Khazar Journal of Science and Technology Volume 3 №1 2019, 58-63
© Khazar University Press 2019 DOI: 10.5782/2520-6133.2019.3.1.58

Diurnal Temperature-Related Dynamics of Glutathione Reductase Activity in Wheat Genotypes Under Drought

Lale Aydinli, Durna Aliyeva
Institute of Molecular Biology and Biotechnologies of ANAS, Laboratory of Bioadaptation and Laboratory of Cell membrane systems
** Corresponding author: aydinlilale@gmail.com*

Abstract

The strategically important wheat plant is the most cultivated of cereals. Drought is one of the main factors adversely affecting its productivity and quality of grain. Components of the ascorbate-glutathione cycle play an important role in the antioxidant defence system. The diurnal dynamics of temperature-related glutathione reductase activity (GR), one of the main components of the antioxidant defence system, were studied in durum (Barakatli 95, Garagylchyg 2) and bread (Gobustan, Tale 38) wheat genotypes of contrasting tolerance, when exposed to sustained soil drought. Glutathione reductase is highly sensitive to glutathione. Leaf samples were taken at the end of the wax ripening phase at three-hourly intervals (8^{00}, 11^{00}, 14^{00}, 17^{00}), frozen in liquid N_2, and kept at -80^0C. The experiments indicated that GR activity increased in stressed, tolerant, (Barakatli 95, Gobustan) and decreased in stressed sensitive varieties (Garagylchyg 2, Tale 38) compared with watered variants. In the samples taken at 11^{00}, enzyme activity increased in all the genotypes, compared with the control. However, in the Gobustan genotype the activity of the enzyme remained almost constant. At the highest temperatures (14^{00}) GR activity decreased by almost 50% both in durum and bread wheat varieties compared with the control. At 17^{00} GR activity increased in durum wheat genotypes and remained at a low level in bread wheat genotypes. Thus, durum wheat genotypes have a stronger defence system against unfavourable environmental conditions compared with bread wheat genotypes.

Keywords: Triticum aestivum L, *Triticum durum Desf., drought,* ascorbate glutathione cycle, glutathione reductase.

Introduction

Strategically important, wheat is one of the world's most cultivated cereals (Cossani et al., 2012), but its productivity and grain yield are affected negatively by drought (Luo et al., 2009). One of the non-specific responses to drought is oxidative stress accompanied by the intensification of ROS synthesis, which disturbs normal cellular function (Farooq et al., 2009). Hydrogen peroxide, used as a marker of degree of stress, is a stable form of ROS (Rhee et al., 2010). The antioxidant defence system, especially components of the ascorbate-glutathione cycle, implements H_2O_2 detoxification in plant cells exposed to the effect of stressors (Farooq et al., 2009; Noctor and Foyer, 1998). According to the results of experiments performed on various agricultural species, plant tolerance of stressors - heavy metal pollutants (Smirnoff, 2000) , radiation (Horemans et al., 2000), drought (Esfandiari et al., 2008) and salinity (Joseph and Jini 2011) - depends on the quantities of ascorbic acid and glutathione. Therefore, a determination of antioxidant enzyme activity may be considered an effective method of screening plant drought tolerance. Currently, components of ascorbate-glutathione are the main targets in developing stress-tolerant tansgen plants (Kang et al., 2013). The main purpose of the research presented was to study the temperature-related dynamics of glutathione reductase, one of the main components of the ascorbate-glutathione cycle, in durum and bread wheat genotypes of contrasting drought tolerance.

Materials and methods

Durum (Barakatli 95, Garagylchyg 2) and bread (Gobustan, Tale 38) wheat genotypes of contrasting productivity and drought tolerance, regionalized in Azerbaijan and collected in the genefund of the Research Institute of Crop Husbandry, were used as objects of the research. Leaf samples were taken at the end of the wax ripening phase at three-hourly intervals (8^{00}, 11^{00}, 14^{00}, 17^{00}), frozen in liquid N_2 and kept at -80^0C.

MDA quantification

The intensity of lipid peroxidation in plants was assessed on the basis of MDA content in drought-exposed leaf samples. MDA content was measured by spectrophotometry at 532 and 600 nm, based on the reaction with tiabarbituric acid (Heath and Packer, 1968) and calculated by the formula: $A_{(mM/g\ fresh\ biomass)} = (D_{532}-D_{600})/46.5$

Enzyme extraction

Leaf samples of 0.5g were ground in liquid nitrogen, homogenized in 100 mM Na-phosphate buffer (pH 7.8) containing 1 mM EDTA-Na, 2 mM FMSF, 1% PVP, 0.1% Triton X-100, and centrifuged at 4^0C, 15000g, for 20 min. The supernatant obtained was used in the analysis of GR.

