

## Effects of drought at different periods of wheat development on the leaf photosynthetic apparatus and productivity

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The problem of drought impact on crop productivity is especially relevant for the leading cereal – wheat, since significant cultivation areas of this crop are located in risky farming zones. The aim of our studies was to compare the peculiarities of drought impact during the period of vegetative growth and reproductive development on the chlorophyll content, activity of chloroplast antioxidant enzymes, the rate of carbon dioxide gas exchange and transpiration as well as productivity of the wheat plants. The studies were carried out on bread winter wheat plants (*Triticum aestivum* L.) of the Astarta and Nataalka varieties grown in a pot experiment. The plants were watered daily to maintain the soil moisture level in the pots in the range 70–60% of field capacity. Drought treatment involving decrease in soil moisture to 30% of the field capacity was applied to some of the plants at late stem elongation stage (BBCH 37-45) and to other plants at heading-anthesis (BBCH 59-65) while control plants were watered as usual. After 7 days of drought, the watering of treated plants was resumed to maintain the soil moisture at the level of control plants until the harvest. The penultimate leaf at the late stem elongation stage and flag leaf at the heading-anthesis period were used for measurements of photosynthetic apparatus response to drought. Plants of both varieties treated at stem elongation stage showed lower decline in the photosynthetic activity, chlorophyll content and grain productivity than those treated at heading-anthesis. The results obtained indicated that photosynthetic apparatus of wheat has a higher drought tolerance at the stem elongation stage than during the heading-anthesis stage. Furthermore, plants stressed at the stem elongation stage revealed better compensatory growth of lateral productive shoots due to superior supply of plants with assimilates after drought termination. Astarta variety had a more drought-tolerant photosynthetic apparatus and higher grain productivity in both well-watered and drought conditions as compared to Nataalka variety. The differences between varieties were more contrasting when drought was applied at heading-anthesis.

**Keywords:** *Triticum aestivum* L.; water deficit; chlorophyll; photosynthesis; superoxide dismutase; ascorbate peroxidase.

### Introduction

Drought is a major limiting factor of crop productivity in many regions of the world. This problem is especially relevant for the leading cereal – wheat, since significant cultivation areas of this crop are located in risky farming zones, which, in particular, include Ukraine (Morgun, 2017). Global climate changes, accompanied by an increase of temperature and sharpening of precipitation distribution irregularity both by regions and during the growing season, increase these risks (Morgun et al., 2010; Lesk et al., 2016; Senapati et al., 2019).

Yield losses under drought are associated with a decline in functional activity of photosynthetic apparatus in consequence of stomata closure and a decrease in leaf conductivity for CO<sub>2</sub> (Pinheiro & Chaves, 2011). In bright sunlight under water stress, when the rate of CO<sub>2</sub> assimilation in leaves is inhibited, there is a risk of oxidative damage to photosynthetic membranes by reactive oxygen species (ROS), which are formed through the accumulation of excess electrons in the electron transport chain (ETC) of chloroplasts and molecular oxygen reduction (Sade et al., 2011; Neto et al., 2017). The most important systems for photosynthetic apparatus protection from oxidative stress caused by ROS are antioxidant enzymes in chloroplasts (Singh et al., 2012; Caverzan et al., 2016).

Therefore, studies of key chloroplast antioxidant enzymes activity changes under the influence of drought are very relevant (Ehonen et al., 2019). Another protective mechanism that prevents the overreduction of ETC components under diminishing CO<sub>2</sub> fixation is photorespiration, the activation of which contributes to photosynthetic apparatus defense from

photoinhibition (Stasik, 2014; Bai et al., 2017; Hanawa et al., 2017). The most studied period in terms of drought impact on wheat productivity is the reproductive development (Lopes et al., 2014). Drought at this time significantly reduces wheat grain number, which leads to considerable yield losses (Senapati et al., 2019). It is also known that drought during the grain filling period worsens its fulfillment, which also negatively affects productivity. At the same time, much less is known about the pattern of water stress influence on plants during the stem elongation stage, when intensive growth of vegetative organs occurs and spike reproductive elements are formed. It should also be noted that at this time the flag leaf is just beginning to appear, and the main donor of assimilates is the penultimate leaf, as it is located in the top tier of the crop canopy and receives the maximal photosynthetically active radiation (PAR). Our previous studies revealed certain differences in the rate and pattern of photosynthetic processes dynamics between the penultimate and flag leaves of wheat plants under conditions of optimal watering (Kiriziy et al., 2017). The aim of this work was to study comparatively the peculiarities of drought effects at the late stem elongation stage and heading-flowering on the chlorophyll content, chloroplast antioxidant enzymes activity, the rate of carbon dioxide gas exchange and transpiration of leaves, as well as on the productivity of wheat plants.

### Materials and methods

The research was carried out on bread winter wheat plants (*Triticum aestivum* L.) of Astarta and Nataalka varieties grown in pots with

10 kg of fertilized soil at natural light. Fertilizers were added in equal quantities ( $N_{80}P_{80}K_{80} + N_{80}P_{80}K_{80}$  mg/kg of soil) when the pots were filled with soil and in the middle of the stem elongation period (BBCH 34). For each variety, 15 pots with 20 plants each were set up.

The pots were watered daily to maintain the soil moisture level within 60–70% of the field capacity (FC). Drought treatment was applied to five pots of each variety at late stem elongation stage (BBCH 37–45) and to another five pots at heading-anthesis (BBCH 59–65) while five pots with control plants were watered as usual. Watering was withheld until the soil moisture reached 30% of the field capacity (FC). This soil moisture level was kept for seven days and then watering was resumed to maintain the soil moisture at the level of control plants until the harvest.

The penultimate leaf at the stem elongation stage and flag leaf at the heading-anthesis period were used for the determinations of water deficit, chlorophyll content, activity of chloroplast antioxidant enzymes, and gas exchange rate. The measurements of leaf parameters were taken on the first day of drought at 30% of FC (third day after cessation of watering) and at the end of the drought period (seventh day at 30% FC). Leaf material for measuring antioxidant enzymes activity was collected at these time points and frozen immediately. Elements of the structure of the plants' grain productivity were determined after achieving the complete grain maturity by weighing the air-dry material.

For the determination of antioxidant enzymes activity, chloroplasts were isolated mechanically at a temperature of 0–4 °C. The sample (2 g) of wheat leaves was homogenized in a 7-fold volume of buffer solution of the following composition: 0.33 M sorbitol, 5 mM  $MgCl_2$ , 0.1% BSA, 4 mM ascorbic acid and 50 mM Tris-HCl (pH 7.5). The homogenate was filtered through two layers of nylon fabric and centrifuged in a centrifuge K-24D at 80 g and a temperature of 0–4 °C for 5 minutes to precipitate heavy particles. The supernatant was poured into other pre-cooled centrifuge tubes and centrifuged at 2000 g for 10 minutes to obtain a fraction of chloroplasts. The chloroplasts sediment was resuspended in isotonic medium with 4 mM ascorbic acid, 50 mM Tris-HCl (pH 7.5) in a volume of 2 mL and subsequently used to determine the activity of superoxide dismutase (SOD), and ascorbate peroxidase (APX).

