Saudi Journal of Biological Sciences (2017) 24, 1529–1533

King Saud University

Saudi Journal of Biological Sciences

www.ksu.edu.sa
www.sciencedirect.com

ORIGINAL ARTICLE

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

Shahniyar Bayramov *

Institute of Botany, Azerbaijan National Academy of Sciences, 40 Patamdar Shosse, AZ-1073 Baku, Azerbaijan

Received 9 June 2015; revised 2 September 2015; accepted 6 September 2015
Available online 14 September 2015

KEYWORDS

Rubisco activase;
Phosphoenolpyruvate carboxylase;
Drought;
Salt;
Wheat

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).

* Tel.: +994 504273377; fax: +994 125102433.
E-mail address: sbayramov@hotmail.com.
Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

http://dx.doi.org/10.1016/j.sjbs.2015.09.013
1319-562X

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

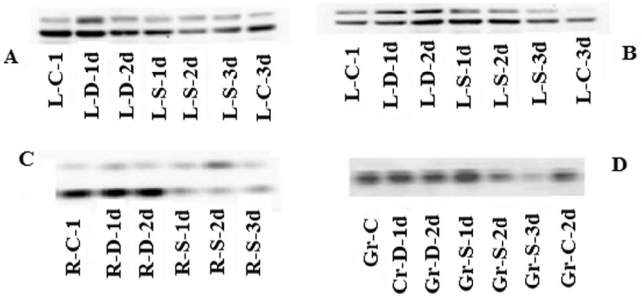

Figure 1 Western-blot analysis of changes in the protein quantities of Rubisco activase isoforms (A) in leaves of early seedlings of different wheat genotypes under normal water supply; (B) in early seedlings of the Barakatli-95 (Bar-) genotype exposed to gradual drought (D-) and 100 mM NaCl (S-); (C) 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.

protein quantity of the 46 kDa isoform remained unchanged in bread wheat genotypes, while the protein quantity of the 42 kDa isoform decreased gradually depending on the duration of stress. However, in durum wheat genotypes the protein quantity of the 46 kDa isoform exceeded several times the protein quantity of the 42 kDa isoform under drought. The protein quantity of the 46 kDa isoform remained stable during the first 2 days of the stress and it began to decrease differently when RWC of leaves reduced and became less than 70%. Thus, this decrease was more pronounced for the Barakatli-95 genotype. In durum and bread wheat genotypes grown in a greenhouse under natural illumination at the end of May when daytime temperature was 28–30 °C amounts of Rubisco activase isoforms changed differently in response to decrease in RWC depending on the duration of stress (Fig. 1C).

The most decrease in RWC was observed in the Azamatli-95 and Giymetli 2/7 genotypes after a 3-day exposure to water stress. On the first and second days of water stress protein accumulations of both Rubisco activase isoforms changed differently compared with the control plants of Giymetli 2/17 and Barakatli-95 genotypes. Protein accumulations of both isoforms were similar in control plants. On the first day of water stress protein accumulations of both isoforms increased in the Giymetli-2/17genotype. But on the second day of stress a decrease in the protein quantity of the 42 kDa isoform was observed. On the third day of stress the protein quantity of the 46 kDa isoform decreased approaching the intensity of the 42 kDa isoform. However, in the Barakatli-95 genotype the protein quantity of the 46 kDa isoform remained unchanged independent of the stress duration, whereas the protein quantity of the 42 kDa isoform decreased markedly since the first days of the stress and then it was not expressed on the following days. In both durum wheat genotypes expression of the 42 kDa isoform was more pronounced and only traces of the 46 kDa isoform were detected. A decrease in the amount of the 42 kDa was observed only on the third day of stress and this change was more pronounced in the Azamatli-95 genotype. Under drought conditions in bread wheat genotypes the expression of the 46 kDa isoform of Rubisco activase was more pronounced contrary to durum wheat genotypes where the 42 kDa isoform was expressed more markedly. The protein quantity of the 42 kDa isoform several times exceeded the protein quantity of the 46 kDa isoform in durum wheat genotypes (Fig. 1D).