Glutathione reductase (GR, EC 1.6.4.2) activity assay

Glutathione reductase activity was determined by spectrophotometry at 340 nm, in the presence of the oxidized form of glutathione (GSSH) based on the oxidation of NADPH for 3 min. A Glutathione Reductase Assay Kit (Sigma-Aldrich) was used for this purpose. Enzyme activity was expressed as u/mg protein min, and the coefficient of molar extinction ε=6.2 $mM^{-1}cm^1$.

Total protein assay

Total protein was determined using the Bradford protein assay (Bradford, 1976). A series of Bovine Serum Albumin (BSA) standards was used in the construction of the calibration curve.

Results and discussion

MDA content was found to increase during the day with rising temperatures in both drought-tolerant and -sensitive varieties exposed to drought, compared with watered varieties, and remained at a high level despite the relative decrease in temperature towards the end of the day (17^{00}).

Table. Time course of malondialdehyde content in leaves of wheat genotypes in conditions of control and drought

Genotypes	MDA, (mM/g fresh biomass)			
	8^{00}	11^{00}	14^{00}	17^{00}
Barakatli 95	1.6±0.08	3.4±0.17	3.9±0.2	3.6±0.18
Garagylchyg 2	2.2±0.11	4.1±0.21	4.9±0.25	3.8±0.19
Gobustan	3.4±0.17	3.7±0.19	5.0±0.25	5.0±0.25
Tale 38	3.4±0.17	4.9±0.25	5.6±0.28	3.6±0.18

At 14^{00} MDA content was at its highest levels in both durum and bread wheat varieties. It remained at a high level in tolerant genotypes (Barakatli 95, Gobustan) at 17^{00}, when the temperature declined, but underwent a relative decrease in sensitive varieties (Garagylchyg 2, Tale 38).

A direct relation between plant drought tolerance and antioxidant system induction is confirmed by numerous facts. Glutathione reductase activity and other parameters characterizing antioxidant status were different in plant varieties with contrasting tolerance. Glutathione reductase is highly sensitive to glutathione and, at the same time, it can catalyse the reduction of other compounds with disulfide bonds. This enzyme detoxifies H_2O_2 by reducing the oxidized form of glutathione using NADPH: GSSG + NADPH + $H^+ \rightarrow$2GSH + $NADP^+$. Analysis of leaf samples taken at 8^{00}, indicated an increase in GR activity in tolerant (Barakatli 95, Gobustan) and a decrease in sensitive genotypes (Garagylchyg 2, Tale 38) exposed to drought (Figure 1). In samples taken at 11^{00}, enzyme activity increased in all genotypes, compared with the control, except the Gobustan genotype, in which activity remained almost constant. At 14^{00} enzyme activity decreased by almost 50% in both durum and bread wheat varieties, compared with the control. At 17^{00}, due to the relative decline in temperature, GR activity increased in durum wheat genotypes, compared with the control, but remained at a low level in the bread wheat genotypes. This confirms that durum wheat genotypes have a stronger defence mechanism against adverse environmental conditions than bread wheat genotypes.