The superoxide dismutase (SOD, EC 1.15.1.1) activity was determined spectrophotometrically using nitrotriazolium blue at a wavelength of 560 nm (Giannopolitis & Ries, 1977). The ascorbate peroxidase (APX, EC 1.11.1.11) activity was measured in the ultraviolet region of the spectrum at 290 nm using the Chen & Asada (1989) method. The content of chlorophyll in the chloroplasts suspension was determined by the Arnon method (Arnon, 1949).

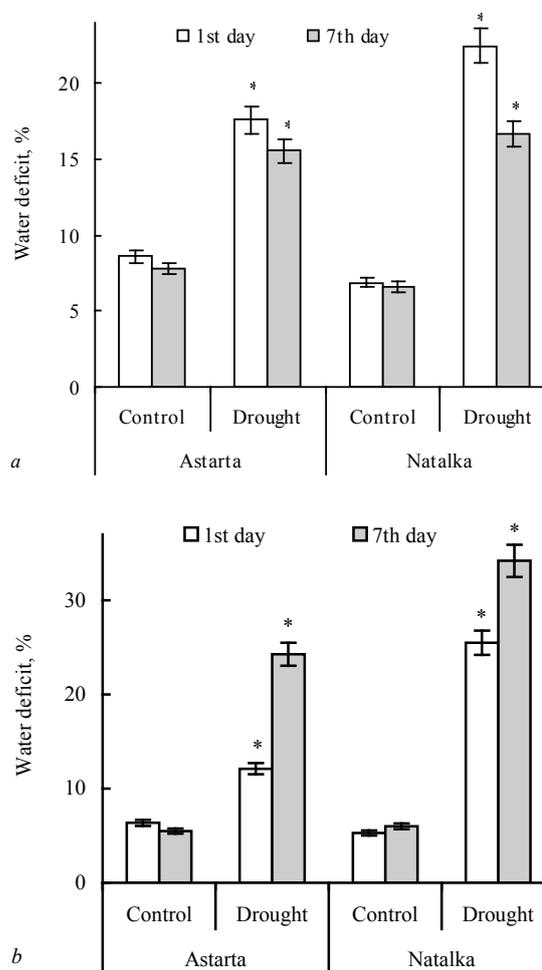
The total chlorophyll content in the leaf lamina was determined by the non-maceration method by extraction with dimethyl sulfoxide, followed by determination of the extracts' extinction coefficients using spectrophotometer (Wellburn, 1994).

The net assimilation ( $A_N$ ) and photorespiration ( $R_{pi}$ ) rate were recorded under controlled conditions by an infrared gas analyzer GIAM-5M. The intact flag leaves (2 in parallel) were placed in a temperature-controlled (+25 °C) chamber (3 × 7 cm) and illuminated (1800  $\mu mol/(m^2 \cdot s)$  PAR) by the TA-11 50W LED spotlights with a light temperature of 5200 K. Atmospheric air was blown through the chamber at a speed of 1 L/min. The photorespiration rate was estimated by the postillumination  $CO_2$  burst from a leaf for 1 min after the light was turned off. The transpiration rate was measured by a thermoelectric micropsychrometer based on the difference in air humidity at the inlet and outlet of the chamber. Gas exchange parameters were calculated according to standard methods (Mokronosov & Kovalev, 1989).

Repeatability of the water deficit determinations was 5-fold, analytical repeatability of photosynthetic pigments content determination using pooled sample of leaves of 5 individual plants – 3-fold, determination of gas exchange and enzymes activity 4-fold. Data on components of grain productivity were determined as average of measurements of 20 individual plants. The obtained data were processed by generally accepted methods of variation statistics. The figures and the table show the arithmetic mean and standard error of the mean. The significance of the difference between controls and treatments were evaluated using ANOVA. Differences were considered significant at  $P < 0.05$ .

## Results

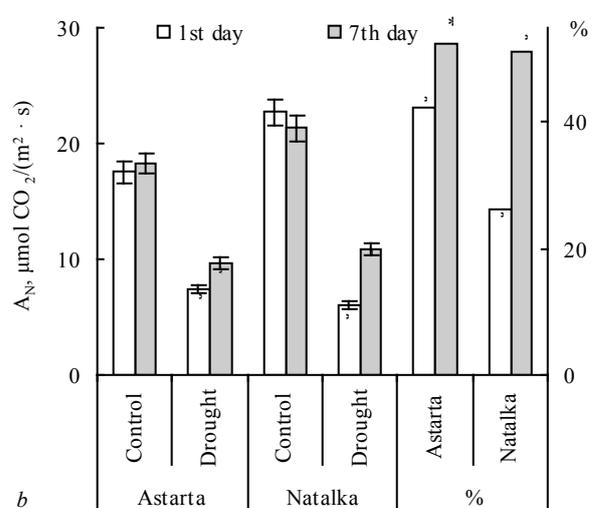
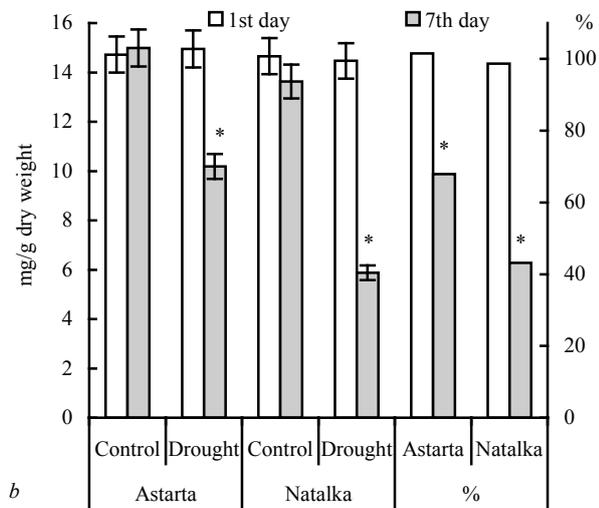
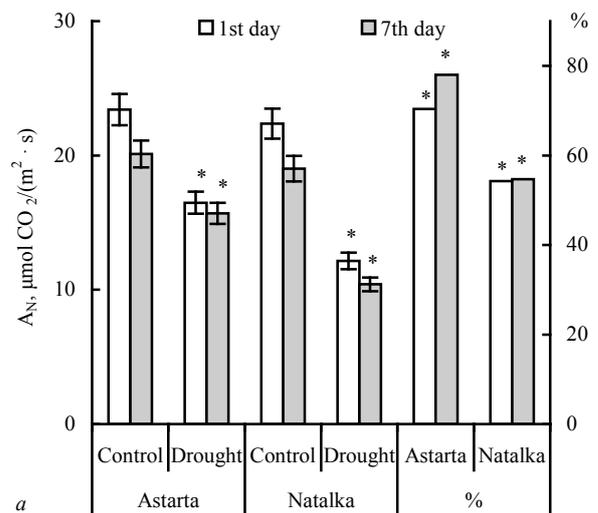
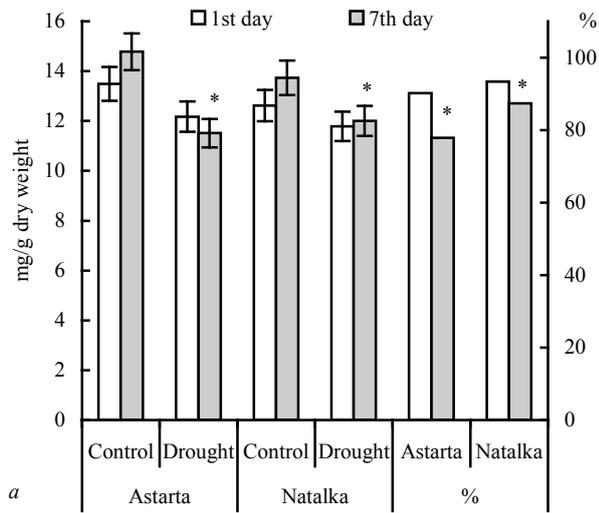
A decrease in soil moisture to 30% FC (1st day of drought) caused a significant increase in water deficit in leaves of both varieties at both studied periods of ontogenesis (Fig. 1). At the same time, in Nataalka variety plants, this parameter increased significantly more than in the Astarta variety (3.3–4.8 and 1.9–2.1 times, respectively). However, in the stem elongation period on the seventh day of drought, there was a slight decrease in penultimate leaf water deficit as compared with the first day of the drought, while in the heading-flowering period this parameter in flag leaf increased.



