

## Assessment of drought tolerance of eight varieties of common winter wheat – a comparative study

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### Abstract

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The ability of eight Bulgarian common winter wheat varieties (Sadovska belya, Lusil, Diamant, Bononia, Tsarevets, Niky, Guinness and Petya) to tolerate drought stress as well as to recover after re-watering were evaluated by following the alterations in the leave pigment content, generation of hydrogen peroxide, lipid peroxidation, and levels of synthesized protective substances (anthocyanins). These parameters were used as fast and sensitive stress markers to assess varieties' susceptibility to drought. Results presented indicated that on the 7<sup>th</sup> day of dehydration alteration in the photosynthetic pigment content occurred as indicated by the decreased chlorophyll to carotenoid ratio showing that the amount of total chlorophylls was changed to a higher extent than that of carotenoids. Data demonstrated that the amount of stress-generated hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) was highest in Guinness, followed by Petya, Lusil and Tsarevets. The extend of membrane damage evidenced by the degree of dehydration-induced lipid peroxidation was highest in varieties Sadovska belya, Petya and Tsarevets. Furthermore, all investigated varieties responded to drought stress by increased synthesis of anthocyanins. Our results also suggested that varieties Guinness and Diamant were suitable for growing in areas with limited water availability as they have a good drought stress resistance and recovery capacity.

**Keywords:** wheat variety; drought stress; photosynthetic pigments; oxidative stress; anthocyanins; recovery

### Introduction

Drought is one of the most important environmental extremes that constraints the plant survival and crop productivity in natural and agricultural habitats (Araus et al., 2002; Passioura, 2002; Chaves et al., 2003), causing oxidative stress which negatively affects crop development and yield (Rampino et al., 2006; Petrov et al., 2019). It is well estab-

lished that drought stress induces significant reductions in plant growth, relative water content, ion homeostasis, photosynthetic pigment content, effectiveness of photosynthetic processes, stomatal conductance, chlorophyll fluorescence, etc. (Chaves et al., 2009; Dulai et al., 2019). One of the most dangerous consequence of drought stress is the enhanced production of reactive oxygen species (ROS) in different cellular compartments, such as chloroplasts, mitochondria

and peroxisomes, resulting in extensive cellular damage and death (Morgan, 1984; Foyer & Noctor, 2003; Cruz de Carvalho, 2008; Izanloo et al., 2008; Xu et al., 2010). This enhanced ROS production is, however, kept under control by accumulation of protective substances, activation of plant enzyme antioxidant system and different signal pathways (Willekens et al., 1997; Izanloo et al., 2008; Xu et al., 2010; Rangani et al., 2018). ROS may play a dual role, causing damage but also serving as stress signaling molecules (Chaves et al., 2009). Furthermore, stress-generated ROS can serve as an alarm signal that triggers defense responses by specific signal pathways that involve  $H_2O_2$  as a secondary messenger (Willekens et al., 1997; Chaves et al., 2003; Foyer & Noctor 2003).

In the last years, a preferential crop genotype selection was applied to combine the advantages of different genes in respect to changing environmental conditions and to improve breeding efficiency in crop plants (Rampino et al., 2006; Kumar et al., 2015; Pieczynski et al., 2017). Breeding of wheat genotypes is a process related to the development of new varieties with optimal combination of different valuable biological and economic properties as well as environment interactions (Chairi et al., 2020). The effects of different genotypes of wheat semi-dwarfing genes (*Rht* genes) and their combination in the wheat tolerance to osmotic stress conditions were also extensively studied (Landjeva et al., 2008; 2011; Petrov et al., 2019). Interspecific hybridization of wheat with wild relatives is an appropriate breeding strategy to improve the stress tolerance (Dulai et al., 2019). Wheat breeding in Bulgaria has been also directed to achieving an increased yield, improved grain quality and increased abiotic stress tolerance (Kolev et al., 2010). Since 1960, more than 130 modern semi-dwarf (*Rht* genes) wheat varieties have been released by the two main breeding centres in Bulgaria – Agricultural Institute in General Toshevo and Institute of Plant Genetic Resources in Sadovo.

