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RESPONSES OF PHOTOSYNTHETIC APPARATUS OF GENETICALLY MODIFIED WHEAT PLANTS CONTAINING A DOUBLE-STRANDED RNA SUPPRESSOR OF THE PROLINE DEHYDROGENASE GENE TO DROUGHT AND HIGH TEMPERATURE

D.A. KIRIZIY, A.S. KEDRUK, O.G. SOKOLOVSKA-SERGIIENKO, O.V. DUBROVNA, O.O. STASIK

Institute of Plant Physiology and Genetics, National Academy of Sciences of Ukraine
31/17 Vasylkivska St., Kyiv, 03022, Ukraine
e-mail: o_stasik@yahoo.com

Peculiarities of drought effects on the water relations, chlorophyll content, chloroplasts' antioxidant enzymes activity, CO_2 assimilation and transpiration at optimal and elevated temperatures as well as on the grain productivity of transgenic bread winter wheat plants containing double-stranded RNA suppressor of the proline dehydrogenase (*pdh*) gene, compared to the original genotype were studied in pot experiment. Drought for plants of both genotypes was applied by reducing soil moisture to the level of 30 % of field capacity (FC) that was maintained for seven days during the heading-anthesis period. Soil moisture for control plants, as well as treated plants, except for the period of drought, was maintained at the optimal level of 60—70 % of FC. Measurements of physiological and biochemical indices were performed on a flag leaf on the first day of soil moisture reached 30 % of FC and on the seventh day of the drought. It was found that seven-day water scarcity decreased the relative water content in the leaves of transgenic plants less than in the plants of the original line. At the same time, drought reduced chlorophyll content more in transgenic plants than in non-transgenic ones. Drought inhibited the CO_2 assimilation and transpiration rate, however in transgenic plants, in contrast to non-transgenic, the degree of decline at the end of the drought was significantly less than at the onset, indicating improved adaptability of the photosynthetic apparatus in transgenic plants. Furthermore, this peculiarity was also confirmed by the formation of cross-tolerance — lessening the inhibitory effect of high temperature (heating at 42 °C) on the CO_2 assimilation in stressed plants after a seven-day drought, compared with its beginning. The reduction in the harmful effect of high temperature on photosynthesis under prolonged drought was stronger in transgenic plants. The presence of a double-stranded RNA suppressor of the *pdh* gene in transgenic plants sustainably increased the activity of chloroplasts' superoxide dismutase in contrast to the activity of chloroplasts' ascorbate peroxidase under optimal irrigation and essentially modified changes in the activity of these enzymes under drought conditions that can be one of reasons for the differences between transgenic and non-transgenic lines in adaptability. Under optimal watering, the studied lines did not differ in plant

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grain productivity, but drought significantly less reduced the weight of grain from the ear of the main shoot (by 21 %) and the whole plant (by 33 %) in transgenic plants than in plants of the original line (40 and 52 %, respectively). Therefore, the presence of a double-stranded RNA suppressor of the proline dehydrogenase gene in transgenic plants of winter wheat plants improves the adaptive properties of the photosynthetic apparatus and reduces the loss of grain productivity under drought conditions.

Key words: *Triticum aestivum* L., transgenic plants, proline, drought, high temperature stress, photosynthesis, cross-adaptation, antioxidant enzymes (SOD, APX), productivity.

Water deficit is one of the most common abiotic environmental factors, which significantly limits the genetic potential of crop productivity in many parts of the world [1, 2]. This problem is especially relevant for the leading cereal — wheat, as large areas of cultivation of this crop are in areas of risky agriculture, which, in particular, includes Ukraine [3, 4]. Global climate change, accompanied by rising temperatures and aggravated uneven distribution of precipitation both by region and during the growing season, enhance these risks [2, 5]. Therefore, the issue of breeding new wheat genotypes with improved drought and heat resistance is becoming increasingly important [4—6].

At the level of the whole plant organism, the deficit of soil moisture leads to a decrease in the photosynthetic activity and plant growth. The main reason for the photosynthetic activity decline under most conditions of water stress is the stomata closure resulting in the reduction of CO_2 diffusion from the atmosphere to the carboxylation sites [7]. The effects of drought on the photosynthetic apparatus, overall metabolism and plant productivity have been studied for a long time, but remain unclear in many aspects. Nowadays, research in this area is conducted on a broad front, as evidenced by the large number of publications [8—10].