Presumably, not only drought, but combined action of water stress and heat stress caused the different changes in the protein accumulation of the isoforms in durum and bread wheat genotypes under natural illumination and at average daytime temperatures of 28–30 °C, which were higher than an optimum temperature for wheat growth. Recently, protein accumulation and gene expression of Rubisco activase have been shown to increase in sugarcane under salt stress (Yang et al., 2012). Protein accumulations of Rubisco activase isoforms changed differently in leaves and green stems of early seedlings of *Brachypodium distachyon* watered with 100 mM NaCl solution and exposed to gradual drought stress. Thus, the protein quantity of RCA large isoform was detected to increase in leaves under water and salt stresses but this increase was more pronounced in salt-stressed seedlings (Bayramov and Guliyev, 2014). Previous studies showed that at the stage of the formation of the 5th and 6th leaves of maize the ratio of Rubisco activase polypeptides 43/41 kDa decreased by 50% under water stress compared with control plants (Ayala-Ochoa et al., 2004). But according to recent researches transcriptional level of RCA gene changed neither under drought nor during rehydration after drought and there is no difference in protein amount of the enzyme between two genotypes of Kentucky bluegrass (*Poa pratensis*) plants with contrasting drought tolerance (Xu et al., 2013).

The comparative study of the dynamics of PEPC subunits changes showed that the protein quantity of the 103 kDa subunit was always more than that of 108 kDa in early seedlings of the Barakatli-95 and Giymetli-2/17 genotypes under normal water supply conditions (Fig. 2). In plants exposed to 100 mM NaCl, protein quantities of both subunits decreased sharply on the first day and then relatively increased during the following two days approaching the control values. Contrarily, after 24-h exposure to drought the protein accumulations of both subunits increased and during the following two days decreased gradually to the level inherent in normal seedlings. However, in roots of early seedlings subjected to gradual drought protein accumulations of PEPC subunits remained stable and a sharp decrease in the protein quantity of the103 kDa subunit was observed when NaCl concentration was 100 mM (Fig. 2C). Similarly, there were no significant changes in protein quantities of PEPC subunits in seeds under drought, but contrary to leaves a gradual decrease was observed in germinating seeds under the influence of 100 mM NaCl (Fig. 2D). The 103 kDa subunit of PEPC was more expressed in germinating as well

Table 1 RWC changes in leaves of drought stressed and control plants of different wheat genotypes depending on time.

Wheat genotypes	I day	II day	III day
Giymetli 2/7 control	90.1	93.0	89.0
Giymetli 2/7 stress	89.1	73.0	50.0
Garagilchig-2 control	97.2	92.5	91.0
Garagilchig-2 stress	82.9	80.2	68.0
Azamatli-95 control	89.5	93.7	91.0
Azamatli-95 stress	86.5	77.2	46.0
Barakatli-95 control	97.0	92.0	86.0
Barakatli-95 stress	94.0	80.0	65.0

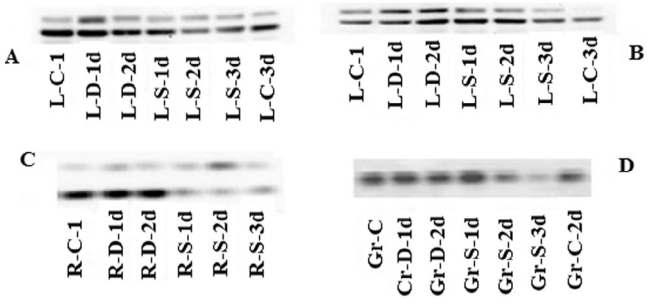

Figure 2 Western-blot analysis of changes in PEPC protein quantities in leaves of early seedlings of the Barakatli-95 (Bar-) (A) and Giymetli-2/17 (Giy-); (B) genotypes and in roots (R) of early seedlings of the Barakatli-95 (Bar-) genotype (C) and germinating seeds (Gr-) (D) under gradual drought and 100 mM NaCl. 15 μg protein of each sample was loaded on 10% SDS polyacrilamide gel to perform electrophoresis.

as developing seeds. In the flag leaves and ear elements of the Barakatli-95 genotype protein quantities of PEPC subunits changed differently depending on time. Beginning from the milk ripeness stage its protein quantity remained stable in flag leaves, but increased gradually in awns and glumes and gradual decrease occurred in maturing seeds (Fig. 3B). But the large subunit of Rubisco was not detected in matured seeds whereas its quantity remained stable in other studied organs. After the fourth day of the anthesis in flag leaves (flaq leaf blade and sheath) and ear elements (exposed penducle and ear penducle, glume, early grain, awn) of the Barakatli-95 genotype the changes in the protein quantity of each isoform were studied comparatively (Fig. 4). The protein quantity of the Rubisco activase large and small isoforms did not differ significantly in awns and glumes contrary to other organs where the protein quantity of the small isoform exceeded that of the large isoform. A positive correlation was observed between changes in the Rubisco large subunit protein quantity and the Rubisco activase total protein quantity in the studied parts of the plant. Thus, the similar changes in the protein quantity of both enzymes occurred in the same organ. Protein levels of both enzymes appeared to be higher in organs and tissues where photosynthetic CO_2 assimilation is active. The most protein quantities of PEPC subunits were observed in ear penducle, exposed penducle, flag leaf blade and sheath.