The aim of the present study was to investigate the response of eight Bulgarian winter common wheat varieties created in the Institute of Plant Genetic Resources in Sadovo, Bulgaria, to drought stress (Sadovska belya, Lusil, Diamant, Bononia, Tsarevets, Niky, Guinness and Petya) and for selection of appropriate genotypes to improve breeding efficiency and choose varieties suitable for specific environmental conditions, in particular under water deficiency. The presented common wheat varieties were exposed to drought stress for 3 and 7 days. Special attention was paid to the ability of the wheat varieties to recover after re-watering of plants. The changes in the content of photosynthetic pigments as fast stress markers, as well as of oxidative stress markers ( $H_2O_2$  and MDA) and synthesis of protective substances (anthocyanins)

anins) were compared in order to classify these eight wheat varieties in respect to their drought resistance for recommendation of potentially suitable genotypes for improving the drought tolerance of common wheat.

## Materials and Methods

### *Plant material*

In this study were included 8 varieties of Bulgarian winter common wheat from the Breeding program of the Institute of Plant Genetic Resources, Sadovo, Bulgaria: Sadovska belya, Lusil, Diamant, Bononia, Tsarevets, Niky, Guinness and Petya (Table 1), obtained by hybridization with the exception of variety Guinness (Ivanov et al., 2018).

### *Plant growth conditions*

Seeds of eight common winter wheat varieties were sown in pots filled with soil, taken from the region of Sadovo, Bulgaria, and grown in a climate chamber under controlled conditions: illumination of  $250 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ , 16/8 h day/night photoperiod, 20/18°C day/night temperature and relative humidity 60% for about 21 days. In every pot ( $d=15.5$  cm,  $h=14.5$  cm) were sown 20 seeds. At the stage of third developed leaf pots were divided into 2 groups – control (10 pots) and subjected to drought stress (10 pots). Watering of the second group of plants was stopped for 7 days followed by a re-watering period of 3 days to evaluate the ability of plants to recover after dehydration stress. Watering of control plants continued during the whole experimental cycle. Leave material (pieces of the second developed leaf from different plants) were collected at the beginning of photoperiod from control (watered) plants and of dehydrated plants at the 3<sup>rd</sup> and 7<sup>th</sup> days of dehydration and after 3 days recovery period, immediately frozen in liquid nitrogen and stored at  $-80^\circ\text{C}$  till processing. Experiments were performed in the period April-July 2019.

### *Pigment analysis*

Photosynthetic pigments were extracted from leave material with ice-cold 80 % acetone (v/v) in dim light as described in Gerganova et al. (2016). Leave material (40 mg) was ground by a mortar and pestle at  $4^\circ\text{C}$  in dim light and the extracts were centrifuged in sealed tubes at 4500 g for 15 min. The clear supernatant was used to spectrophotometrically (UV-VIS Specord 210 Plus, Analytic Jena, Jena, Germany) determine the pigment content of chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*) and carotenoids (Car) as described in Lichtenthaler (1987). At every time point four parallel samples were collected from plants after 3 and 7 day of dehydration and after 3

**Table 1 Characteristics of 8 Bulgarian common wheat varieties used in the study**

Variety	Hybridization	Properties of varieties established by field tests
Sadovska belya	♀Lada X ♂Sadovo	Medium drought and excellent cold resistance. High productive potential with a stable yield. Highly plastic and adaptive.
Lusil	♀Yantar X ♂Medven	Good drought and cold resistance. Excellent adaptability and suitable for growing throughout the country, including more intensive technology
Diamant	♀ Jubiley X ♂Sadovo	Very good drought and excellent cold resistance. Highly adaptable with excellent productivity and grain quality, suitable for growing in areas with colder climates.
Bononia	♀Krasnodarsky karlik X ♂Ludogorka	Excellent winter hardiness and medium to low drought resistance. High productivity with very good balance between yield and quality of grain.
Tsarevets	♀Zebrets X ♂Katya	Tolerant to winter hardiness and drought resistance. Highly productive and has very large spikes, with high number of spikelets and grains.
Niky	♀Sadovo X ♂Pobeda, followed by treatment of F1 seed generation with 1mM NaN <sub>3</sub>	Good cold and a medium drought resistance.
Petya	♀Thessee X ♂Carmen	Good drought and cold resistance. It has a complex resistance to diseases and provides high and stable yield.
Guinness	Induced mutagenesis of variety Katya with gamma rays 50 Gy.	Excellent drought and a good cold resistance. Very high yield potential based on high and balanced productive tillering. Extremely high drought resistance and adaptability.