**Fig. 1.** Water deficit indices in leaves of control and drought-treated wheat plants (1st and 7th days at 30% FC) at stages of stem elongation (a) and heading-flowering (b);  $x \pm SE$ ,  $n = 5$ ; \* – significant difference compared to the control ( $P < 0.05$ )

The total chlorophyll content in the penultimate leaves of plants of both varieties under the drought during the stem elongation period showed a tendency to decrease by 7–10% already on the first day of soil moisture reaching 30% FC (Fig. 2a). On the seventh day of the experiment, this parameter remained almost unchanged in treated plants, and in control plants it tended to increase, which led to a certain decrease in the treatment/control ratio. In general, there were no significant differences between the treated plants of both varieties in terms of chlorophyll content in the penultimate leaves.

During the experiment in the heading-flowering period, the value of chlorophyll content in flag leaves of control plants of both varieties did not differ significantly (Fig. 2b). On the first day of drought 30% FC, the chlorophyll content in the treated plants did not change compared to the control, however, on the seventh day, its sharp decline was observed. It was much stronger in plants of the Nataalka variety than in Astarta (respectively by 57 and 32% relative to control).



**Fig. 2.** The total chlorophyll content in the leaves of wheat of the control and drought-treated plants (1st and 7th days at 30% FC) at stages of stem elongation (a) and heading–flowering (b): the additional ordinate axis shows the values for the treated plants in % relative to the corresponding control; n = 3; see Fig. 1

**Fig. 3.** Net assimilation rate ( $A_N$ ) in wheat leaves of control and drought-treated plants (1st and 7th days at 30% FC) at stages of stem elongation (a) and heading–flowering (b): the additional ordinate axis shows the values for the treated plants in % relative to the corresponding control; n = 4; see Fig. 1

Measurements of the gas exchange parameters in wheat leaves revealed that the net  $\text{CO}_2$  assimilation rate ( $A_N$ ) in the penultimate leaves of plants of the studied varieties at stem elongation stage under conditions of sufficient moisture supply did not differ (Fig. 3a). Drought at this period significantly reduced  $A_N$ , and decline was more pronounced in Nataalka variety plants than in Astarta variety. In addition, a general declining tendency in the  $A_N$  of the studied varieties during the experiment both under conditions of normal soil moisture and during drought can be noted. This is probably due to age-related changes in physiological and biochemical processes in the leaf.

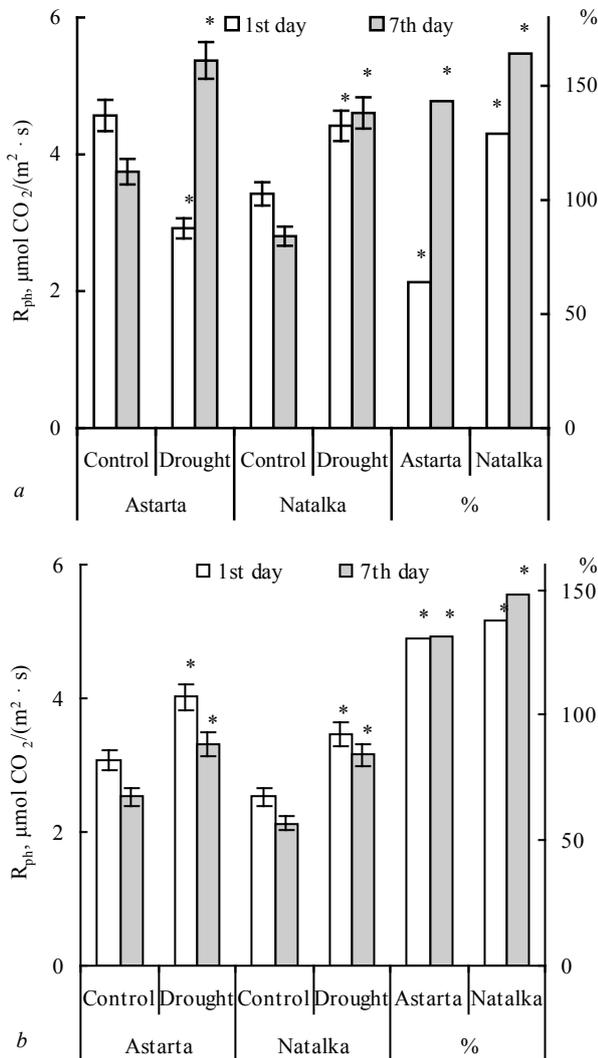
The  $A_N$  in the flag leaves of Astarta variety plants during the heading–flowering period under optimal water supply was less than that of the penultimate leaves in the stem elongation period, whereas in Nataalka variety plants these parameters were almost the same (Fig. 3b). That is, during the heading–flowering period, the  $A_N$  in flag leaf of Nataalka variety control plants was higher than that of the Astarta variety. After the irrigation of treated plants was stopped, on the first day of soil moisture reaching 30% FC, significant inhibition of  $A_N$  was observed in leaves of both varieties: more than two times in Astarta and three times in Nataalka variety. As a result, the  $A_N$  in leaves of the first variety during drought was significantly higher than in the second. On the seventh day of the drought,  $A_N$  became somewhat more active compared with the first day in leaves of both varieties, which can be considered as evidence of the photosynthetic apparatus adaptation to the effect of the stressor.