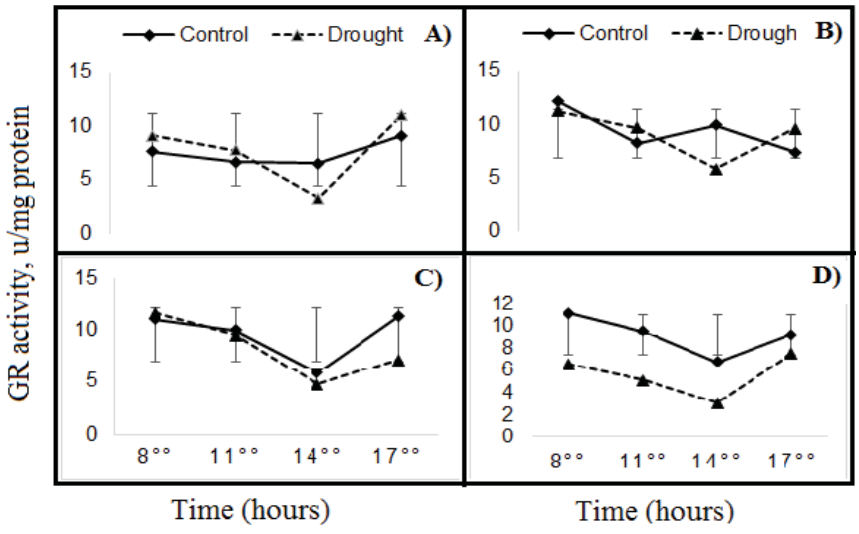

Fig. 1. Time course of total GR activity in leaves of wheat genotypes grown under conditions of control and drought stress: A) – Barakatli 95, B) - Garagylchyg 2, C) – Gobustan, D) – Tale 38

Lascano et al. (2001) reported an increase in glutathione reductase (GR) activity in tolerant wheat genotypes and a greater decline in reduced glutathione (GSH), ascorbate content and less oxidative damage than in susceptible genotypes.

Conclusion

According to the results obtained, drought changes the balance between the synthesis of reactive oxygen species and the antioxidant defence system in wheat genotypes exposed to drought. Components of the ascorbate-glutathione cycle play the major role in the detoxification of H_2O_2 formed in wheat genotypes due to the effect of drought. Study of the diurnal temperature-related dynamics of glutathione reductase activity demonstrated that the defence system in hexaploid wheat genotypes is weaker than that in tetraploid wheat genotypes.

Acknowledgment

This work was supported by the Science Development Foundation under the President of the Republic of Azerbaijan - Grant № EİF-KETPL-2-2015-1(25) - 56/35/3.

References

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analyt. Biochem. 72:248-254.

Cossani, C. M., & Reynolds, M. P. (2012). Physiological traits for improving heat tolerance in wheat. Plant physiology. 160(4):1710-1718.

Esfandiari, E., Shakiba, M. R., Mahboob, S. A., Alyari, H., & Shahabivand, S. (2008). The effect of water stress on antioxidant 427 content, protective enzyme activity, proline content and lipid 428 peroxidation in wheat seedling. Pak. J. Biol. Sci. 11 (15), 1916– 429 1922.

Farooq, M., Wahid, A., Kobayashi, N., Fujita, D., & Basra, S. M. A. (2009). Plant drought stress: effects, mechanisms and management. Agron. Sustain.Dev., 29(1), 185–212.

Heath, R. L., & Packer, L. (1968). Photoperoxidation in isolated chloroplast. I. Kinetics and stoichiometry of fatty acid peroxidation. – Arch. Physiol. Biochem. 125: 189-198, 1968.

Horemans, N., Foyer, C. H., Potters, G., & Asard, H. (2000). Ascorbate function and associated transport systems in plants. Plant Physiology and Biochemistry 38: 531–540.

Joseph, B., & Jini, D. (2011). Development of Salt Stress-Tolerant Plants by Gene Manipulation of Antioxidant Enzymes. Asian Journal of Agricultural Research, 5, 17-27.

Kang, N. Y., Cho, C., & Kim, J. (2013). Inducible expression of Arabidopsis response regulator 22 (ARR22), a type-C ARR, in transgenic Arabidopsis enhances drought and freezing tolerance. PLoS One. 8(11): e79248.

Lascano, H. R., Antonicelli, G. E., Luna, C. M., Melchiorre, M. N., Gomez, L. D., Racca, R. W., Trippi, V. S., Casano, L. M. (2001). Antioxidant system response of different wheat cultivars under drought: Field and in vitro studies. Aust J Plant Physiol, 28:1095-1102.

Luo, R., Wei, H., Ye, L., Wang, K., Chen, F., Luo, L., Liu, L., Li, Y., Crabbe, M. J., Jin, L., Li, Y., & Zhong, Y. (2009). Photosynthetic metabolism of C3 plants shows highly cooperative regulation under changing environments: a systems biological analysis. Proceedings of the National Academy of Sciences. 106(3):847-852.

Noctor, G., & Foyer, C. H. (1998). Ascorbate and glutathione: keeping active oxygen under control. Annu Rev Plant Physiol Plant Mol Biol., 49(1), 249–279.

Rhee, S., Chang, T. S., Jeong, W., & Kang, D. (2010). Methods for detection and measurement of hydrogen peroxide inside and outside of cell. Mol. Cells, 29(6), 539–549.

Smirnoff, N. (2000). Ascorbic acid: metabolism and functions of a multifaceted molecule. Current Opinion in Plant Biology 3: 229–235.