days re-watering (recovered) plants. At every time point parallel samples were taken from control, normally watered plants. Mean values ( $\pm$ SE;  $n = 4$ ) were calculated and expressed on a dry weight basis (mg pigment  $g^{-1}$  DW).

#### **Determination of lipid peroxidation and $H_2O_2$ content**

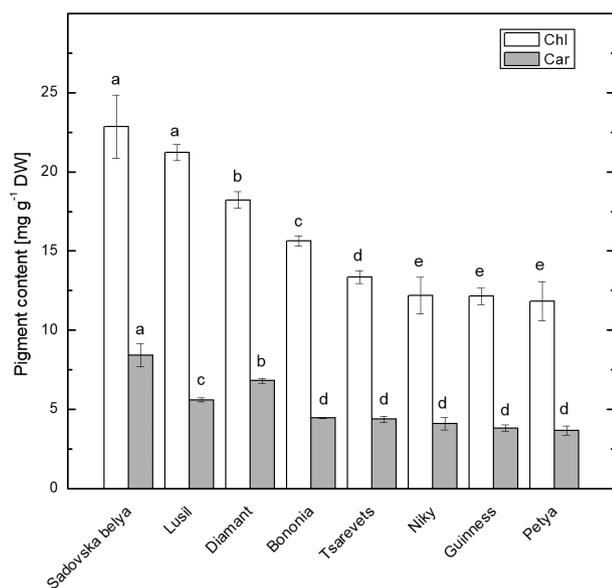
The extent of lipid peroxidation in the 8 investigated wheat varieties was determined by the malondialdehyde (MDA) content following the thiobarbituric acid method (TBA) (Esterbauer & Cheeseman, 1990). Leave material (100 mg) was homogenized in 3 ml 0.1% (w/v) trichloroacetic acid (TCA) at 4°C. After centrifugation at 4500 g for 15 min at 4°C 1 ml of the clear extract was mixed with 1 ml of 20% TCA containing 0.5% thiobarbituric acid (TBA). The mixture was boiled in a water bath for 25 min. After cooling the absorbance was recorded at 532 and 600 nm (UV-VIS Specord 210 Plus). The absorbance at 600 nm was read to correct for unspecific turbidity. The amount of formed 2-thiobarbituric acid-reactive metabolites (aldehydes, mainly MDA and endoperoxides) was calculated using the extinction coefficient of 155 000  $M^{-1} cm^{-1}$  and expressed on a dry weight basis ( $\mu$ mol MDA  $g^{-1}$  DW). Mean values ( $\pm$ SE,

$n = 4$ ) were calculated from four parallel samples for each time point of every experiment.

For determination of  $H_2O_2$  content 0.5 ml of the supernatant after centrifugation of homogenized leave material with 0.1% TCA was mixed with 0.5 ml K phosphate buffer (pH 7.0) and 1 ml 1 M KI. After 2 hours in the dark and several times vortex-mixing the absorbance at 390 nm was spectrophotometrically determined by UV-VIS Specord 210 Plus spectrophotometer. The amount of  $H_2O_2$  was determined using a standard curve (Velikova et al., 2000) and expressed on a dry weight basis as ( $\mu$ mol  $H_2O_2$   $g^{-1}$  DW). The mean values ( $\pm$ SE,  $n=4$ ) were calculated from four parallel samples for each time point of every experiment.