Under natural conditions, drought is often accompanied by high temperature, and photosynthetic apparatus of the leaves suffers from overheating because of reduced leaves ability to self-cool due to transpiration suppression. High temperatures provoke oxidative stress in wheat leaves [11], cause loss of photosynthetic pigments, and reduce assimilation rate [8, 12]. This is accompanied by accelerated aging of leaves [13] that leads to a decrease in grain weight.

Although the dependence of photosynthesis on temperature has been well studied in many plant species, the mechanism that reduces the CO_2 assimilation rate at temperatures above the optimum remains currently controversial [14, 15]. It is suggested that the decrease in the CO_2 assimilation with increasing temperature may be a manifestation of temperature dependence of the particular photosynthetic processes (reverse changes) and/or the consequence of damage to certain structural elements of the photosynthetic apparatus (relatively or completely irreversible changes) [8].

A recent review of more than 120 studies of crops response to the combined effects of drought and heat stress showed that combination of these factors significantly affects yields due to reduced harvest index, shor-

tening plant life cycle, and changes in seed number, size and composition [16]. These negative effects are more severe when a combination of stresses occurs during the reproductive stage of plant development. The authors emphasize the need to focus of further research and breeding approaches on yield response of crops to the combination of drought and heat at the reproductive stage. At the same time, the studies of adaptation mechanisms that can counteract such negative effects on productivity by increasing drought and thermotolerance of the whole plant and its photosynthetic apparatus are especially relevant [10, 14, 17].

Today, one of the promising areas for the creation of new genotypes of agricultural plants, including wheat, resistant to biotic and abiotic environmental stressors is the use of genetic engineering methods [18, 19]. It is known that resistance to drought, salinity and temperature stress are complex traits, and the full set of genes that determine such phenotype is not known [20]. At the same time, there are a number of studies linking these features with the content of free L-proline in plant tissues, which is actively synthesized in response to various stressors [21, 22].

It is believed that proline regulates the acidity of the cytosol and maintains the NAD/NADH ratio, helps to preserve the photochemical activity of photosystem II in thylakoid membranes, and reduces lipid peroxidation. Additional synthesis of this amino acid increases the overall resistance of plants to abiotic stresses, as proline protects membranes, macromolecules and structural elements of the cell, thus increasing non-specific resistance [21]. In plants, the accumulation of proline during stress occurs both by increasing the rate of its synthesis and by suppressing its catabolism [22]. The proline dehydrogenase gene, related to proline catabolism, is of practical importance for genetic engineering, as partial inhibition of its expression may lead to an increase in free proline content and, as a consequence, in plant resistance to abiotic stress [23].

It has been established that the use of vector constructs in which a double-stranded RNA suppressor is located as an inverse repeat is promising for partial suppression of the proline dehydrogenase gene [24]. It is assumed that such a construct due to RNA interference is effective for increasing the proline level. In some cases, a correlation between the proline content and increasing the level of transgenic plants drought resistance has been proven [25].

Therefore, the study of physiological characteristics of transformed wheat plants with suppressed proline catabolism, in particular the response of their photosynthetic apparatus to soil drought, is relevant in terms of assessing the prospects of their involvement in the selection process to create drought-resistant varieties.

The aim of this work was to study the peculiarities of drought effects at heading—anthesis period on the water relations, chlorophyll content, chloroplast antioxidant enzymes activity, CO_2 assimilation and transpiration at optimal and elevated temperatures as well as on the grain productivity of transgenic wheat plants containing double-stranded RNA suppressor of the proline dehydrogenase (*pdh*) gene, compared to the original genotype.

Materials and methods

The study was performed on winter bread wheat plants (*Triticum aestivum* L.) line UK065 (wild type) and obtained on its basis transformants (generation T_3) with double-stranded RNA suppressor of proline dehydrogenase (*pdh*) gene (transgenic plants) [26], which were 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 at the middle of the stem elongation period (GS 34). For each genotype, 10 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 genotype at heading—anthesis period (GS 59—65) while five pots with control plants were watered as usual. Watering was withheld until the soil moisture reached 30 % FC (first day of drought). 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. Soil moisture in the pots was monitored gravimetrically twice a day.

