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APPLICATION OF FAST BIOCHEMICAL STRESS MARKERS FOR EVALUATION OF DROUGHT TOLERANCE OF FOUR COMMON WINTER WHEAT VARIETIES

Anelia Dobrikova, Preslava Borisova, Ekaterina Yotsova, Radoslav Chipilski*, Evgeniy Dimitrov*, Zlatina Uhr*, Elena Todorovska, Antoaneta V. Popova#**

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Abstract

The aim of the present work was to compare the drought tolerance of three new Bulgarian common winter wheat varieties (Sashez, Ginra and Gizda) with one older variety Fermer, that is sensitive to drought stress. The sensitivity of investigated varieties to 3 and 7 days dehydration and their ability to recover after a period of 3 days re-watering were evaluated by following the alterations in the leaf pigment content, lipid peroxidation (MDA), generation of reactive oxygen species (H_2O_2) and levels of protective substances (anthocyanins). Results revealed that varieties Ginra and Gizda were the most resistant to applied dehydration, Sashez had medium resistance, while Fermer showed high sensitivity to water deprivation. Variety Sashez responded to 7 days of water deprivation with a significant increase of lipid peroxidation, but after re-watering for 3 days recovered successfully. After 3 days of dehydration, the level of protective anthocyanins was enhanced in Sashez, Ginra and Gizda, while during the whole experimental setup their level was very low in Fermer. Data demonstrated as well that the dehydration-induced lipid peroxidation and levels of H_2O_2 were highest in variety Fermer. The photosynthetic pigments were decreased after

#Corresponding author.

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7 days dehydration in all varieties, except in Ginra, and recovered after re-watering. The results obtained in respect to drought resistance and ability to recover after the stress of the investigated wheat varieties will be of interest to breeders for the development of new high-yielding drought tolerant wheat and achievement of sustainable agriculture in water-limited environments.

Key words: wheat variety, drought stress, photosynthetic pigments, anthocyanins, lipid peroxidation, hydrogen peroxide

Introduction. Drought is one of the most important environmental problems limiting the survival and productivity of crop plants in natural and agricultural habitats [1,2], including also wheat yields [3]. It is well established that drought causes oxidative stress inducing significant reductions in the plant growth, relative water content, ion homeostasis, photosynthetic pigment content, photosynthetic processes, stomatal conductance, chlorophyll fluorescence, etc. [2,4]. One of the most dangerous consequences of drought stress is the enhanced production of reactive oxygen species (ROS) in different cellular compartments resulting in extensive cellular damage and death [2,5]. The enhanced ROS production is, however, kept under control by activation of the plant antioxidant systems and different signal pathways [2,5,6]. Moreover, ROS may also serve as stress signalling molecules [5].

The harmful effects of drought stress on plant productivity could be reduced by selection of stress-tolerant genotypes [3,4,7,8]. Breeding of wheat genotypes is a process related to the development of new varieties with optimal combination of different valuable biological and economic traits as well as environment interactions [8]. Recently, the effects of different genotypes of wheat semi-dwarfing genes (*Rht* genes) and their combination in the wheat tolerance to osmotic stress conditions have also been studied [4].

Three new cultivars, Ginra, Gizda and Sashez, obtained by mutagenesis and intervarietal hybridization at the Institute of Plant Genetic Resources, Sadovo, Bulgaria, were used in this study. These varieties were selected as high-yielding breeding lines after several years of field experiments, including also few dry years (2017, 2019), due to their ability to withstand water deficit in accordance with agronomic and physiological evaluation performed according to the methodology described in the study of Chipilski and Uhr [9]. As a control in these experiments was included the older variety Fermer which was characterized as drought-sensitive [10]. The aim of the study was to compare the tolerance of new varieties to water stress at the early stage of their development under controlled conditions. Special attention was paid to their ability to recover after re-watering. Changes in the content of photosynthetic pigments, of oxidative stress markers (H_2O_2 and MDA) and synthesis of protective substances (anthocyanins) were compared in order to determine the level of tolerance and identify appropriate genotypes with better performance under drought stress conditions.

Materials and methods. ***Plant material and growth conditions.*** Four varieties of common winter wheat (*Triticum aestivum* L.) with high productive potential and stable yields were used in this study as follows: Fermer (mutant line of variety Pobeda, obtained by treatment of dry seeds with gamma rays 50 Gy); Gizda (mutant line of variety Pobeda, obtained by treatment of dry seeds with 1 mM NaH_3), Sashez (obtained by intervarietal hybridization of ♀Fermer × ♂Yunak), and Ginra (obtained by intervarietal hybridization of ♀Sadovo 1 × ♂Guinness/1322).