As the values of measured leaf gas exchange parameters in control and treated plants can vary during the observation period upon influence of environmental factors other than water supply and also on account of intervariety differences, for assessing the drought tolerance of the photosynthetic apparatus of wheat plants, it is reasonable, in our opinion, to calculate relative changes under drought conditions compared with the corresponding control values.

The  $A_N$  in penultimate leaves of Astarta variety plants on the first day of soil moisture reaching 30% FC at the stem elongation period was 70% of the control (Fig. 3). On the seventh day of drought,  $A_N$  tended to increase, but still was significantly lower than the control values. In Nataalka variety plants, the  $A_N$  of penultimate leaves under drought conditions declined in regard to respective control values much more than in Astarta variety, and amounted to about 55% of control at both periods of measurement. The  $A_N$  in flag leaves of Astarta variety plants on the first day of drought at the heading–flowering period was 42% of the control (significantly less than the corresponding relative parameter in penultimate leaf). On the seventh day of drought, we also observed the tendency to increase of  $A_N$  relative values in flag leaves. At the same time, in Nataalka variety plants the  $A_N$  in flag leaves on the first day of drought was only 26% of the control, which is much lower compared to the same parameter for the penultimate leaf of this variety, and for the flag leaves of Astarta variety plants.

The photorespiration rate ( $R_{ph}$ ) in penultimate leaves of Astarta variety plants under optimal moisture supply was higher than that of Nataalka variety (Fig. 4a). Under drought conditions in the stem elongation period,

this parameter increased significantly, especially in Astarta variety plants on the seventh day of the stressor impact. Meanwhile, the difference between the varieties remained: Astarta variety plants exceeded the Natalka variety in the  $R_{ph}$  in penultimate leaves. At the same time, in the second variety the absolute values of this parameter during drought remained almost stable.



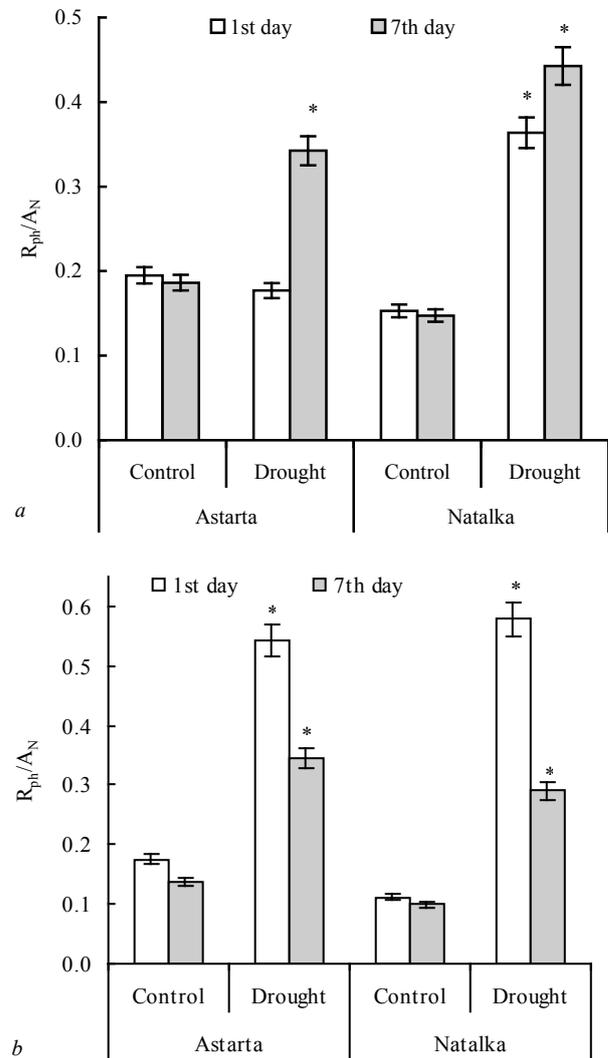
**Fig. 4.** Photorespiration rate ( $R_{ph}$ ) in wheat leaves of control and drought-treated plants (1st and 7th days at 30% FC) at stages of stem elongation (a) and heading-flowering (b): the additional ordinate axis shows the values for the treated plants in % relative to the corresponding control;  $n = 4$ ; see Fig. 1

Generally, the  $R_{ph}$  in flag leaves was slightly lower than that in the penultimate (Fig. 4b). However, common trends remained: in Astarta variety plants this parameter was higher than in Natalka variety both under optimal water supply and drought conditions, and in the latter case there was a significant increase in the  $R_{ph}$  in both periods of wheat development.

When calculating the changes of  $R_{ph}$  relative to control values in penultimate leaves during drought, it is evident that on the seventh day in Natalka variety plants this parameter also was higher compared to the first day. In the flag leaves of plants of both varieties, during the experiment it was more stable and exceeded the control in Astarta variety plants by 31% and in Natalka variety by 38–48%.

Calculations of the ratio of  $R_{ph}$  to  $A_N$  revealed a significant rise in the fraction of the photorespiration in carbon dioxide gas exchange budget in the light under drought conditions (Fig. 5). Under normal water supply, this parameter was higher in plants of Astarta variety, both in the penultimate and flag leaves. Under the drought, the ratio  $R_{ph}/A_N$  in the penultimate leaf was higher in Natalka variety plants. The same was observed for the flag leaf on the first day of reaching soil moisture of 30% FC after