The flag leaf was used for the determinations of relative water content, 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 plant grain productivity were determined after achieving the complete grain maturity by weighing the air-dry material.

The CO_2 net assimilation (A_N) rate was recorded under controlled conditions by an infrared gas analyzer GIAM-5M. The intact flag leaves (2 in parallel) were placed in a temperature-controlled chamber (3×7 cm) and illuminated at 1800 μmol/(m^2 · s) PAR by the TA-11 50W LED spotlights with a color temperature of 5200 K. Atmospheric air was blown through the chamber at a speed of 1 l/min. The atmospheric CO_2 concentration and air humidity at the inlet and outlet of the chamber were measured with a portable gas analyzer EGM-5 (PP Systems, USA). Gas exchange parameters were calculated according to standard methods [27, 28].

The gas exchange chamber was designed in such a way that its upper part with a glass window for illuminating the leaf was hermetically separated from the lower by a thin transparent film, over which water from the ultrathermostat of a given temperature was constantly circulating. The leaves in the chamber were fixed so that the adaxial surface of the leaf was in close contact with the film, and atmospheric air was blown at through the lower half of the chamber, where the abaxial surface was oriented. Thus, it was possible to set the leaf surface temperature with a precision of 0.2 °C.

Recording of gas exchange parameters was started at 25 °C 40—50 min after placing the leaves in the chamber, when they adapted to the measurement conditions. After that, the temperature was raised at a rate of 1 °C/min, and 40 min after reaching a temperature of 42 °C, which was

previously selected as a test temperature [29], gas exchange rates were recorded again.

Leaf relative water content was determined by standard methods [30]. To determine the dry weight, the samples were fixed at 105 °C for 30 min and dried to constant weight at 65 °C. 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 extract extinction coefficients using spectrophotometer [31] and calculated on the dry weight.

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 nitrotetrazolium blue at a wavelength of 560 nm [32]. 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 method [33]. The content of chlorophyll in the chloroplasts suspension was determined by the Arnon method [34].

At the end of the growing season, the dry weight of the aboveground part and the elements of grain productivity of plants were determined.

Repeatability of the relative water content determinations was 5-fold, analytical repeatability of photosynthetic pigments content determination using pooled sample of leaves of 5 individual plants — 5-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 using Microsoft Excel. The figures and the tables 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 $p \leq 0.05$.

Results and discussion

It is known that period of heading—anthesis is the most vulnerable in terms of the impact of drought on wheat productivity [35]. Drought at this time significantly reduces the graininess of plants, which leads to significant crop losses [5]. Therefore, in our experiments, the plants were subjected to drought at this period.

Relative water content in flag leaves significantly decreased already on the first day of drought at 30 % FC compared to control plants grown

*Table 1. Relative water content and total chlorophyll content in the flag leaves of control (70 % FC) and drought-stressed (30 % FC) winter wheat plants of the original line UK065 (wild type) and obtained on its basis transgenic plants with double-stranded RNA suppressor of the proline dehydrogenase gene (transformant) on the first and seventh day of drought (x±SE, n = 5; * — significant difference compared to the control at p < 0.05)*

Genotype	Variant	Relative water content, %		Chlorophyll content, mg/g dry weight	
		1st day	7th day	1st day	7th day
UK065 wild type	Control	90.1±1.2	87.9±0.9	14.9±0.3	12.5±0.3
	Treatment	76.1±1.0*	49.1±1.8*	15.2±0.4	10.1±0.4*
UK065 transformant	Control	90.2±0.6	87.5±0.8	14.3±0.2	14.4±0.3
	Treatment	68.4±2.1*	57.4±2.5*	14.3±0.5	7.4±0.4*