Seeds were sown in pots (about 20 seeds per pot) filled with soil, obtained from the region of Sadovo, containing 11.4% humus, 486 mg/kg K_2O, 654 mg/kg P_2O_5 and 265 mg/kg total N (pH 7.6). Wheat plants were grown in growth chamber under controlled conditions: illumination of 250 µmol photons m^{-2} s^{-1}, 16/8 h day/night photoperiod, 20/18 °C day/night temperature and relative humidity 60% for about 21 days. At the third leaf stage of the plants' development the pots were divided into two groups – control (3 pots) and subjected to drought stress (5 pots). Watering of the second group of plants was stopped for 7 days followed by a re-watering period for 3 days to evaluate the ability of plants to recover after dehydration stress. Watering of control plants continued during the whole experimental cycle. Plant material (pieces of the second developed leaf from different plants) was collected at the end of the dark period from the control and subjected to dehydration plants on the 3rd and 7th days of dehydration and after 3 days recovery period. The plant material was immediately frozen in liquid nitrogen and stored at −80 °C till processing. At each time point four parallel samples were collected from the control, 3 and 7 days dehydrated, and 3 days recovered plants. Mean values (±SE; $n = 4$) were calculated and expressed on a dry weight (DW) basis.

Pigment analysis. Photosynthetic pigments were extracted from leaves (40 mg) with ice-cold 80% acetone (v/v) and the content of chlorophyll $a + b$ (Chl $a + b$) and total carotenoids (Car) were determined spectrophotometrically (UV-VIS Specord 210 Plus, Analytic Jena, Jena, Germany) as described in [4] and expressed as [mg pigment g^{-1} DW].

Lipid peroxidation and H_2O_2 content analysis. The extent of lipid peroxidation in the investigated four wheat varieties was determined by the malondialdehyde (MDA) content as described in Esterbauer and Cheeseman [11]. The absorbance was recorded at 532 and 600 nm (UV-VIS Specord 210 Plus). The amount of MDA was calculated using the extinction coefficient of 155 000 M^{-1} cm^{-1} and expressed as [nmol MDA g^{-1} DW]. The amount of H_2O_2 was determined according to Velikova et al. [12] and as [µmol H_2O_2 g^{-1} DW].

Determination of anthocyanins content. Samples (50 mg leaf material) were homogenized in 6 ml methanol/HCl/H_2O (79/1/20, v/v/v) and centrifuged at 10 000 × g for 15 min at 4 °C. The absorbance of the supernatant was recorded at 535 and 653 nm. Anthocyanins were determined using the equation: A =

($A_{535} - 0.24 \times A_{653}$) [13] and extinction coefficient of 33 000 M^{-1} cm^{-1} and as [µmol A g^{-1} DW].

Results and discussion. ***Changes in the oxidative stress markers under drought stress and recovery.*** Exposure of plants to abiotic stress can lead to generation of ROS and oxidative stress [5]. One of the most damaging effects of different types of ROS is the peroxidation of membrane lipids, especially of chloroplasts membranes, that are characterized by a high level of polyunsaturated fatty acid chains, leading to increased permeability, intensive leakage and finally to cell death [5,6]. Similarly, under conditions of drought stress, the content of MDA and H_2O_2 in blackberry leaves have increased in a linear manner with the stress progression [14].

In order to evaluate the degree of suffered injury after water deprivation and recovery period, the content of H_2O_2 and degree of lipid peroxidation (MDA) were followed as typical stress indicators. The dehydration-induced generation of H_2O_2 was best expressed on the 7th day of drought stress for Fermer, while in the other three varieties there was no increase of H_2O_2 content. After re-watering of plants for 3 days the amount of generated H_2O_2 was reduced in Sashez and Gizda, remaining highest in Fermer, indicating the inclusion of various protective mechanisms leading to significant recovery with exception of variety Fermer (Fig. 1).

In order to determine the degree of membrane lipid peroxidation that perturbs membrane structure and functions, MDA content was used as a criterion of the stress-induced oxidative damage [15] (Fig. 2). Data showed that the extent of lipid peroxidation was significantly increased on the 7th day of dehydration in varieties Fermer and Sashez. For Fermer, the high degree of lipid peroxidation correlated with the increased amount of generated H_2O_2 (Fig. 1), while in Sashez it was most probably due to an inclusion of other ROS molecules [2]. Complete recovery after re-watering was observed for Gizda and Sashez followed by Ginra (Fig. 2). At the same time, for the variety Fermer no decrease in lipid peroxidation was observed after applied drought stress, although the level of H_2O_2 was significantly reduced after re-watering, which indicated that some stress-related processes were still functioning after re-set of watering. On the other hand, the two varieties Ginra and Gizda showed high resistance to long-term drought stress in respect to membrane's lipid peroxidation. This probably indicates that various antioxidant defense mechanisms function in these varieties, which will be the subject of a future research.