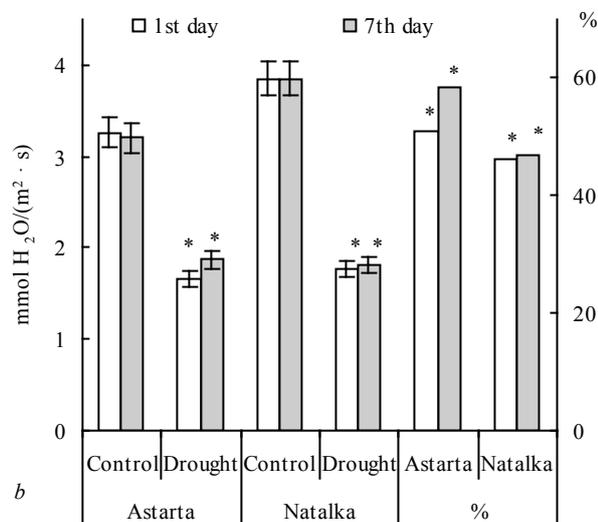
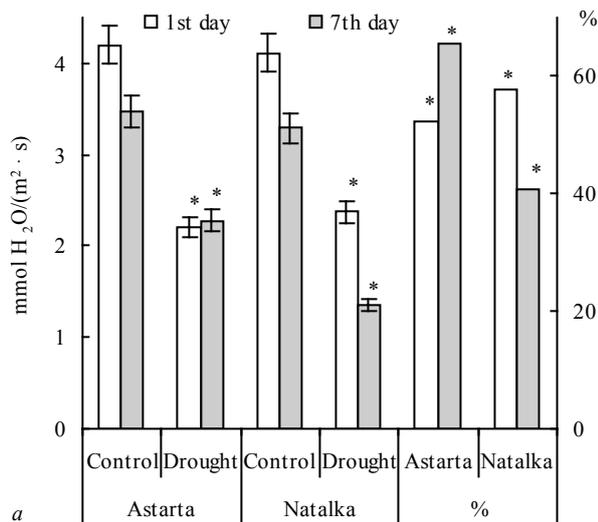
watering ceased. Only on the seventh day of drought, a tendency to a decrease in this parameter in the flag leaves of Natalka variety plants compared to Astarta variety was observed. The observed superiority of Natalka variety plants over Astarta variety in terms of  $R_{ph}/A_N$  is rather related to more pronounced decrease in the  $A_N$  under drought conditions than to an increase in the  $R_{ph}$  (Fig. 3, 4).



**Fig. 5.** The ratio of photorespiration to assimilation rate ( $R_{ph}/A_N$ ) in wheat leaves of control and drought-treated plants (1st and 7th days at 30% FC) at stages of stem elongation (a) and heading-flowering (b): the additional ordinate axis shows the values for the treated plants in % relative to the corresponding control;  $n = 4$ ; see Fig. 1

The transpiration rate of wheat leaves under drought conditions is, on the one hand, an indicator of moisture losses, and, on the other hand – a quantitative characteristic of stomata conductivity for carbon dioxide. According to this parameter in the penultimate leaves of control plants at stem elongation stage, the studied varieties practically did not differ (Fig. 6). On the first day of the drought at 30% FC, the transpiration rate in treated plants decreased almost by half, but no varietal differences were observed. On the seventh day of drought, in Astarta variety plants this parameter remained almost unchanged compared to the first day, and in Natalka variety plants it decreased significantly.

The transpiration rate of the control plants' flag leaves during the heading-flowering period in Natalka variety was somewhat higher than in Astarta variety (Fig. 6b). Under drought conditions, this parameter in the flag leaf dropped almost twofold as in the penultimate leaf. At the same time, the transpiration rate of the flag leaves of control and treated plants of both varieties was quite stable during the observation period, and in most cases its values were lower than those of the penultimate leaves.

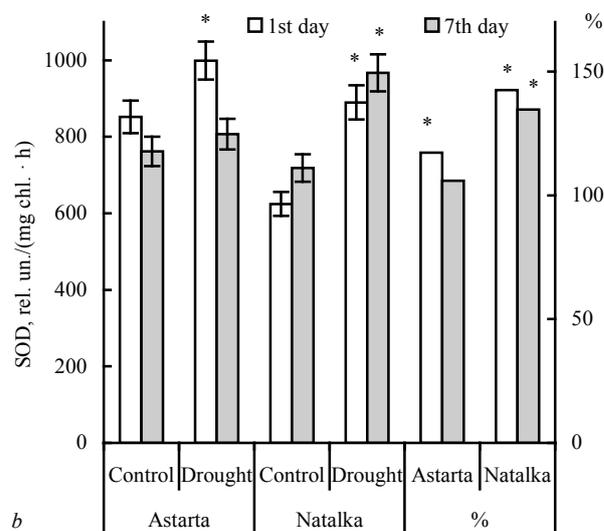
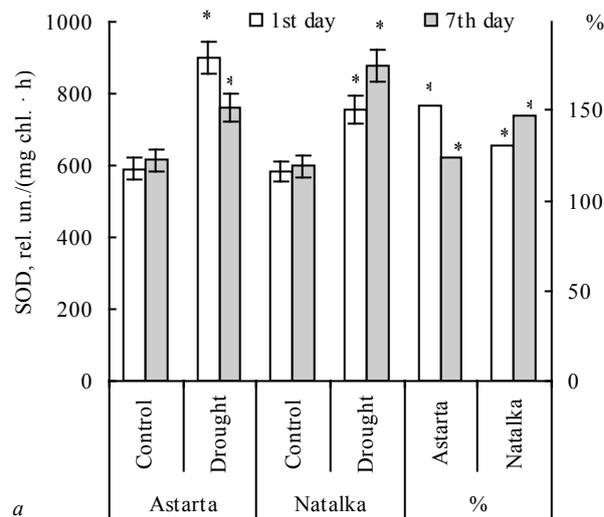


**Fig. 6.** Transpiration rate of wheat leaves of the control and drought-treated plants (1st and 7th days at 30% FC) at stages of stem elongation (a) and heading-flowering (b): the additional ordinate axis shows the values for the treated plants in % relative to the corresponding control; n = 4; see Fig. 1

Calculations of changes in transpiration rate during drought relative to control values revealed that on the seventh day of drought in the leaves of Astarta variety plants, this parameter tended to increase compared with the first day in both periods of the experiment (Fig. 6). However, it decreased under drought in Natalka variety plants at the stem elongation stage, and remained unchanged during heading-flowering.

At the stem elongation stage, the activity of chloroplastic SOD in control plants leaves of both varieties was similar (Fig. 7a). At the first day of drought, the SOD activity in treated plants significantly increased compared to the control: by 53% in Astarta variety and by 30% in Natalka variety. On the seventh day of drought, this parameter in Astarta variety plants slightly decreased remaining 24% higher than control, while the Natalka variety showed a further increase up to 47% superiority to control.

Differences in the APO activity in control plants leaves during this period were also insignificant (Fig. 8a). Drought led to an increase in this parameter. Meanwhile, on the first day of drought, the chloroplastic APO activity in leaves of both varieties was almost the same and exceeded the control by 41–44%. On the seventh day of drought, the absolute value of this parameter in Astarta variety plants practically did not change, while it increased significantly in Natalka variety. Against the background of a slight increase in APO activity during this period in control, in relative terms, the Astarta variety showed a tendency toward a decline in APO activity and the Natalka variety showed an upward trend.