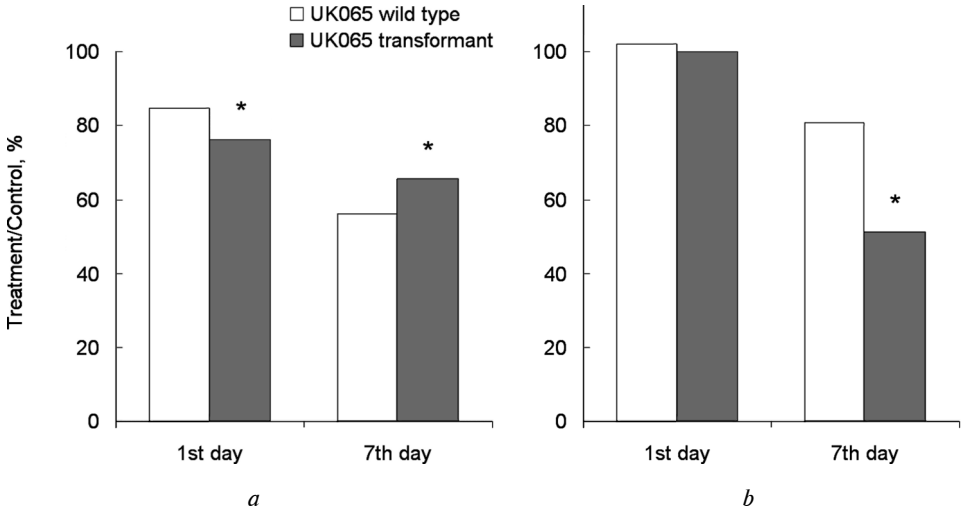

Fig. 1. Changes relative to the corresponding control at 70 % FC in relative water content (*a*) and total chlorophyll content (*b*) in the flag leaves of drought-stressed (30 % FC) wheat plants of the original line UK065 (wild type) and obtained on its basis transgenic plants with double-stranded RNA suppressor of the proline dehydrogenase gene (transformant) on the first and seventh day of drought ($n = 5$; * — significant difference compared to the wild type at $p < 0.05$)

under 70 % both in wild-type and transgenic plants (Table 1). This effect was more pronounced in transgenic plants than in wild-type (Fig. 1, *a*). On the seventh day of drought, the relative water content lowered even more, and this decline was substantially bigger in wild-type than in transgenic plants. As a result, the relative water content in flag leaf lamina of latter was higher than in the former. This may indicate that in transgenic plants, the processes of adaptation to drought proceeded more efficiently than in wild-type plants. One of the reasons contributing to the better adaptation of transgenic plants to drought is obviously the increased content of an osmotically active substance, proline, which increases the water-holding capacity of cells [25]. In addition, it is possible to assume a gain in the sucking power of the roots that also contributes to the improvement of plant water relations under drought conditions (although this issue requires an additional study).

*Table 2. The indices of gas exchange in flag leaves of control (70 % FC) and drought-stressed (30 % FC) winter wheat plants of the original line UK065 (wild type) and obtained on its basis transgenic plants with double-stranded RNA suppressor of the proline dehydrogenase gene (transformant) on the first and seventh day of drought measured under 25 and 42 °C (x±SE, n = 4; * – significant difference compared to the control at $p < 0.05$; # – significant difference compared to 25 °C at $p \le 0.05$)*

Genotype		Variant	Photosynthesis, μmol $CO_2/(m^2 \cdot s)$		Transpiration, mmol $H_2O/(m^2 \cdot s)$		Stomatal conductance for CO_2, (g) mmol/$(m^2 \cdot s)$		Intercellular CO_2 concentration (C_i), ppm	
			25 °C	42 °C	25 °C	42 °C	25 °C	42 °C	25 °C	42 °C
UK065 wild type	1st day	Control	15.76±0.47	8.05±0.24$^{\#}$	2.24±0.07	4.52±0.14$^{\#}$	283±8	124±4$^{\#}$	322±10	321±10
		Treatment	8.17±0.25*	2.88±0.09*,$^{\#}$	1.22±0.04*	1.81±0.05*,$^{\#}$	121±4*	42±1*,$^{\#}$	324±10	329±10
	7th day	Control	13.80±0.41	7.13±0.21$^{\#}$	2.37±0.07	5.18±0.16$^{\#}$	318±10	150±5$^{\#}$	335±10	341±10
		Treatment	7.13±0.21*	3.91±0.12*,$^{\#}$	1.39±0.04*	2.48±0.07*,$^{\#}$	137±4*	58±2*,$^{\#}$	342±10	333±10
UK065 transformant	1st day	Control	15.07±0.45	8.05±0.24$^{\#}$	2.10±0.06	4.66±0.14$^{\#}$	252±8	129±4$^{\#}$	319±10	324±10
		Treatment	7.94±0.24*	3.11±0.09*,$^{\#}$	1.03±0.03*	1.90±0.06*,$^{\#}$	102±3*	46±1*,$^{\#}$	314±9	337±10
	7th day	Control	13.16±0.39	6.67±0.20$^{\#}$	1.97±0.06	5.39±0.16$^{\#}$	234±7	159±5$^{\#}$	323±10	348±10
		Treatment	8.74±0.26*	4.14±0.12*,$^{\#}$	1.32±0.04*	3.39±0.10*,$^{\#}$	128±4*	84±3*,$^{\#}$	322±10	350±11