Previously, it has been shown that in re-watered plants (after 3-days recovery period) the content of MDA and H_2O_2 also decreased compared to the controls, indicating drought tolerance which was accompanied by increased SOD and APX activity [6,16]. The fast recovery of the three varieties (Sashez, Ginra and Gizda) suggested that plants may have developed complex antioxidant mechanisms to sense water deficiency and reprogramme their metabolism.

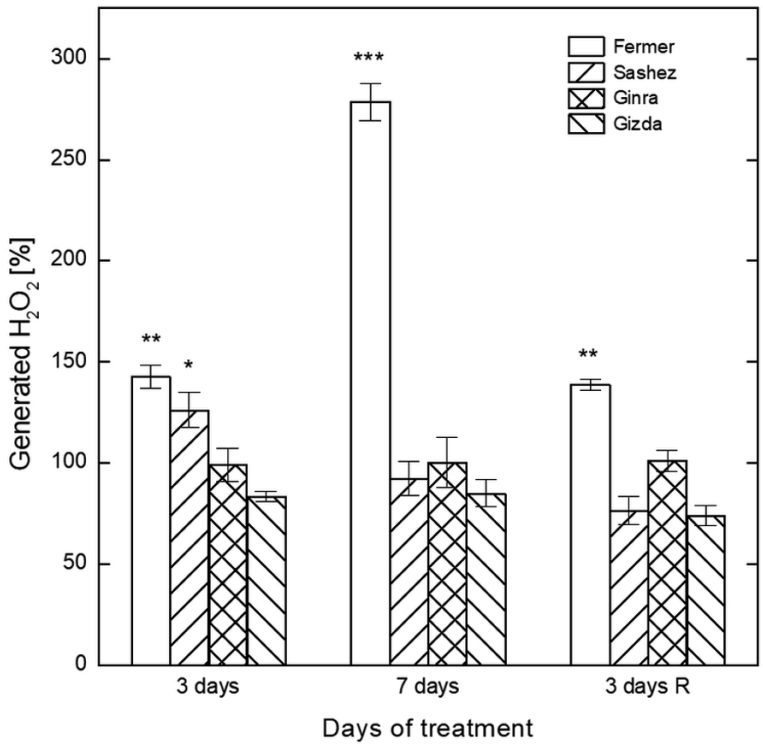

Fig. 1. Time-dependent generation of H_2O_2 in leaves of four varieties of common winter wheat, induced by dehydration and after re-watering for 3 days (R). Results were presented as percentage from the respective normally watered control plants. Means (±SE, $n = 4$) were calculated from four parallel samples at each time point. Significant differences in comparison to control values were estimated by Student's t-test ($^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$)

Alterations in photosynthetic pigment contents. Plants respond differently to abiotic stress, especially to dehydration, with alteration in their pigment content [4,17]. Photosynthetic pigments (chlorophylls and carotenoids) participate in the absorption, transmission, and distribution of photosynthetic light energy and also play a key role in plant growth and development [4,18,19]. Chlorophyll degradation is a common response in plants under drought stress [17,18]. Previous study on the drought-resistant plant *C. procera* [16] has shown that only carotenoid content increased in drought stressed plants, while Chl *a* decreased, compared to the control. Furthermore, after the recovery period, Chl *a*, Chl *b* and Car increased in re-watered plants compared to the control suggesting fast recovery and ability to tolerate drought stress. However, in another study [14] chlorophyll content was greatly increased in leaves of blackberry cultivar while the content of Chl *b* gradually increased during drought stress period.