#### **Determination of anthocyanins content**

Leave material (50 mg) was collected at every time point from control (normally watered), treated and recovered plants of eight wheat varieties for evaluation of anthocyanins content. Samples were homogenized in 6 ml solution containing methanol/HCl/ $H_2O$  (79/1/20, v/v/v) and centrifuged at 10 000 g for 15 min at 4°C. The absorbance at 535 and 653 nm was recorded spectrophotometrically by UV-VIS Specord 210



**Fig. 1. Pigment content (Chl (a+b) and Car (total carotenoid content)) of control plants of eight common wheat varieties, arranged in respect to decline of pigment content. Means ( $\pm$ SE, n=4) were calculated from 4 parallel samples. Same letters indicate non-significant differences, while significant differences were indicated with different letters according to LSD test.**

Plus spectrophotometer in the clear supernatant. The absolute values of anthocyanins were determined using the equation:  $A = (A_{535} - 0.24 \times A_{633})$  (Murray and Hackett, 1991) and the extinction coefficient of  $33\,000\text{ M}^{-1}\text{ cm}^{-1}$  (Hodges & Nozzolillo, 1996) and expressed on a dry weight basis ( $\text{nmol g}^{-1}\text{ DW}$ ). Mean values ( $\pm$ SE, n=4) were calculated from four parallel samples for each time point of every experiment.

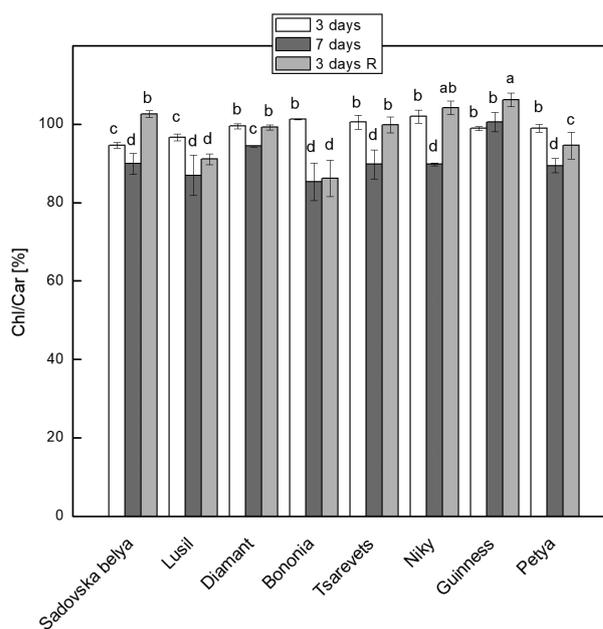
### Statistics

Data were presented as mean values  $\pm$  SE. Mean values were calculated from 4 parallel samples for each time point. Comparison of means was performed by the Fisher least significant difference (LSD) test at  $P \leq 0.05$  following ANOVA. A statistical software package (StatGraphics Plus, version 5.1 for Windows, USA) was used. Same letters indicate non-significant differences, while significant differences were indicated with different letters according to LSD test.

## Results and Discussion

### Alterations in the photosynthetic pigment content

In Figure 1 are arranged the values of total chlorophyll (Chl a+b) and total carotenoid (Car) content of the control