No significant differences were observed in the total chlorophyll content in flag leaves of wild-type and transgenic plants on the first day of drought (see Table 1 and Fig. 1, *b*). On the seventh day of drought, this index decreased in comparison to the control in both wild-type and transgenic plants; however, in the latter, this decline was more pronounced. As a result, in absolute and relative terms, the chlorophyll content in the leaves of drought-stressed transgenic plants was lower than that in wild-type plants.

At the same time, the CO_2 assimilation rate under 25 °C in flag leaves of wild-type and transgenic plants on the first day of drought practically did not differ, and on the seventh day of stressor impact in the latter it was even slightly higher than in the former (Table 2). From this point of view, a sharper decrease in the chlorophyll content on the seventh day of drought in the leaves of transgenic plants as compared to the wild type can be regarded as an adaptive response. Its meaning consists in a radical reduction in the size of the light-collecting antenna under conditions of CO_2 assimilation inhibition during drought, which helps to reduce the overload of the chloroplasts electron transport chain and reduces the risk of formation of an excessive amount of reactive oxygen species (ROS).

Already on the first day of reaching soil moisture content of 30 % FC, the net assimilation rate (A_N) in flag leaves under a temperature of 25 °C in plants of both genotypes decreased by almost half (see Table 2). Wherein, if on the first day of drought the differences between the wild type and transformants in the relative decrease in the A_N compared to the control were insignificant, then on the seventh day the ratio of treatment/control was higher for the latter (i.e., the decrease was less), than for the wild type. This indicates the development of adaptation proces-