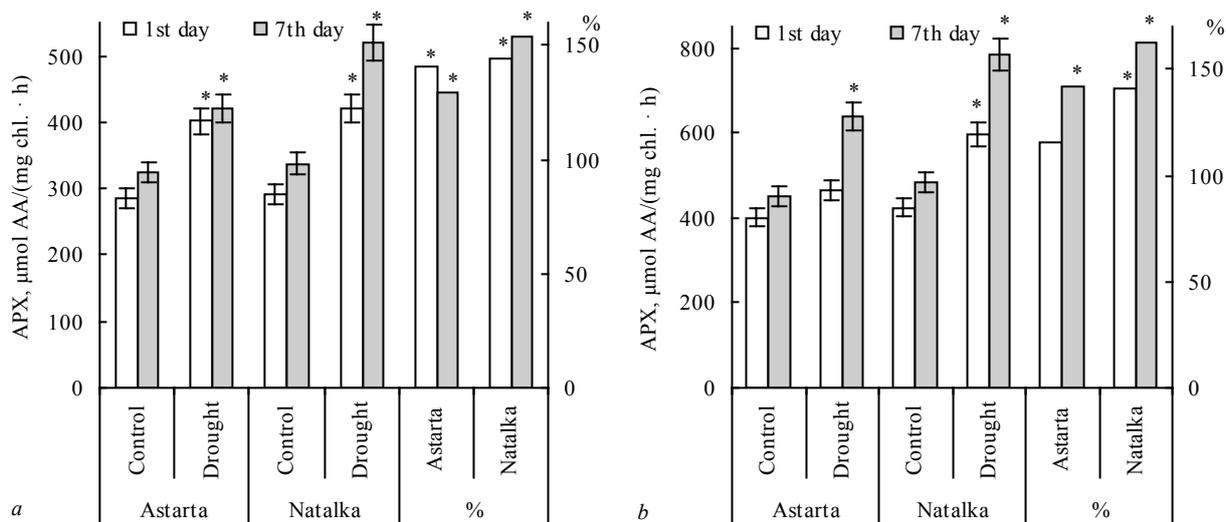


**Fig. 7.** The chloroplastic superoxide dismutase (SOD) activity in wheat leaves of control and drought-treated plants (1st and 7th days at 30% FC) at stages of stem elongation (a) and heading-flowering (b): the additional ordinate axis shows the values for the treated plants in % relative to the corresponding control; n = 4; see Fig. 1

During the heading-flowering period, the chloroplastic SOD activity in flag leaves of control plants in Astarta variety was higher than in Natalka variety (Fig. 7b). The drought at this period led to an increase in SOD activity in leaves of both varieties which was more significant in Natalka variety than in Astarta variety. The relative to the control changes in this parameter on the seventh day of drought decreased insignificantly in both varieties.

The chloroplastic APO activity in flag leaves of control plants during the heading-flowering period was practically the same in both varieties (Fig. 8b). On the first day of drought, this parameter increased by 16% in Astarta variety plants, and by 41% in Natalka variety plants. On the seventh day of drought, a further increase in APO activity was observed, by 42% and 63% relative to the control values, respectively.

The negative effect of drought on the physiological and biochemical processes, and primarily on photosynthetic CO<sub>2</sub> assimilation, led to a significant decrease in both biological and grain productivity of plants (Table 1, 2). In general, the grain productivity of control and treated Astarta variety plants was higher than Natalka variety, which corresponds to their passport characteristics (Morgun et al., 2015). It should be noted that drought subjected plants of Astarta variety showed a smaller decrease relative to the control in such parameters as total aboveground mass, grain mass, number of grains compared to Natalka variety.



**Fig. 8.** The chloroplastic ascorbate peroxidase (APX) activity in wheat leaves of control and drought-treated plants (1st and 7th days at 30% FC) at stages of stem elongation (a) and heading-flowering (b): AA – ascorbic acid; the additional ordinate axis shows the values for the treated plants in % relative to the corresponding control; n = 4; see Fig. 1

**Table 1**

Productivity (calculated on dry matter) of main shoot of wheat plants subjected to drought at stages of stem elongation (BBCH 37-45, drought 1) and heading-flowering (BBCH 59-65, drought 2) ( $\bar{x} \pm SE$ ; n = 20)

Variety	Variant	Total weight, g	Grain weight, g	Number of grains, pcs.	1000 grains weight, g
Astarta	Control	4.58 ± 0.13	2.65 ± 0.07	48.2 ± 1.4	55.6 ± 1.8
	Drought 1	3.36 ± 0.16	1.83 ± 0.12	39.5 ± 2.3	47.4 ± 2.1
	Drought 2	3.52 ± 0.18	1.95 ± 0.13	42.0 ± 1.5	45.9 ± 1.9
	% relative to control for 1	73.4*	69.1*	81.9*	85.2*
	% relative to control for 2	76.7*	73.5*	87.2*	82.4*
Nataalka	Control	4.43 ± 0.16	2.12 ± 0.10	44.8 ± 1.2	47.0 ± 1.5
	Drought 1	2.69 ± 0.19	1.32 ± 0.11	29.6 ± 2.0	44.2 ± 2.3
	Drought 2	3.12 ± 0.11	1.44 ± 0.07	33.7 ± 1.6	42.8 ± 2.9
	% relative to control for 1	60.7*	62.2*	66.2*	93.9
	% relative to control for 2	70.4*	67.9*	75.3*	91.0

Note: \* – significant difference compared to the control (P < 0.05).

**Table 2**

Productivity (calculated on dry matter) of whole wheat plants subjected to drought at stages of stem elongation (BBCH 37-45, drought 1) and heading-flowering (BBCH 59-65, drought 2) ( $\bar{x} \pm SE$ ; n = 20)

Variety	Variant	Total weight, g	Grain weight, g	Number of grains, pcs.	1000 grains weight, g	Harvest index	Number of productive shoots, pcs.
Astarta	Control	8.93 ± 0.41	4.94 ± 0.23	103.3 ± 5.2	48.4 ± 1.2	0.55 ± 0.01	2.57 ± 0.15
	Drought 1	7.72 ± 0.39	3.92 ± 0.23	98.9 ± 5.6	39.7 ± 0.6	0.50 ± 0.01	3.32 ± 0.19
	Drought 2	5.35 ± 0.30	2.70 ± 0.17	58.9 ± 3.1	45.9 ± 1.7	0.50 ± 0.01	1.81 ± 0.10
	% relative to control for 1	86.4*	79.3*	95.7	82.0*	91.1	129.4*
	% relative to control for 2	59.9*	54.7*	57.0*	94.7	91.1	70.7*
Nataalka	Control	7.92 ± 0.56	3.85 ± 0.28	87.2 ± 5.6	43.9 ± 0.7	0.49 ± 0.01	2.15 ± 0.13
	Drought 1	5.94 ± 0.47	2.68 ± 0.23	75.4 ± 5.7	35.9 ± 1.8	0.45 ± 0.02	3.33 ± 0.22
	Drought 2	3.72 ± 0.22	1.53 ± 0.09	35.8 ± 1.7	42.8 ± 2.8	0.40 ± 0.01	1.20 ± 0.09
	% relative to control for 1	75.1*	69.7*	86.5*	81.6*	92.4	155.0*
	% relative to control for 2	47.0*	39.7*	41.1*	97.5	82.6*	55.8*

Note: see Table 1.