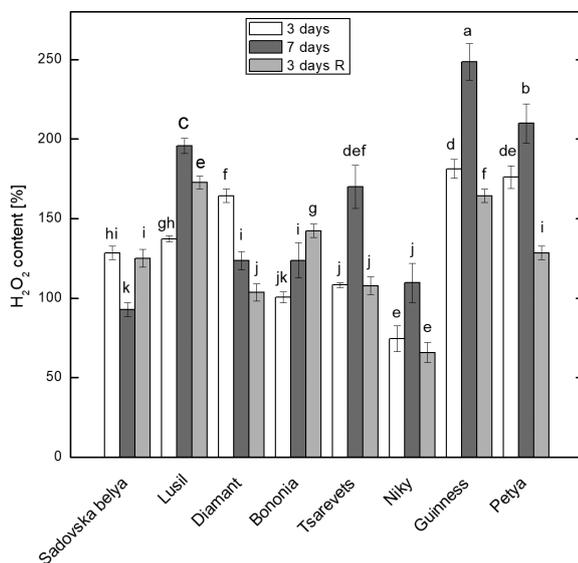
**Fig. 2. Drought-induced alterations in the Chl/Car ratio of eight Bulgarian common winter wheat varieties after 3 and 7 days of dehydration followed by a recovery period of 3 days (R). The values of the total chlorophyll (Chl a+b) to carotenoids (Car) ratio were calculated as (mg pigment g<sup>-1</sup> DW) and results were presented as percent from the respective control. Mean values ( $\pm$ SE, n=4) were calculated from 4 parallel samples for every time point of each experiment. Same letters indicate non-significant differences, while significant differences were indicated with different letters according to LSD test.**

plants of investigated eight wheat varieties. The highest content was observed in Sadovska belya. Pigment content of Lusil, Diamant, Bononia and Tsarevets gradually decreased. Pigment content in the control plants of Niky, Guinness and Petya was the lowest and very similar. Therefore, it can be assumed that the effectiveness of photosynthetic processes was at the highest level in Sadovska belya, decreasing gradually in Lusil, Diamant, Bononia and Tsarevets, while in Niky, Guinness and Petya these processes were performed in low and comparable rate (Khanal et al., 2017).

In Figure 2 are included the results of dehydration-induced alterations in the pigment content of the eight wheat varieties, represented as the ratio of total chlorophyll content to total carotenoid content (Chl/Car), calculated as percent from the respective watered control on the 3<sup>rd</sup> and 7<sup>th</sup> day of water deprivation and after 3 days re-watering. Pigment analysis of leaves from plants subjected to dehydration as a fast stress indicator revealed that during the first 3 days

of water deficit, a slight decrease in the ratio Chl/Car was observed only for two wheat varieties: Sadovska belya and Lusil (Figure 2). After 7 days of drought stress the ratio Chl/Car decreased to a different extent for all varieties, except for Guinness. However, the most prominent decline was observed for Sadovska belya, Lusil and Bononia, with around 15%. On re-set of watering, the ratio Chl/Car was restored, except for Lusil, Bononia and Petya. The observed reduction of Chl/Car ratio was due to a more significant decrease in the amount of total chlorophyll (Chl *a+b*) than that of the total carotenoid content. The decrease in photosynthetic pigment content indicated that either the synthesis of pigments was retarded by lack of water, or the process of their degradation was accelerated.

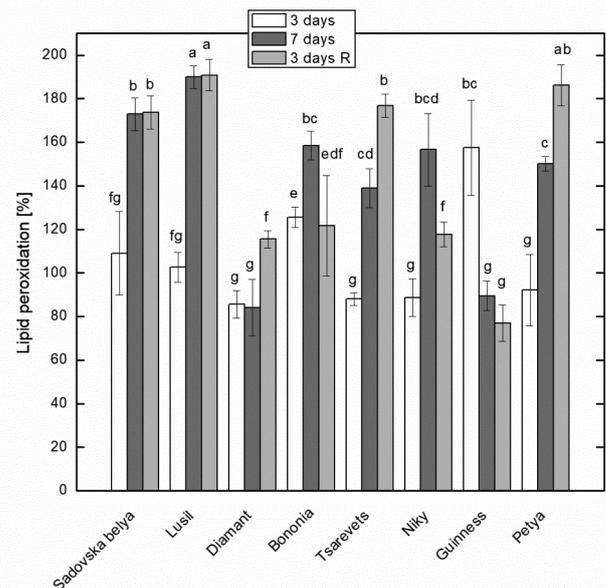
The decline in availability of photosynthetic pigments could have a negative impact on performing the effective photosynthetic process and also on crop yields (Rivas et al., 2017). Longer period of dehydration (7 days) affected all investigated varieties except Guinness. The ability of the eight investigated varieties to recover after the 7 days of dehydration was demonstrated by restoration of Chl/Car ratio to control values. Only for varieties Lusil, Bononia and Petya the