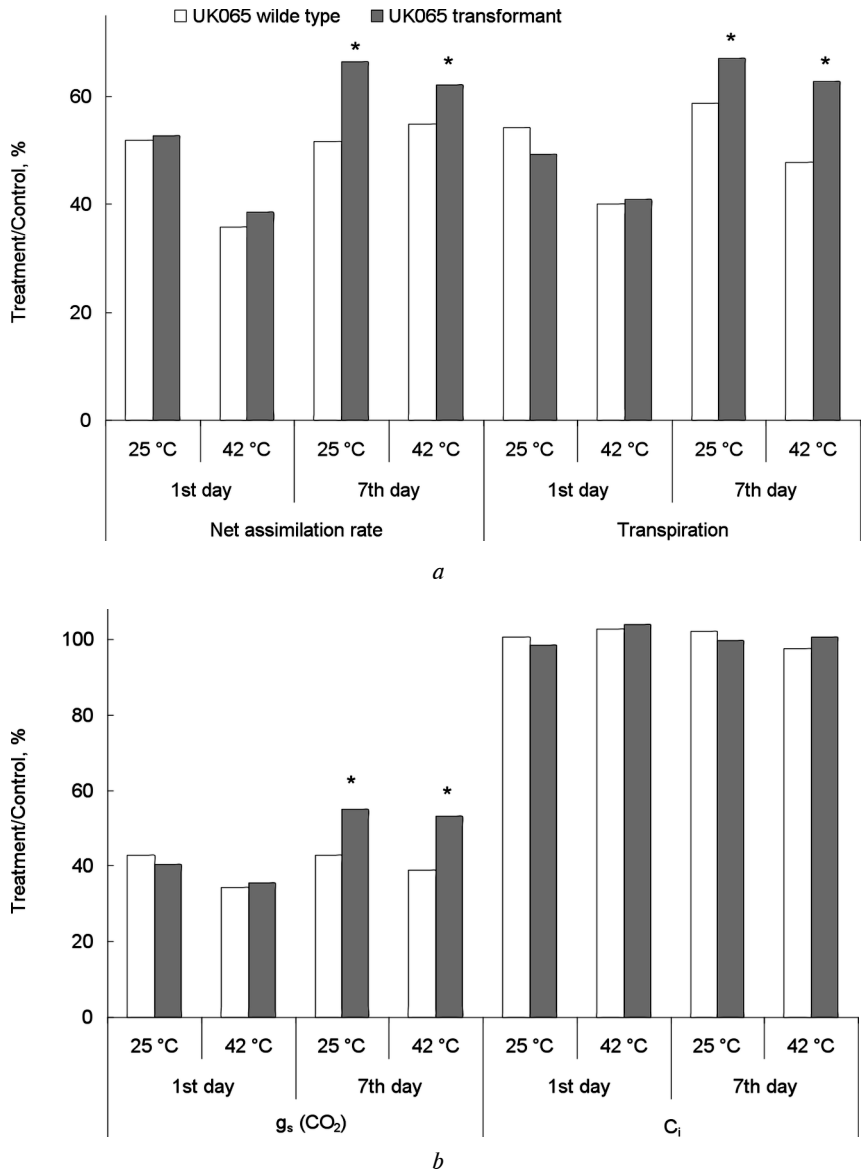

Fig. 2. Changes relative to the corresponding control at 70 % FC in net assimilation rate and transpiration (*a*), stomatal conductance (g_s) and intercellular CO_2 concentration (C_i) (*b*) of the flag leaves of drought-stressed (30 % FC) wheat plants of the original line UK065 (wild type) and obtained on its basis transgenic plants with double-stranded RNA suppressor of the proline dehydrogenase gene (transformant) on the first and seventh day of drought ($n = 4$; * — significant difference compared to the wild type at $p < 0.05$)

ses in transgenic plants, and their better acclimation to drought conditions (Fig. 2, *a*).

Heating the leaves of control plants to 42 °C also led to an almost twofold decrease in the A_N compared to 25 °C (see Table 2), and to an even greater decrease in this index in plants exposed to drought. As a result, on the first day of drought, the treatment/control ratio under 42 °C in plants of both genotypes was even lower than at 25 °C (see Fig. 2, *a*). Obviously, this is due to the simultaneous cumulative effect of two stress factors on the photosynthetic apparatus, when the defense mechanisms have just begun to be activated. There are evidences in the literature that the simultaneous effect of drought and high temperature significantly inhibits the vital processes of plants and reduces productivity in comparison with the influence of each stressor separately [36, 37].

However, on the seventh day of drought, this ratio under 42 °C was practically equal to that under 25 °C (although the absolute values of indices in the control and treated plants in the first case were lower than in the second). At the same time, transgenic plants retained the advantage previously noted for 25 °C.

The transpiration rate in plants subjected to drought decreased by 1.5—2 times as compared to control ones (see Table 2). On the first day of drought, under 25 °C the ratio treatment/control in plants of both genotypes did not differ significantly, and on the seventh day there was a tendency to an excess of this index in transgenic plants compared to the wild type (see Fig. 2, *a*). Heating the leaves of control plants to 42 °C led to a more than twofold increase in the transpiration rate (in contrast to the photosynthesis). In plants subjected to drought, transpiration also increased under heating, but to a lesser extent than in control.

On the first day of drought, heating up the leaves of plants of both genotypes led to a much stronger decrease in transpiration compared to the control values than under 25 °C, at the same time no genotypic differences were observed. On the seventh day of drought, the inhibitory effect of high temperature on this parameter was weaker than on the first day, however, in wild-type plants it was still stronger than under 25 °C, and in transgenic plants, the difference in the ratio treatment/control under 25 °C and 42 °C was insignificant. Consequently, the relative inhibition of transpiration by high temperature in transgenic plants was less (the ratio treatment/control was higher) than in the wild type, as was also observed for the photosynthesis.

