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RESPONSE OF ANTIOXIDANT DEFENSE SYSTEM TO DROUGHT AND RE-WATERING IN *Triticum aestivum* L. VARIETIES

L.M.Aydinli, D.R.Aliyeva, Academician of ANAS I.M.Huseynova

Two local bread wheat varieties (Gobustan, drought tolerant and Tale 38, drought sensitive) cultivated under normal irrigation conditions after drought exposure were rewatered and leaf samples were taken after 3 and 7 day rehydration. Level of malone dialdehyde (MDA), photosynthetic pigments, activity and isoenzyme content of catalase enzyme were determined, plant post-drought recovery performance were studied comparatively in the watered and drought variants. Chlorophyll content was found to increase slightly under drought stress, whereas amounts of carotenoids increased in both variants and after the rehydration returned to the previous level. Catalase enzyme activity increased 1.3 time in Gobustan variety and 1.5 time Tale-38 variety under water deficiency compared with watered plants. Only one isoform was detected for the catalase enzyme extracted from wheat leaves. Obtained results suggest that both genotypes can preserve recovery ability after rewatering, but the processes of recovery are faster in the tolerant genotype compared with the sensitive one.

Keywords: *Triticum aestivum* L., *antioxidant defense system, malonedialdehyde, catalase, photosynthetic pigments, drought, re-watering*

Introduction

Drought is one of the main factors negatively affecting plant productivity and grain quality all over the world. Being the major factor causing a decline in wheat productivity, it causes the formation of ROS- $O_2^{-\cdot}$, $\cdot OH$, $^1O_2$, H_2O_2, etc. The balance between the forming and scavenging of ROS is one of the main conditions for plant growth under stress. Response of the plant antioxidant defense system to stress depends on species and physiological status of plants, and also severity and duration of stress [1]. According to modern climate models, frequency, intensity and durability of drought will soon increase [2].

The study of the drought tolerance mechanisms of plants and evaluation of the plant post-drought recovery is considered to be the major problem of the modern science. Currently, proteome and metabolome changes in plants caused by adverse environmental conditions have been extensively investigated by biologists, psychologists and biochemists. The number of researches focused on reactive oxygen species, which cause oxidative stress, violations of the antioxidant system function and cell metabolism and even lead to apoptosis, have increased. Recently, genotypes tolerant to abiotic stressors or transgenic plants having genes coding enzymes involved in biosynthesis or catabolism of antioxidant enzymes and low molecular weight antioxidants have been used in the study of the plant adaptation mechanisms [3,4]. In this point of view, the study of proteome and metabolome changes induced by oxidative stress in vegetative organs of wheat plants with contrasting stress tolerance and the function of antioxidant defense system is of great importance.

The main purpose of the presented work was to study the effects of drought stress and re-watering on the antioxidant and osmoprotective systems of bread wheat varieties with contrast drought tolerance.

Materials and methods

Plant materials. The objects of the study were two local bread wheat (*Triticum aestivum* L.) varieties, Gobustan and Tale 38, contrasting in drought tolerance. Pre-soaked in distilled water

wheat seeds were planted in 5l vessels containing the mixture of sand and soil (2:1). Plants cultivated under well watered conditions were exposed to drought stress and then rewatered. Leaf samples taken after 3 and 7 day rehydration were used for the analysis.

Determinaation of malondialdehyde (MDA) content. The intensity of lipid perooxidation was determined based on the MDA level in wheat leaves. MDA was quantified according to the reaction performed with tiabarbituric acid at 532 nm and 600 nm, using the spectrophotometric method [5].

Extraction of the enzyme. Leaf sample (0.5g) was ground in liquid N_2. 100 mM Na-phosphate (pH 7.8) buffer containing 1 mM EDTA, 2 mM PMSF, 1% PVP and 0.1% Triton X-100 was used as a homogenization medium. Then the sample was centrifuged at 40^0C, for 20 min, at 15,000 g and the obtained supernatant was used for the analysis of catalase.

Determination of catalase (CAT) activity. Catalase activity (CAT, EC1.11.1.6) was measured spectrophotometrically at 240 nm for 1 min based on the hydrogen peroxide destruction rate [6]. The reaction medium was 60 mM Na-phosphate (pH 7.8) buffer containing 15mM H_2O_2 and 100 µl of the enzyme extract. $\varepsilon = 39.5\ mM^{-1}\ cm^{-1}$.

Determination of catalase isoenzyme content. The qualitative analysis of the enzyme was performed using native electrophoresis in 7% PAAG, for 3 hours, at 4°C and at constant current (30 mA). When electrophoresis was completed, the gel was stained using the medim containing 1% (w/v) K3[Fe(CN)6] and 1% (w/v) $FeCl_3$ [7].