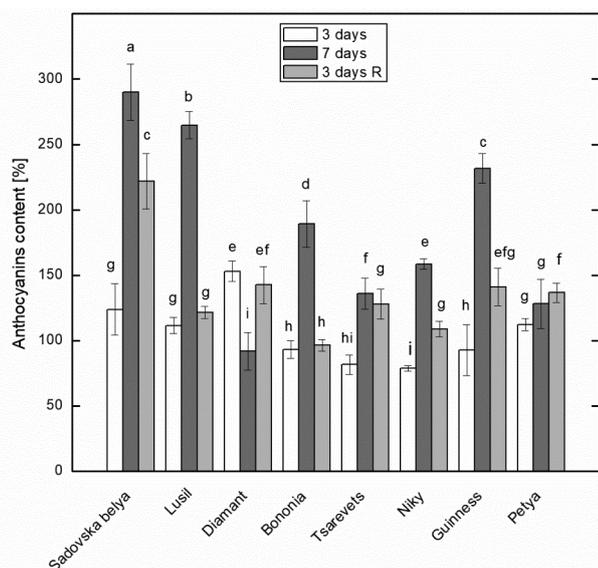
**Fig. 3.** Time-dependent generation of H<sub>2</sub>O<sub>2</sub> in leaves of eight Bulgarian varieties of common winter wheat, induced by dehydration. Results were calculated as ( $\mu\text{mol H}_2\text{O}_2 \text{ g}^{-1} \text{ DW}$ ) and presented as percent from respective control. Means ( $\pm\text{SE}$ ,  $n = 4$ ) were calculated from 4 parallel samples at each time point of every experiment. Same letters indicate non-significant differences, while significant differences were indicated with different letters according to LSD test.

ratio did not recover completely. In respect to the drought-induced alterations in the pigment content, the most sensitive varieties were Bononia and Lusil that sense the water deficiency in the early stage of dehydration and did not restore to their control values after 3 days re-watering. Results showed that the varieties Guinness followed by Diamant were the most resistant in terms of photosynthetic pigment content.

Chlorophyll degradation is a common response in plants under drought stress (Guerfel et al., 2009; Liu et al., 2011). Previous study of the drought-resistant plant *C. procera* (Rivas et al., 2017) has shown that only Car content increased during drought stress, while the Chl *a* content decreased in comparison 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. Moreover, Yang et al. (2019) reported that chlorophyll content was greatly increased in the leaves of blackberry resistant cultivar as the content of Chl *b* gradually increased during the drought stress period.



**Fig. 4.** Level of lipid peroxidation in leaves of eight varieties of Bulgarian common winter wheat, exposed for 3 and 7 days to dehydration and after 3 days of re-watering. At every time point four parallel samples were processed from control and treated plants. Means ( $\pm\text{SE}$ ,  $n = 4$ ) were calculated from 4 parallel samples at every time point. Results were calculated as ( $\mu\text{mol MDA g}^{-1} \text{ DW}$ ) and expressed as percent from respective normally watered control. Same letters indicate non-significant differences, while significant differences were indicated with different letters according to LSD test.



**Fig. 5. Drought-induced accumulation of anthocyanins in leaves of eight varieties of common winter wheat, exposed for 3 and 7 days to dehydration followed by 3 days re-watering (R). Results were calculated as (nmol anthocyanins g<sup>-1</sup> DW) and expressed as percent from respective control. Means ( $\pm$ SE, n = 4) were calculated from 4 parallel samples for each independent experiment at every time point. Same letters indicate non-significant differences, while significant differences were indicated with different letters according to LSD test.**