## Discussion

The results of determining water deficit in leaves provide grounds for arguing, that the water regime of wheat plants is better adapted to the lack of moisture at the stem elongation period than at the heading-flowering period. Obviously, in the stem elongation period, when the growth processes of vegetative organs have not yet been completed, under drought conditions the growth of the above-ground part, especially the main shoot, is inhibited (which will be shown in the discussion of productivity parameters). On the one hand, this helps to save water in the plant, and on the other hand, more resources can be used to enhance the water absorption capacity of the roots, which together leads to a decrease of water deficit in the leaves on the seventh day of drought. Undoubtedly, the leading role in

this is played by the phytohormonal regulation of growth processes. During the flowering period, the growth of vegetative organs of wheat practically ends and the plant's ability to regulate the shoot/root ratio becomes very limited. At the same time, the leaf area reaches a maximum, which, despite the stomata closure during drought, increases the uncontrolled loss of water by the plant through the remaining stomatal apertures and by the cuticle. As a result, the water deficit in leaves continues to increase with lengthening period of the stressor's action. It should be noted that water deficit in leaves of Astarta variety plants during drought was less than that of Nataalka variety.

Drought, especially prolonged, significantly influenced the assimilation activity of plants, reducing the chlorophyll content (Fig. 2), net assimilation rate (Fig. 3) and transpiration (Fig. 6) of leaves. It should be noted

that the penultimate leaves of plants of the studied wheat varieties under drought conditions during the stem elongation period showed higher assimilation rates than the flag ones under drought during the heading-flowering period. This finding implies an increased sensitivity of wheat plants to the impact of drought during the flowering stage compared with the stem elongation stage. At the same time, in these parameters Astarta variety proved to be more drought tolerant than Natalka variety.

Under drought conditions promoting stomatal closure, photorespiration plays a protective role as a source of CO<sub>2</sub> inside the cell, which helps to maintain somewhat the functioning of the Calvin cycle. On the other hand, photorespiration is an alternative electron sink for chloroplastic ETC (Hanawa et al., 2017). Both of these features of photorespiration contribute to the “unloading” of ETC components from electrons, which reduces the formation of reactive oxygen species. However, photorespiration itself is a source of hydrogen peroxide. Therefore, photorespiration can play a protective role provided that antioxidant defense systems function effectively. Otherwise, this role can be insignificant or photorespiration will even increase the negative influence of drought on the photosynthetic apparatus due to the additional ROS formation (Stasik, 2014; Morgun et al., 2016). Interestingly, the ratio  $R_{\text{pt}}/A_N$  increased on the seventh day of drought in the penultimate leaves, while in the flag leaves, on the contrary, it decreased (Fig. 5). The latter can be explained by a decrease in pools of intermediate products of metabolic cycles as a result of inhibition of CO<sub>2</sub> assimilation processes through stomata closure under conditions of lack of moisture in the soil.

Based on the experimental data, it can be assumed that Astarta variety plants have a higher net assimilation rate in leaves under drought conditions compared to Natalka variety due to the better functioning mechanisms of the photosynthetic apparatus protection, including photorespiration. During drought, plants of Natalka variety increased unproductive losses of assimilated carbon, and the protection of the photosynthetic apparatus was less effective than that in Astarta variety plants.

It is known that even at the early stages of water stress, stomata conductivity sharply decreases under the control of a drought-induced signaling system, the main factor of which is considered ABA (Sade et al., 2011; Mittler & Blumwald, 2015). ABA is synthesized in large quantities in the roots and aboveground organs of plants with a decrease in moisture supply (Boursiac et al., 2013; McAdam & Brodribb, 2018). A decrease in CO<sub>2</sub> input into the leaf due to stomata closure under drought inhibits Calvin cycle functioning, which reduces the use of primary products of photosynthesis – ATP and NADP•H<sub>2</sub> and leads to the superreduction of ETC components (Ehonen et al., 2019). This induces the electrons from ETC to reduce molecular oxygen to its reactive species (ROS), primarily superoxide radical anions (Mehler reaction) (Neto et al., 2017). Oxygen superoxide radical anions are the source of the formation of hydroxyl radicals and hydrogen peroxide.

ROS can lead to serious functional impairments as a result of damage to various components of the cells (Sade et al., 2011). An example is the initiation of lipid peroxidation in biological membranes, which leads to a violation of their structure and increase of permeability. ROS damage the photosynthetic apparatus, cause nucleotide modifications, and disrupt the functioning of cells genetic apparatus, inhibit cell division.

Among the most important systems for protecting the photosynthetic apparatus from oxidative stress caused by ROS are the antioxidant enzymes of chloroplasts – SOD and APO (Kolupaev & Kokorev, 2019). SOD catalyzes the reaction of disproportionation of superoxide radical anions to oxygen and hydrogen peroxide, while APO inactivates hydrogen peroxide. Therefore, an increase in the activity of chloroplast SOD and APO under conditions of soil drought can be considered as a protective response of the photosynthetic apparatus to oxidative stress.

At the stem elongation stage, the activity of SOD and APO during drought response development changed synchronously (Fig. 7, 8). A decrease in enzymes' activity on the seventh day of drought in the Astarta variety plants can be considered as the evidence of their adaptation to the stressor influence. At the same time, a further increase in the chloroplast antioxidant enzymes activity in the Natalka variety plants suggests the growing dynamics of disturbances in the photosynthetic apparatus and augmentation of oxidative stress, which necessitates the activation of protective ROS detoxification systems.

In the Astarta variety in addition to maintenance of higher stomatal conductivity and CO<sub>2</sub> assimilation activity, it is possible that mechanisms of regulation of photosynthetic processes are also more effective, which reduce the probability of ROS formation in chloroplasts under drought conditions, for example, a decrease in the superreduction of ETC components due to increased non-photochemical dissipation of absorbed light energy, as was shown in other experiments (Kiriziy et al., 2014).

