of drought stress protein content of some Calvin cycle and photorespiration enzymes changed differently (Wingler et al., 1999). While Rubisco and plastidic FBP remained unchanged the content of SBP and NADP-GPDH decreased. The photorespiratory enzyme - chloroplastic glutamine synthetase remained unchanged as stress developed but the content of NADH-dependent hydroxypyruvate reductase increased.

In vitro assays of the content of key Calvin cycle enzymes involved in the regeneration of RuBP, by Western blot analyses, indicated that UV-B irradiation of mature leaves of oilseed rape induced a reduction in the content of SBP, but not FBP or PRK on a leaf area basis (Allen et al., 1998).

Rubisco activase has been reported to be particularly sensitive to inactivation by high temperature stress, and most of the Rubisco activase is sequestered to the thylakoid membrane from the soluble stromal fraction during high temperature stress (Rokka et al., 2001). Recently in SBP overexpression rice plant showed that the content of RuBP and Rubisco activase in the soluble stroma fractions decreased significantly under salt stress, and such a decrease was more pronounced in wild-type plants than in transgenic plants (Feng et al., 2007). Analyses of Western blotting also showed that there were no changes in the content of Rubisco and PRK in wild-type and transgenic plants during salt stress. In contrast, initial Rubisco and PRK activities clearly decreased with increasing salt concentration in rice leaves (Feng et al., 2007). Recently Xue et al. (2008) have reported that expression levels of most genes encoding chloroplast enzymes involved in carbon fixation (Calvin cycle) were reduced in the bread wheat leaves during prolonged drought stress.

The change of protein content of Calvin cycle enzymes under drought observed in our experiments were similar to that under influence of salt, heat and UV-B radiation. Therefore, we conclude that this change is independent of the stress sources. The sharp decrease of tested enzymes in an early stage of development in the ear elements in contrast with a flag leaf verified its more sensitiveness to water deficit. Obtained results suggest that water stress effects on protein content of Calvin cycle enzymes differently depending on the development stage of the plant and also its separate organs. These results would promote to choose the right irrigation time.

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