#### *Changes in the oxidative stress markers under drought and recovery*

It is well documented that drought stress causes a marked increase in oxidative damage of plants (Yue et al., 2012; Pynngrope et al., 2013). As a result of different abiotic stress factors generation of ROS occurs leading to oxidative stress including oxidation of biological molecules as lipids, proteins, pigments and DNA (Asada, 2006). One of the most damaging effects of different types of ROS is the peroxidation of membrane lipids, especially of thylakoid membranes, that are characterized by a high level of polyunsaturated fatty acid chains (Pospíšil & Yamamoto, 2017) triggering chain reactions that seriously disturb the structural and functional organization of membranes, leading to increased permeability, intensive leakage and finally to cell death (Imlay, 2008; Smirnov & Arnaud, 2018). ROS production including H<sub>2</sub>O<sub>2</sub> may cause membrane damage but can also perform a role of a stress signalling molecule (Chaves et al., 2009). Under drought stress, it has been shown that the content of H<sub>2</sub>O<sub>2</sub>, lipid peroxidation (MDA

content), and protective substances as soluble sugars in the leaves increased in a linear fashion with the progression of stress (Yang et al., 2019).

In order to evaluate the degree of suffered injury in the course of dehydration the content of H<sub>2</sub>O<sub>2</sub> and the degree of lipid peroxidation were followed as typical stress indicators. The dehydration-induced generation of H<sub>2</sub>O<sub>2</sub> was best expressed on the 7<sup>th</sup> day of drought stress. The highest value of accumulated H<sub>2</sub>O<sub>2</sub> was observed for Guinness, followed by Petya, Lusil and Tsarevets. After re-watering of plants for 3 days the amount of generated H<sub>2</sub>O<sub>2</sub> was reduced, indicating significant ability for recovery after dehydration with exception for Sadovska belya and Bononia.

It is well documented that drought stress causes a marked increase in oxidative damage to plants (Yue et al., 2012; Pynngrope et al., 2013). Under conditions of abiotic stress, the generated ROS can lead to peroxidation of membrane lipids that perturb membrane structure and functions. MDA is the decomposed product of polyunsaturated fatty acids in bio-membranes and usually used as the criterion for stress-induced oxidative damage of membranes (Yue et al., 2012).

Under control conditions the values of registered MDA in investigated wheat varieties were ranging between 5.82 $\pm$ 0.56  $\mu$ mol MDA g<sup>-1</sup> DW in Guinness and 10.47 $\pm$ 1.12  $\mu$ mol MDA g<sup>-1</sup> DW in Sadovska belya. In Figure 4 are presented the results of stress-induced lipid peroxidation, determined by the MDA method. For every wheat variety the values were presented as percent from the respective control (plants that were the same age and watered regularly) – after 3 and 7 days of deprivation of water supply and after 3 days re-watering. The extent of lipid peroxidation was significantly increased on 7<sup>th</sup> day of dehydration in majority of wheat varieties, with exception of Diamant and Guinness. Significant degree of recovery after re-start of watering was observed only in Bononia, Niky and Guinness (Figure 4). For all other varieties the degree of lipid peroxidation was either comparable with 7<sup>th</sup> day of dehydration (Sadovska belya and Lusil), or even higher (Diamant, Tsarevets, Petya), indicating that some stress-related processes were still functioning after re-set of watering of plants.

Although the highest values of H<sub>2</sub>O<sub>2</sub> were registered in Guinness (Figure 3), the amount of MDA (Figure 4) was at the lowest level in this variety after 7 days of water deprivation. For Diamant we also observed high content of H<sub>2</sub>O<sub>2</sub> and low level of lipid peroxidation (Figures 3 and 4). This is an indication of inclusion of various protective mechanisms against drought stress. Further research with these two varieties will give a detailed description of the underlying defense mechanisms.

Previously it has been shown that in re-watered plants, after 3 days recovery period the content of MDA and  $H_2O_2$  decreased compared to the controls, indicating drought tolerance which was accompanied with increased SOD and APX activity (Rivas et al., 2017). Fast recovery indicates that plants might have evolved complex mechanisms to sense water availability and re-program their metabolism and that the applied dehydration stress was not severe and was completely reversible on water availability.