According to earlier studies conducted with bread wheat seedling, roots showed almost double the level of PEPC activity of shoots and the treatment with NaCl and LiCl induced PEPC expression in roots (González et al., 2003). Nhiri et al. (Nhiri et al., 2000) showed that the PEPC protein amount and the degree of the enzyme phosphorylation decreased many times in sorghum seeds grown in the medium containing NaCl, while the amount of the enzyme PEPC kinase performing its phosphorylation remained unchanged. The relative quantity of Rubisco, determined with immunoblotting method, was shown to be higher in flag leaves of rice than in awns during milk and wax ripeness phases. However, the quantity of Rubisco appeared to decrease in flag leaves and awns at the beginning of the wax ripeness phase. In the milk ripeness phase the quantity of PEPC changed similarly in both organs, while at the beginning of the wax ripeness phase its quantity remained stable in flag leaves and increased in awns (Lopes et al., 2006). Zang et al. (Zhang et al., 2008) detected a high PEPC activity in the ear organs of wheat. They showed that this activity positively correlated with the total protein concentration in glumes and seeds and with grain mass. They assumed that PEPC facilitated C and N metabolisms in seeds

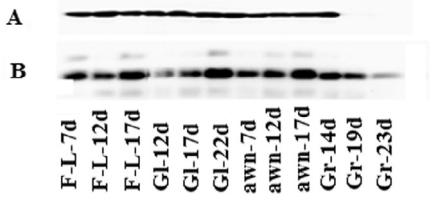

Figure 3 Western-blot analysis of dynamics of the changes in the protein quantity of Rubisco large subunit (A) and PEPC subunits (B) in flag leaves (F-L-), glumes (Glum-), awns (awn-) and germinating seeds (Gr-) of the Azamatli-95 genotype grown under normal conditions, after the seventh day of anthesis.

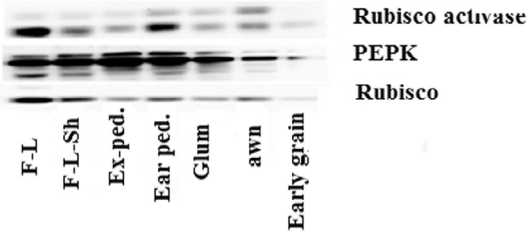

Figure 4 Comparative analysis of protein quantities of RCA (10 μg protein), PEPC (15 μg protein) and large subunit of Rubisco (5 μg protein) in flag leaves and ear elements of the Barkatli-95 genotype after the fourth day of anthesis.

and involved in the manipulation of the reserve protein synthesis.

The previous studies showed that ear organs of wheat contributed greatly to grain yield (Araus et al., 1993), especially during drought periods (Abbad et al., 2004). Recently, dynamics of the changes in PEPC protein accumulations have been studied in maturing seeds of rice. The results confirmed a positive correlation between the changes in PEPC protein accumulations and the enzyme activity (Yamamoto et al., 2014). Thus, at the tillering stage the protein accumulations of the Rubisco activase isoforms changed differently in the studied durum and bread wheat genotypes under the combined action of heat and water stresses. While the total quantity of the isoforms decreased when RWC became lower than 70%. Protein quantities of both PEPC subunits did not significantly change either in leaves, roots and germinating seeds of the studied wheat genotypes under drought. However, when these seedlings were grown in the medium containing 100 mM NaCl, protein quantities of the PEPC subunits decreased and this change was more pronounced in roots and germinating seeds of the early seedlings.

4. Conclusion

The general changes in the protein quantities of Rubisco activase and the large subunit of Rubisco were similar in the same organ. The obtained results suggested the involvement of the enzymes fulfilling the initial carboxylation step – Rubisco and PEPC – in the adaptation mechanisms of wheat genotypes against water and salt stresses.

Acknowledgment

This work was financially supported by the Post-doctoral European Scholarships for Young Scientific Researchers from the Azerbaijan National Academy of Sciences.

References

Abbad, H., El Jaafari, S., Bort, J., Araus, J.L., 2004. Comparative relationship of the flag leaf and ear photosynthesis with the biomass and grain yield of durum wheat under a range of water conditions and different genotypes. Agronomie 24, 19–28.

Aliev, J.A., Guliev, N.M., Kerimov, S.Kh., Hidayatov, R.B., 1996. Photosynthetic enzymes of wheat genotypes differing in productivity. Photosynthetica 32 (1), 77–85.

Araus, J.L., Brown, H.R., Febrero, A., Bort, J., Serret, M.D., 1993. Ear photosynthesis, carbon isotope discrimination and the

contribution of respiratory CO_2 to differences in grain mass in durum-wheat. Plant, Cell Environ. 16, 383–392.