Determination of photosynthetic pigment content. Content of pigments were determined spectrophotometrically according to Sims and Gamon (2002) using ULTROSPEC 3300 PRO ("Amersham", USA) [8]. Leaf samples were extracted in 80% acetone-Tris medium (80:20, pH 7.8), optical density was measured and the following formulae were used for calculations:

Chl *a* (µmol/ml) = 0.01373 A663 – 0.000897 A537 – 0.003046 A647

Chl *b* (µmol/ml) = 0.02405 A647 – 0.004305 A537 – 0.005507 A663

Carotenoids (µmol/ml) = (A470 – (17.1 x (Chl *a*+ Chl *b*) – 9.479 x anthocyanin)) / 119.26

Statistic analysis. The obtained results were analyzed using the computer programs Microsoft Office Word 7 and Excel 7.

Results and discussion

Cessation of watering causes a decline in soil humidity and thereby some morphological and physiological alterations in plants. Thus, long-term water deficiency results in twisted leaves, decreased leaf surface areas, retarded stem growth and shortened ears. These parameters were more pronounced in the Gobustan variety compared with Tale 38 (Fig. 1).

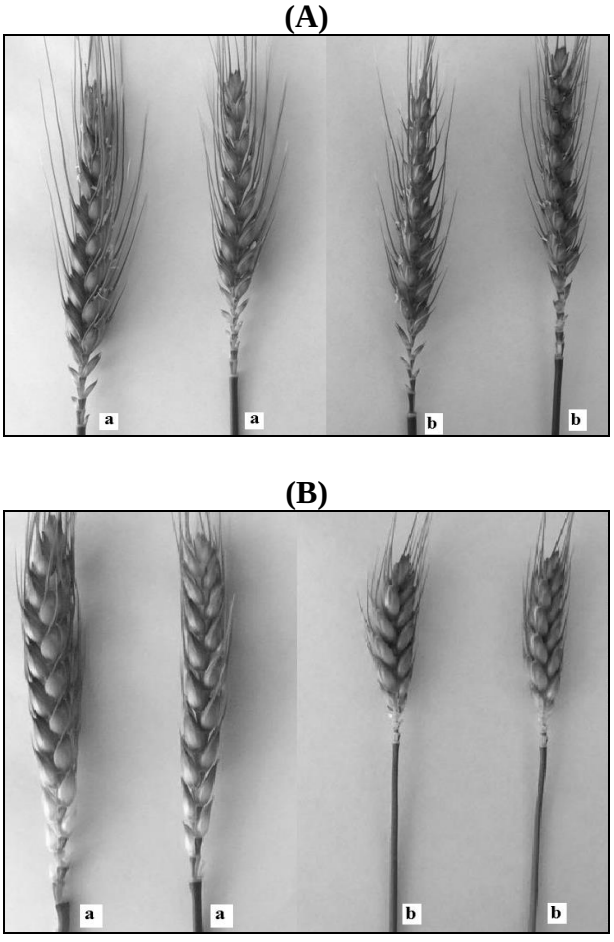

Fig. 1. The effect of drought stress on the ear growth parameters of wheat varieties with contrasting drought tolerance. A – Gobustan, B – Tale 38; *a* – watered, *b* – rewatering

Long term drought leads to lipid peroxidation in plant cells and a change in the MDA content is considered as the index of lipid peroxidation. We observed an increase in MDA levels under drought which confirms the effect of oxidative stress. Within three days of re-watering this index declined and after 7 days the value of this parameter approached the control (Fig.2).

Drought stress did not cause significant changes in the leaf chlorophyll content (Table 1). Thus, although chlorophyll *a* and *b* contents decreased slightly, this parameter increased upon re-watering and after 7 days it approached to control. However, carotenoids contents significantly increased in both varieties under drought, which is attributed to the osmoprotective role of these compounds.

One of the antioxidant enzymes playing the major role against oxidative stress is catalase. This enzyme provides rapid utilization of H_2O_2 [9]. A high CAT activity was observed in both genotypes exposed to drought stress. Under water deficiency catalase activity increased 1.3 times reaching 15.93±0.79 μmol/mg min in the Gobustan variety and it increased 1.5 times and became 16.97±0.84 μmol/mg min in the Tale 38 variety. High catalase activity under drought confirms its defensive role against stress. Catalase is a chromoprotein having an oxidized heme as a prosthetic (nonprotein) group. H_2O_2 formed during exchange reactions at certain concentrations has a toxic effect on the cell. Catalase scavenges H_2O_2 converting it into water and inactive molecular oxygen [10].