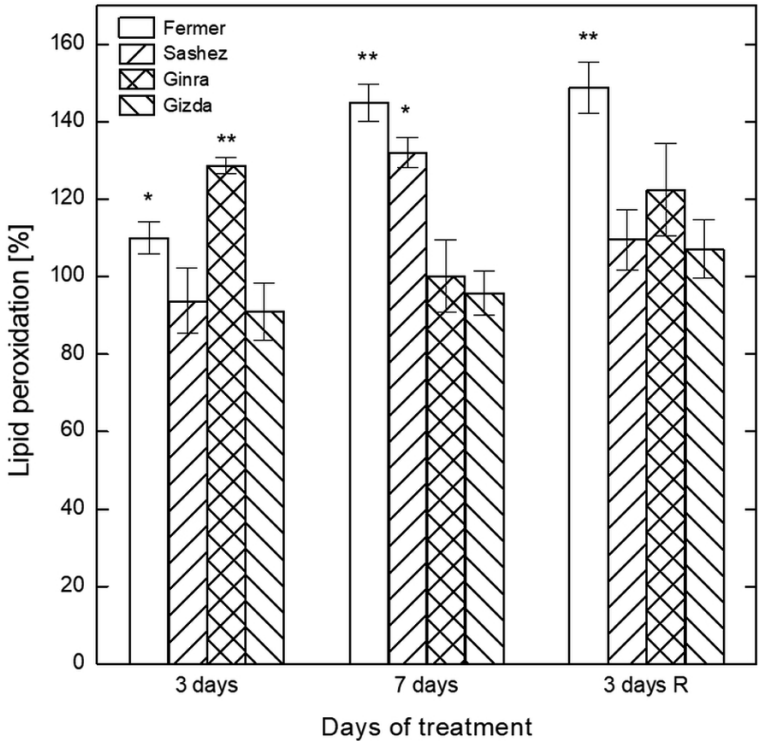

Fig. 2. Level of lipid peroxidation in leaves of four common winter wheat varieties, exposed for 3 and 7 days to dehydration and after re-watering for 3 days (R). Means (±SE, $n = 4$) were calculated from four parallel samples at every time point. Results were expressed as percentage from the respective normally watered control plants. Significant differences in comparison to control values were estimated by Student's t-test ($^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$)

The altered values of total chlorophyll (Chl $a+b$), and total carotenoid (Car) content of investigated four wheat varieties in comparison to control plants are presented in Fig. 3. After 3 days of dehydration an increased pigment content was observed only in Fermer, however, after 7 days treatment the pigments decreased most pronounced in Fermer followed by Gizda. After the recovery period of 3 days the Chl $(a + b)$ and carotenoids content in Ginra and Gizda was comparable to that of control plants. It can be assumed that the effectiveness of photosynthetic processes was at the highest level in Ginra and Gizda, while in Fermer and Sashez the recovery of photosynthetic pigments was not complete after dehydration.

Content of anthocyanins. As a response to unfavourable environmental conditions, plants increase synthesis of protective substances in leaves including anthocyanins. These substances belong to a vast and diverse flavonoid group that are located in plant vacuole, realize a protective effect mainly by screening excessive light and/or acting as effective antioxidants with activities, higher than those of well-recognized antioxidants ascorbic acid and vitamin E [20].

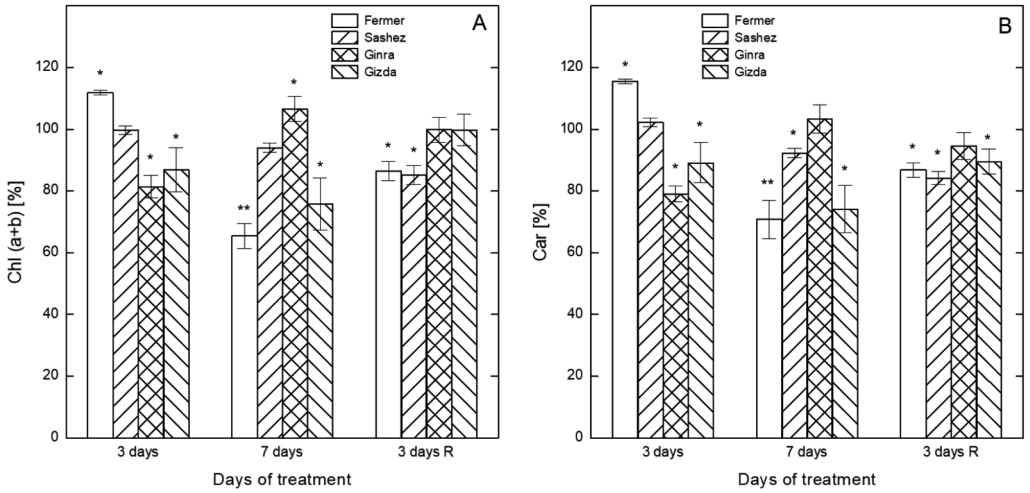

Fig. 3. Pigment content of Chl $(a+b)$ (A) and total carotenoids (Car) (B) of dehydrated plants for 3 and 7 days and after re-watering for 3 days (R). Mean values (±SE, $n = 4$) were calculated from four parallel samples at every time point and expressed as percentage from the respective normally watered control plants. Significant differences in comparison to control values were estimated by Student's t-test ($^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$)

Under short dehydration for 3 days varieties Sashez, Ginra and Gizda responded with increased amounts of anthocyanins (by 20–30%) while in variety Fermer a decline by 35% was observed (Fig. 4). Under longer time of dehydration (7 days) increased synthesis of anthocyanins was observed only in Gizda. After recovery period of 3 days the level of anthocyanins was increased only in variety Ginra. For the whole experimental set-up variety Fermer demonstrated low levels of synthesized anthocyanins. It can be supposed that the high level of synthesized anthocyanins at the beginning of dehydration (3 days) plays a very important role in assuring high resistance to drought stress.