Ayala-Ochoa, A., Vargas-Suárez, M., Loza-Tavera, H., León, P., Jiménez-García, L.F., Sánchez-de-Jiménez, E., 2004. In maze, two distinct ribulose 1,5-bisphosphate carboxylase/oxygenase activase transcripts have different day/night patterns of expression. Biochimie 86, 439–449.

Barrs, H.D.Weatherley, 1962. A re-examination of the relative turgidity technique for estimating water deficit in leaves. Aust. J. Biol. Sci. 15, 413–428.

Bayramov, Sh., Guliyev, N., 2014. Changes in Rubisco activase gene expression and polypeptide content in *Brachypodium distachyon*. Plant Phys. Biochem. 81, 61–66.

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein–dye binding. Anal. Biochem. 72, 248–254.

Carmo-Silva, A.E., Salvucci, M.E., 2013. The regulatory properties of Rubisco activase differ among species and affect photosynthetic induction during light transitions. Plant Physiol. 161, 1645–1655.

González, M.C., Sánchez, R., Cejudo, F.J., 2003. Abiotic stresses affecting water balance induce phosphoenolpyruvate carboxylase expression in roots of wheat seedlings. Planta 216, 985–992.

Holaday, A.S., Martindale, W., Alred, R., Brooks, A., Leegood, R.C., 1992. Changes in activities of enzymes of carbon metabolism in leaves during exposure of plants to low temperature. Plant Physiol. 98, 1105–1114.

Laemmli, U.K., 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227, 680–685.

Lopes, M., Cortadellas, N., Kichey, T., Dubois, F., Habash, D., Araus, J., 2006. Wheat nitrogen metabolism during grain filling: comparative role of glumes and the flag leaf. Planta 225 (1), 165–181.

Maroco, J.P., Rodrígues, M.L., Lopes, C., Chaves, M.M., 2002. Limitations to leaf photosynthesis in field-grown grapevine under drought – metabolic and modelling approaches. Funct. Plant Biol. 29, 451–459.

Nhiri, M., Bakrim, N., Bakrim, N., El Hachimi-Messouak, Z., Echevarría, C., Vidal, J., 2000. Posttranslational regulation of phosphoenolpyruvate carboxylase during germination of Sorghum seeds: influence of NaCl and L-malate. Plant Sci. 151, 29–37.

O'Leary, B., Park, J., Plaxton, W.C., 2011. The remarkable diversity of plant PEPC (phosphoenolpyruvate carboxylase): recent insights into the physiological functions and post-translational controls of nonphotosynthetic PEPCs. Biochem. J. 436, 15–34.

Portis Jr, A.R., 2003. Rubisco activase – Rubisco's catalytic chaperone. Photosynth. Res. 75, 11–27.

Shi, J., Yi, K., Liu, Y., Xie, L., Zhou, Z., et al, 2015. Phosphoenolpyruvate carboxylase in arabidopsis leaves plays a crucial role in carbon and nitrogen metabolism. Plant Physiol. 167, 671–681.

Spreitzer, R.J., Salvucci, M.E., 2002. Rubisco: structure, regulatory interactions, and possibilities for a better enzyme. Ann. Rev. Plant Biol. 53, 449–475.

Tambussi, E.A., Bort, J., Guiamet, J.J., Nogues, S., Araus, J.L., 2007. The photosynthetic role of ears in C3 cereals: metabolism, water use efficiency and contribution to grain yield. Crit. Rev. Plant Sci. 26, 1–16.

Xu, L., Yu, J., Han, L., Huang, B., 2013. Photosynthesis enzyme activities and gene expression associated with drought tolerance and post-drought recovery in Kentucky bluegrass. Environ. Exp. Bot. 89, 28–35.

Yamamoto, N., Tatsuya, K., Takehiro, M., Naomasa, Sh., Kunisuke, T., Toshio, S., Yoshikiyo, O., 2014. Molecular cloning, gene expression and functional expression of a phosphoenolpyruvate carboxylase Osppc1 in developing rice seeds: implication of involvement in nitrogen accumulation. Seed Sci. Res. 24, 23–36.

Yang, Z., Lu, Q., Wen, X., Chen, F., Lu, C., 2012. Functional analysis of the rice Rubisco activase promoter in transgenic Arabidopsis. Biochem. Biophys. Res. Commun. 418, 565–570.

Zhang, Y.H., Wang, Z.M., Huang, Q., Shu, W., 2008. Phosphoenolpyruvate carboxylase activity in ear organs is related to protein concentration in grains of winter wheat. J. Cereal Sci. 47, 386–391.