#### **Content of protective substances (anthocyanins)**

As a response to unfavorable environmental conditions, especially to high light illumination, plants increase synthesis of protective substances including anthocyanins. These substances belong to a vast and diverse flavonoid group that are located in plant vacuole and realize a protective effect mainly by screening excessive light and/or acting as effective antioxidants (Gould et al., 2002; Hernandez et al., 2009; Landi et al., 2015). It had been shown that *in vitro* anthocyanins demonstrate antioxidant activities, higher than of well-recognized antioxidants ascorbic acid and vitamin E (Gould et al., 2002; Hernandez et al., 2009).

Under control conditions the eight investigated wheat varieties form two groups in respect to anthocyanins content – Niky, Tsarevets, Lusil and Bononia contain twice higher amounts of anthocyanins (in the range between 1.2 and 1.5  $\mu\text{mol g}^{-1}$  DW) than Petya, Guinness, Sadovska belya and Diamant (at around 0.5  $\mu\text{mol g}^{-1}$  DW). Wheat varieties, subjected to dehydration for 7 days showed increased synthesis of anthocyanins, which was best expressed in Sadovska belya and Lusil followed by Guinness and Bononia, Niky and Tsarevets. Only Diamant did not show increase in synthesis of anthocyanins (Figure 5). After the recovery period of 3 days (re-watering), the level of anthocyanins in majority of wheat varieties showed a tendency of decrease in comparison to 7 days stress with exception for Diamant.

Although the drought-induced content of anthocyanins in Sadovska belya was at the highest level and in Diamant at the lowest level, both varieties showed a high resistance to drought stress as indicated by their low level of lipid peroxidation (Figure 4). This probably indicates that in these two varieties were included different defense mechanisms which could be the subject of a future research.

#### **Conclusion**

Data presented indicated that the most pronounced dehydration-induced alterations in eight Bulgarian common winter wheat varieties in respect to pigment content, generation

of  $H_2O_2$ , lipid peroxidation and accumulation of protective substances (anthocyanins) were observed after 7 days of water deprivation. On the 7<sup>th</sup> day of dehydration alterations in photosynthetic pigment content occurred, except for variety Guinness, as indicated by the decrease of ratio Chl/Car pointing out that the amount of total chlorophyll decreased to a higher extent than that of carotenoids. In addition, drought stress induced pronounced oxidative damage in the leaves of all eight common winter wheat varieties.

Dehydration accelerated the generation of ROS ( $H_2O_2$ ), provoked membrane lipid peroxidation (MDA), and disrupted the balance of chlorophyll metabolism. Based on the comparative analysis of 8 varieties of common winter wheat, it can be concluded that the degree of dehydration-induced lipid peroxidation is high for almost all varieties: Lusil, Sadovska belya, Bononia, Niky, Tsarevets and Petya with exception for Guinness and Diamant. After the recovery period only Bononia, Niky and Guinness showed a recovery on re-set of watering.

Furthermore, all investigated varieties responded to dehydration stress by increased synthesis of anthocyanins, best expressed in Sadovska belya followed by Lusil and Guinness. In addition, drought stress induced pronounced oxidative damage in the leaves of all wheat varieties. It is suggested that drought stress tolerance of wheat varieties was associated with their ability to maintain higher chlorophyll content and lower MDA levels as well as to increase synthesis of protective substances like carotenoids and anthocyanins.

All these suggested that the most stable varieties in respect to dehydration were Guinness followed by Diamant that showed a good drought stress resistance and recovery capacity after re-watering as evidenced by no change in Chl/Car ratio in the course of dehydration, low level of lipid peroxidation and high amounts of anthocyanins. The fact that the dehydration-induced  $H_2O_2$  was high could indicate that other mechanisms are involved in maintaining low level of membrane damage. Our results support the idea that these two varieties are suitable for growing in areas with limited water availability. Further investigation of the involved defense mechanisms on functional and metabolic level of these two varieties (Guinness and Diamant) are needed for better understanding the nature of their resistance towards dehydration.

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