Conclusions. Our results indicated that new wheat varieties Ginra and Gizda demonstrated good resistance to drought stress and recovery capacity after re-watering at early stage of plant development, which makes them suitable for growing in fields with limited water availability. For these two varieties the stress markers – lipid peroxidation and generated H_2O_2 were not altered after 3 and 7 days of dehydration. The amounts of photosynthetic pigments (Chl $a + b$ and Cars) were decreased to a different extent in Fermer, Sashez and Gizda on the 7th day of dehydration, but after re-watering their content was completely recovered only in Ginra and Gizda. The variety Sashez showed increased level of lipid peroxidation on the 7th day of dehydration, however, these changes were not extreme and after re-watering it recovered successfully indicating its good adaptability and plasticity. Data obtained by application of the fast biochemical stress markers confirm the high sensitivity of Fermer as revealed by high level of

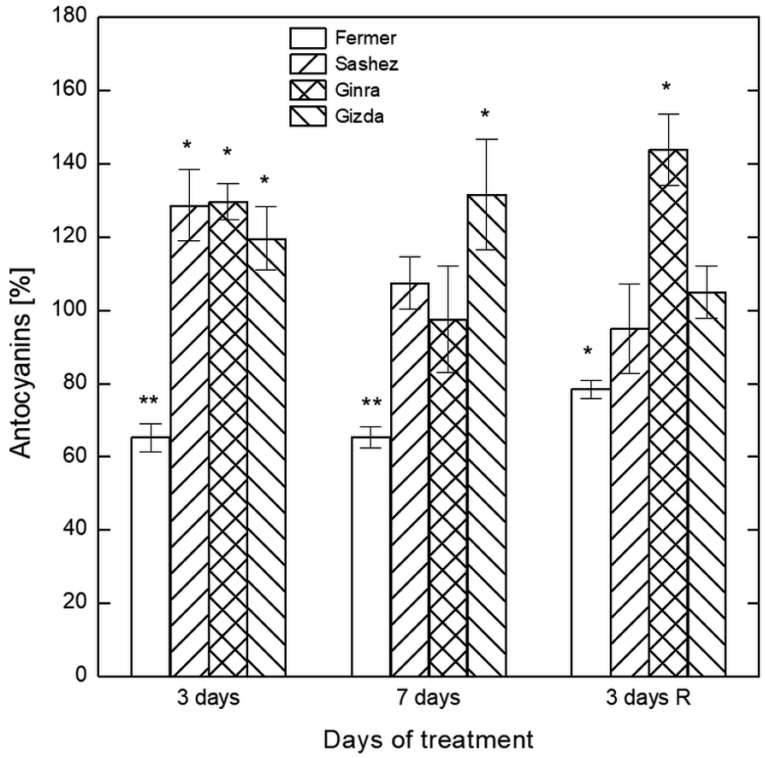

Fig. 4. Drought-induced accumulation of anthocyanins in leaves of four varieties of common winter wheat, exposed for 3 and 7 days to dehydration followed by 3 days re-watering (R). Results were expressed as percentage from the respective control plants. Means (±SE, $n = 4$) were calculated from four parallel samples. Significant differences in comparison to control values were estimated by Student's t-test ($^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$)

generated H_2O_2 and lipid peroxidation, decreased amounts of photosynthetic pigments and reduced synthesis of the protective substances (anthocyanins). All this suggests that the applied fast stress markers can be used as a reliable screening of stress tolerance.

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Institute of Biophysics
and Biomedical Engineering
Bulgarian Academy of Sciences
Akad. G. Bonchev St, Bl. 21
1113, Sofia, Bulgaria
e-mails: popova@bio21.bas.bg
aneli@bio21.bas.bg
preslavab12345@gmail.com
ekaterina_yotsova@abv.bg

Institute of Plant Genetic Resources
"Konstantin Malkov"
Agricultural Academy
4122 Sadovo, Bulgaria
e-mails: radoch@abv.bg
genipg@abv.bg
zlatinaguhr@abv.bg

**AgroBioInstitute*
Agricultural Academy
8 Dragan Tsankov Blvd
1164 Sofia, Bulgaria
e-mail: e.g.todorovska.abi@gmail.com