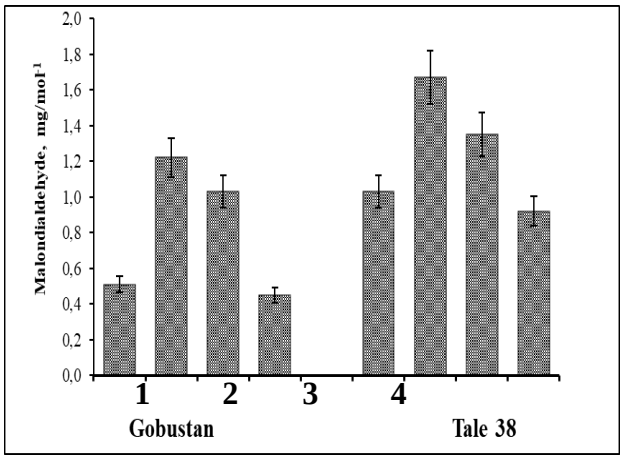

Fig. 2. Changes in MDA content in the leaves of Gobustan and Tale 38 varieties subjected to drought, followed by 3- and 7-day re-watering:
1 – Gobustan (control); *2* – Gobustan (stress); *3* – Gobustan (3 day re-watering); *4*-Gobustan (7 day re-watering); *5* – Tale-38 (control); *6* – Tale-38 (stress); *7* – Tale 38 (3 day re-watering); Tale 38 (7 day re-watering)

Table 1

Changes in the content of Chl *a*, Chl *b* and Carotenoids in bread wheat genotypes during drought and re-watering periods. C-control, D-drought, R_1-3 day rehydration, R_2-7 day rehydration

Varieties	Variants	Chl *a* (mol*ml^{-1})*10^{-2}	Chl *b* (mol*ml^{-1})*10^{-2}	Car (μmol*ml^{-1})*10^{-2}
Gobustan	C	0.32±0.016	0.94±0.047	36.21±1.81
	D	0.24±0.012	0.65±0.033	39.43±1.97
	R1	0.28±0.014	0.73±0.037	37.63±1.88
	R2	0.36±0.018	1.32±0.066	36.91±1.85
Tale 38	C	0.27±0.014	0.75±0.038	29.46±1.47
	D	0.19±0.009	0.60±0.03	37.68±1.88
	R1	0.23±0.012	0.65±0.33	36.45±1.82
	R2	0.27±0.014	0.71±0.036	29.84±1.49

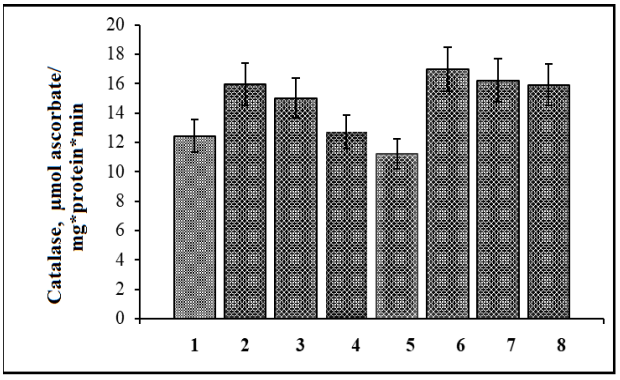

Fig. 3. Effects of drought stress and re-watering on catalase activity in leaves of wheat varieties contrasting in drought tolerance. *1* - Gobustan (watered), *2* - Gobustan (drought), *3* - Gobustan (3 day re-watering), *4* - Gobustan (7 day re-watering), *5* - Tale 38 (watered), *6*- Tale 38 (drought), *7* - Tale 38 (3 day re-watering), *8* - Tale 38 (7 day re-watering)

There were no pronounced quality differences (appearing or disappearing of additional bands in the electrophoregram) in the electrophoretic spectra of the studied wheat varieties. However, the intensity of isoforms in wheat leaves exposed to stress increased compared with the control variants (Fig. 3).

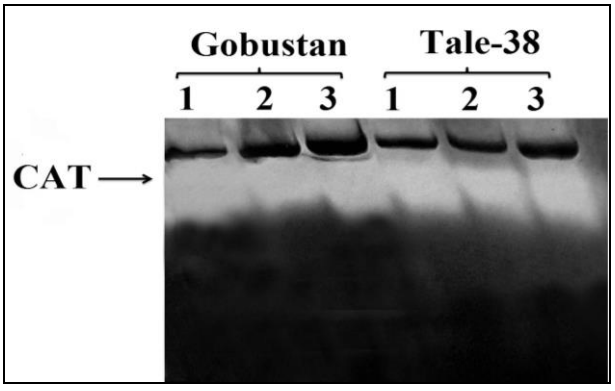

Fig. 4. Effect of drought stress and re-watering on isoenzyme content of catalase in leaves of wheat varieties with contrasting drought tolerance. *1*-watered, *2*-drought, *3*-re-watering. 35µg protein was added to each well of the gel

As seen in the figure, one isoform of catalase was observed in the enzymatic extract from wheat leaves. The intensity of this isoform was higher in plants exposed to drought and rewatering compared with the watered variant, which can be attributed to the higher activity of this enzyme in these variants. Analogical results were reported previously [11, 12]. The obtained data show that Tale 38 is more tolerant to drought than Gobustan. On the other hand, both genotypes could preserve self-recovery ability after rewatering, which can be attributed to the reversible character of the damage caused by drought. The results of the researches can play a theoretical role in the creation of new test systems for the assessment of drought tolerance of plants.

