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ORIGINAL ARTICLE

Changes in protein quantities of phosphoenolpyruvate carboxylase and Rubisco activase in various wheat genotypes

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Abstract In early seedlings of wheat genotypes two isoforms of Rubisco activase with molecular weights of 42 and 46 kDa are expressed. Amounts of both isoforms significantly increase in early seedlings of the durum wheat genotype Barakatli-95 exposed to salt stress. But at the beginning of the tillering stage, the changes in quantities of both RCA isoforms are different in durum and bread wheat genotypes subjected to a 3-day drought stress. In the leaves of the early seedlings of the studied wheat genotypes exposed to drought stress quantities of PEPC subunits increase compared to the control but they remain relatively stable in early roots and germinating seeds. However, quantities of its subunits decrease sharply in roots and germinating seeds of early seedlings under the influence of 100 mM NaCl. In flag leaves and ear elements of the Barakatli-95 genotype grown under normal water supply conditions protein quantities of PEPC subunits change differently depending on time. Changes in protein quantities of RCA, PEPC and Rubisco enzymes have been studied comparatively in ear elements and flag leaves after the fourth day of anthesis.

1. Introduction

Activating the ubiquitous enzyme Ribulose-1,5-bisphosphate carboxylase/oxygenase (EC 4.1.1.39) (Rubisco), which catalyzes the initial reaction of photosynthetic carbon assimilation, Rubisco activase plays an important role in the regulation of plant growth. So, Rubisco activase (RCA) adjusts the conformation of the active center of Rubisco removing tightly bound inhibitors thus contributing to the enzyme rapid carboxylation (Portis, 2003; Spreitzer and Salvucci, 2002; Carmo-Silva and Salvucci, 2013).

Phosphoenolpyruvate carboxylase (PEPC) catalyzing irreversible carboxylation of phosphoenolpyruvate and converting it to oxaloacetate plays an important role in carbon and nitrogen metabolism of C3 plants. Acting in cytoplasm of plant cells PEPCs play different physiological roles depending on the developmental phases of plants. Nonphotosynthetic PEPCs are generally less well described in terms of their genetic origin and post-translational controls (O'Leary et al., 2011; Shi et al., 2015).

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According to our previous research, activities of the enzymes-PEPC and Rubisco changed simultaneously in flag leaves and ear elements of different wheat genotypes under normal water supply conditions and positive correlation was observed between the levels of the enzyme activities and grain yield (Aliev et al., 1996). Holadey et al. (Holaday et al., 1992) observed reduced total activity of Rubisco in wheat flag leaves during anthesis under drought. Total protein and chlorophyll amounts were also shown to be decreased under drought. As drought occurs gradually under field conditions, photosynthetic carbon assimilation and also distribution and consumption of formed assimilates are probably limited simultaneously with the decrease in CO_2 diffusion rate. So, in Summer when natural drought takes place partial closure of stomata results in limited transportation of CO_2 to chloroplasts causing thereby a decline in the Calvin cycle enzyme activities (Maroco et al., 2002). Tambussi et al. remarked a less decrease in RWC and water potential in wheat ear elements compared with flag leaves under drought. They also detected a higher rate of photosynthesis in ear elements than in flag leaves at the last stages of grain filling (Tambussi et al., 2007). Therefore, the comparative study of protein accumulation of the enzymes catalyzing the initial CO_2 carboxylation – PEPC, Rubisco and RCA participating in the catalytic activation of Rubisco in wheat genotypes contrasting in drought tolerance is of great theoretical and practical importance.

2. Materials and methods

2.1. Plant materials and treatments

Currently wheat genotypes chosen for the study are being extensively used in sown fields of Azerbaijan and they manifest different tolerance to drought and salt stresses. Two local bread wheat (*Triticum aestivum*) genotypes differing in drought-resistance (Giymatli-2/17 – drought-sensitive, and Azamatli-95 drought-tolerant) and two local durum wheat (*Triticum durum*) genotypes (Barakatli-95 – drought tolerant, and Garagylchyg-2-drought sensitive) were used as the study objects. Seeds were sterilized and sown in vegetation vessels filled with peat-soil mixture. Plants were kept in a growth chamber with controlled temperature and illumination regimes with 10 h/14 h of dark/light period at 24/18 °C, respectively. Plants were watered daily with 50% Hoagland solution. Early seedlings as well as matured plants were used in the study. Plants were exposed to drought and salt stresses during different stages of the vegetation.

2.2. Immunoblotting method

Immunoblotting analysis was used in the study (Bayramov and Guliyev, 2014). Denaturing (SDS–PAGE) polyacrylamide gel electrophoresis was performed according to the Laemmli method (Laemmli, 1970). Western blots were developed using the enhanced chemiluminescence (ECL) peroxidase system.

2.3. Relative water content

Relative water content (RWC) was determined in flag leaf as per the method of Barrs and Weatherley (1962). For the determination of RWC, fresh leaves were weighed to get fresh weight (FW). Later, floated on distilled water at 4 °C overnight, weighed again (TW), and dried at 70 °C for 48 h, after which, dry mass was determined (DW). RWC was calculated as:

$$RWC = [(FW - DW)/(TW - DW)] \times 100$$

2.4. Protein measurement

The protein concentration was determined by the Bradford method with bovine serum albumin as a standard (Bradford, 1976).

2.5. Statistical analysis

Each result shown in figures was the mean of at least three replicated measurements. The intensities of bands in Western blots were quantified with an image analysis program (ImageJ1.37v).

3. Results and discussion

In early seedlings of wheat genotypes grown under normal water supply conditions Rubisco activase isoforms with molecular weights of 42 and 46 kDa were expressed. When the initial seedlings of the Barakatli-95 genotype were transferred to the medium containing 100 mM NaCl, protein quantity of both Rubisco activase isoforms increased significantly depending on the duration of salt stress. This increase was more pronounced for the 46 kDa isoform. However, no marked changes occurred in protein quantities of both isoforms in seedlings exposed to drought (Fig. 1). But at the beginning of the tillering stage protein quantities of both Rubisco activase isoforms in leaves of durum and bread wheat genotypes changed differently in parallel with the decrease of RWC after a 3-day exposure to drought (Table 1). During the first days of stress the

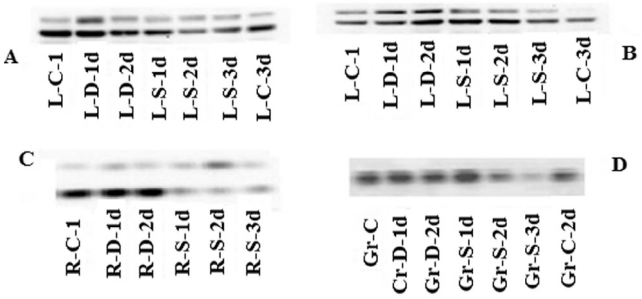

Figure 1 Western-blot analysis of changes in the protein quantities of Rubisco activase isoforms (A) in leaves of early seedlings of different wheat genotypes under normal water supply; (B) in early seedlings of the Barakatli-95 (Bar-) genotype exposed to gradual drought (D-) and 100 mM NaCl (S-); (C) in bread wheat (Azamatli-95 (Aza-) and Giymetli-2/17 (Giy-)) genotypes and (D) in durum wheat genotypes (Barakatli 95 (Bar-) and Garagilchig-2 (Gar-) at the middle of the tillering stage 10 μg protein of each sample was loaded on 10% SDS polyacrylamide gel to perform electrophoresis.