During the heading-flowering period, we can also suggest the synchronous functioning of the chloroplast antioxidant protection enzymes, at least in the sense that in Natalka variety plants a greater increase in SOD activity during drought corresponded to a larger increase in APO activity, compared to the Astarta variety. A slight increase in the last parameter on the seventh day of drought is probably due to the activation of other sources of hydrogen peroxide under these conditions, for example, photorespiration (Ehonen et al., 2019).

In general based on the dynamics of absolute values and relative to control values of SOD and APO activity under the drought impact, it can be concluded that photosynthesis in Astarta variety plants is more efficiently regulated compared to the Natalka variety, which allows the plants to maintain greater stomata openness and CO<sub>2</sub> assimilation activity under conditions of lack of moisture, which, in turn, mitigates the energy imbalance in chloroplasts and reduces the ROS formation.

It should also be noted that in most cases, the absolute values of enzymes activity in flag leaves during the experiment in the heading-flowering period were higher than in the penultimate ones during the stem elongation period. This indirectly indicates an increase in ROS formation in flag leaves, which may be related both to the above mentioned differences in the functioning systems of their generation control, and to environmental conditions, e.g. higher daily average temperatures.

Taken together, these results indicate that the photosynthetic apparatus of Astarta variety plants is more resistant to drought compared to the Natalka variety. This may be related to a presence of wheat-rye translocation 1BL.1RS in the genome of the Astarta variety (Morgun, 2016). The data obtained also show a stronger damaging effect of drought on the photosynthetic apparatus during heading-flowering compared to the stem elongation stage. In the latter case, the differences can be explained, at least in part, by the greater drought tolerance of penultimate leaves than flag ones, which is caused by both structural and functional features of the leaves themselves and differences in their functioning in the source-sink system during the studied periods of plant development. The flag leaf during the heading-flowering period, when the vegetative growth has already stopped, but grains as the main sink of assimilates are not yet formed, may be more vulnerable to external influence due to a greater tension in the source-sink system. Besides this, the noted differences in the effect of soil drought can to some extent be associated with different air temperatures. Thus, the average daily air temperature during the experiment at stem elongation stage was 15.9 °C, and during the heading-flowering it was 20.7 °C ([www.pogodaiklimat.ru](http://www.pogodaiklimat.ru)).

The plant response to drought as regards its performance markedly differed depending on the period of application and the judged parameters of productivity. So, if we consider the parameters for the main shoot, then one can note in both varieties a tendency to more severe inhibition of ear productivity under the drought treatment at the stem elongation stage than at the heading-flowering period (Table 1, 2). Only by mass of 1000 grains did plants subjected to drought in the last term have some advantage. If we take into account parameters for the whole plant (together with the lateral shoots), then the pattern changes to the opposite – more reduction in all productivity elements (except for the mass of 1000 grains) is observed in plants subjected to drought at the heading-flowering.

The key to resolving this contradiction is the index of productive tillering, which in plants subjected to drought at the stem elongation stage not only did not decrease (unlike other parameters), but exceeded the control by 30% in the Astarta variety and by 55% in the Natalka variety. In plants subjected to drought during the heading-flowering period, the number of lateral productive shoots was lower than in control. It was due to the increase in productive tillering that plants drought-stressed at the stem elongation stage demonstrated an advantage in productivity over ones treated with drought at the heading-flowering stage. The increase in tillering under drought at the stem elongation stage in comparison with heading-

flowering period is undoubtedly associated with the above-mentioned stronger inhibition of the main shoot growth. It occurred as result of both a decrease in the supply of assimilates as photosynthesis declined and a change in the phytohormonal balance related to increased ABA synthesis. After restoration of normal watering under inhibited growth of the main shoot, the lateral shoots gained an advantage in the distribution of assimilates in the whole plant source-sink system, which led to an increase in productive tillering even in comparison with the control. The ending of drought at the early period of ontogenesis allowed the plants to form a high grain productivity of lateral shoots.

In the case of drought during the heading-flowering period, when the growth of the main shoot has already finished, the lack of assimilates and the disruption of the phytohormonal balance affected the development of productive lateral shoots more, which led to a significant decrease in their number. Therefore, the number of grains per plant was greatly reduced, although, after the termination of drought and restoration of the leaves' photosynthetic function, the supply of grains with assimilates (which were not distracted to the lateral shoots) increased.

Thus, the drought at the stem elongation period strongly inhibited the growth of the main shoot, while with the restoration of normal water supply, the development of lateral productive shoots was promoted, which to some extent compensated the negative impact of stress on the whole plant's grain productivity. When a plant was exposed to drought during the heading-flowering period, both the productivity of the main shoot (albeit to a lesser extent than in the previous case) and the number of productive shoots decreased. This led to a stronger relative decrease in whole plant grain productivity, compared with the drought impact at the stem elongation stage. The lack of assimilates under drought at the stem elongation stage obviously more seriously disrupted the flowers' formation, therefore the main shoot ear grain number in plants stressed at the heading-flowering period was greater compared to the effect of drought at the stem elongation stage, although this led to some decrease in the mass of 1000 grains. One can note that under field conditions, the degree of these effects may be modified by other factors, for example, a large number of plants per area unit may increase the competition for light, and mineral nutrition elements and limit the formation of lateral shoots or delay their maturation.

It is worth noting that the Astarta variety bearing wheat-rye translocation 1BL.1RS has higher whole plant grain productivity and all its components in both well-watered and drought conditions as compared to the Natalka variety. This advantage may be brought about by Astarta's ability to maintain active photosynthetic apparatus longer during the grain filling period (stay-green phenotype). It was shown that 1BL.1RS translocation can be associated with the stay-green phenotype and higher productivity (Luo et al., 2009). The stay-green traits are considered as pivotal for maintaining yield performance during drought and useful physiological markers for drought tolerance breeding in wheat (Christopher et al., 2016; Ghodke et al., 2019).

## Conclusion

Thus, our pot experiment data show clearly that wheat plants are potentially more resistant to drought during the stem elongation stage than during the heading-flowering stage due to the more pronounced ability to compensatory growth of lateral productive shoots. A study of the leaves' physiological and biochemical characteristics – the chloroplast antioxidant enzymes' activity, the photosynthetic pigments' content, and gas exchange parameters – also indicates that the photosynthetic apparatus of wheat has a higher drought tolerance during the stem elongation than during the heading-flowering period. This promotes the better assimilation supply of the plants and better lateral shoots growth, especially after the termination of drought, which contributes to the compensation of negative impact of drought. The Astarta variety has a more drought-tolerant photosynthetic apparatus and higher grain productivity in both well-watered and drought conditions as compared to the Natalka variety. The differences between varieties are more contrasting under drought applied at heading-anthesis stage, which makes this period appropriate for comparative studies of different genotypes and selection for drought tolerance.

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