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Institute of Molecular Biology and Biotechnologies of ANAS
huseynova-i@botany-az.org

Triticum aestivum L. BUĞDA SORTLARINDA ANTİOKSİDANT MÜDAFİƏ SİSTEMİNİN QURAQLIĞA VƏ YENİDƏN SUVARILMAYA CAVAB REAKSİYALARI

L.M.Aydınlı, D.R.Əliyeva, İ.M.Hüseynova

Normal suvarılma şəraitində becərilən iki yerli yumşaq buğda sortu (Qobustan - quraqlığadavamlı və Tale-38 - quraqlığa davamsız) quraqlıq stresinə məruz qaldıqdan sonra yenidən suvarılmış, suvarmadan 3 və 7 gün sonra götürülmüş yarpaq nümunələrində malondialdehidinin (MDA), fotosintetik piqmentlərin miqdarı, katalaza fermentinin aktivliyi və izoenzim tərkibi təyin edilərək, bərpa prosesləri suvarılan və quraqlıq variantlarla müqayisəli şəkildə tədqiq edilmişdir. Müəyyən olunmuşdur ki, stress zamanı xlorofilin miqdarında cüzi azalma müşahidə olunsa da, karotinoidlərin miqdarı quraqlığın təsirindən hər iki variantda əhəmiyyətli dərəcədə artmış, yenidən suvarılmadan sonra isə əvvəlki səviyyəsinə qayıtmışdır. Qobustan sortunda normal suvarılan bitkilərlə müqayisədə su qıtlığı zamanı katalazanın fəallığı 1,3 dəfə, Tale-38 sortunda isə 1,5 dəfə artmışdır. Buğda yarpaqlarından alınmış ferment ekstraktında katalazanın yalnız bir izoforması müşahidə olunmuşdur. Əldə olunan nəticələrdən belə qənaətə gəlmək olar ki, hər iki sort yenidən suvarmadan sonra özünübərpa qabiliyyətini qoruyub saxlaya bilir və bərpa prosesləri davamlı sortdahəssasla müqayisədə daha sürətlə gedir.

Açar sözlər: *Triticum aestivum* L.*, antioksidant müdafiə sistemi, malondialdehidi, katalaza, fotosintetik piqmentlər, quraqlıq, yenidən suvarılma*

РЕАКЦИЯ АНТИОКСИДАНТНОЙ ЗАЩИТНОЙ СИСТЕМЫ У МЯГКИХ СОРТОВ ПШЕНИЦЫ (*Triticum aestivum* L.) НА ЗАСУХУ И ПОВТОРНОЕ ОРОШЕНИЕ

Л.М.Айдынлы, Д.Р.Алиева, И.М.Гусейнова

Два местных сорта мягкой пшеницы (Гобустан – засухоустойчивый и Тале-38 – чувствительный к засухе), культивируемые в условиях орошения, после 2-х недельного воздействия засухой, повторно орошались. В качестве материала исследования использовались листья, взятые непосредственно после засухи, а также после 3-х и 7-дневного повторного орошения. Изучалось содержание малонового диальдегида (МДА), фотосинтетических пигментов, а также активность и изоферментный состав каталазы. Выявлено, что в обоих вариантах, несмотря на незначительное снижение содержания хлорофилла во время стресса, содержание каротиноидов под воздействием засухи в значительной степени возросло, а после повторного орошения приблизилось к первоначальному уровню. Активность каталазы по сравнению с нормально поливаемыми растениями у сорта Гобустан увеличилась в 1,3 раза, а у сорта Тале-38 – в 1,5 раза. В электрофореграмме обнаружилась лишь одна изоформа каталазы в листьях пшеницы. Полученные данные позволяют предположить, что оба сорта сохранили способность к восстановлению после возобновления полива, а процессы восстановления в устойчивом сорте по сравнению с чувствительным происходят быстрее.

Ключевые слова*: Triticum aestivum* L.*, защитная система антиоксидантов, малондиальдегид, каталаза, фотосинтетические пигменты, засуха, повторное орошение*