Both drought and high temperature sharply reduced stomatal conductance (see Table 2). Undoubtedly, in the case of high temperature, the observed increase in water evaporation by leaves of the control and treated plants was due not to physiological, but to physical mechanisms. In the latter case, the leading role was played by a 2.6-fold (from 3.17 to 8.21 kPa) increase in the pressure of saturated water vapors in the intercellular spaces, and as a consequence — an increase in the release of water vapor to the outside, despite a decrease in stomatal conductance because of a reduce in the stomatal aperture under the stressors impact.

In general, the dynamics of treatment/control ratio for stomatal conductance was similar to that of photosynthesis (see Fig. 2, *b*). On the first

day of drought, no differences were observed between wild-type and transgenic plants; under 42 °C this ratio was less than under 25 °C. On the seventh day of drought, in the wild type the treatment/control ratio under 42 °C was equal to that under 25 °C, and in transgenic plants exceeded wild type both under 25 °C and 42 °C. This once again indicates that the processes of leaves photosynthetic apparatus adaptation in transgenic plants proceeded more efficiently than in the wild type. It is possible that the ability to maintain a higher stomatal conductance (and, consequently, the transpiration rate) in transgenic plants under drought conditions is caused by their lower water deficit because of an increase in the sucking power of roots due to an increased level of proline [25].

Calculations of the intercellular CO_2 concentration (C_i) showed that in our experiments this parameter was practically stable and did not differ significantly between genotypes under soil moisture of both 70 and 30 % FC, independently of temperature conditions. From this, at least two conclusions can be drawn: firstly, the flag leaves stomatal apparatus of plants of both genotypes was adequately matched with its main function of maintaining a constant C_i in accordance with the assimilation rate in mesophyll cells [38], and strongly depended on the water relations in the plant. Secondly, differences in the degree of drought and high temperature impacts on the net CO_2 assimilation rate between wild-type and transgenic plants were rather brought about by the differences in the damage to the chloroplasts' photosynthetic machinery than stomatal regulation.

The reduction in the A_N inhibition under heating the flag leaves of the drought-stressed plants as compared to the control a week after the onset of drought can be explained by processes of priming and cross-adaptation, which cause an increase in resistance to a certain stress as a result of the previous action of another stressor [38—40]. It has been found that the influence of the first stressor activates non-specific protective mechanisms that retain increased activity for some time after its cessation, or accelerates the induction of protective mechanisms under repeated exposure to the same or another stressor («stress memory»). This causes, under the action of the second stress factor, maintaining the functional state of cells and plant as a whole at a higher level than in the case when there was no previous stress. In our experiments, drought was the first stress factor that induced adaptation processes, and high temperature was the second.

It is known that the adaptation of the photosynthetic apparatus to drought includes significant physiological and metabolic changes which can increase thermotolerance, in particular, the accumulation of osmotically active substances (proline, glycinebetaine, soluble carbohydrates), increasing the proportion of unsaturated fatty acids in the membranes [42], activating the synthesis of chaperone proteins and other protective proteins, as well as ROS level control systems [39]. These changes, obviously, in our experiments led to an increase in the activity of the photosynthetic apparatus of treated plants on the seventh day of drought compared to the first, which occurred against the background of reduced chlorophyll and decreased relative water content in leaf tissues, and are convincing evidence of photosynthetic apparatus adaptation to drought.

It should also be noted that cross-adaptation was detected only when protective mechanisms were activated in response to the first stressor (in

our experiment on the seventh day of drought), while at the beginning of the first stressor action (drought), under the simultaneous influence of second stressor (high temperature), was recorded stronger inhibition of photosynthesis. The presence of a certain period of delay (lag phase) between the action of the stress factor and the formation of cross-tolerance (in other words, cross-resistance) is considered as the characteristic feature of priming [41]. It can be assumed that an important role in the formation of such response of photosynthesis and transpiration is played by drought-induced accumulation of abscisic acid (ABA), which, on the one hand, leads to stomata closure and reduced their conductivity, and on the other — activates adaptive restructuring of metabolism and synthesis of a number of stress-protective proteins [43].

According to modern ideas, one of the key factors in the formation of cross-tolerance is the ROS homeostasis control system [44—46]. The effect of the vast majority of abiotic stressors on plants is accompanied by the formation of a large number of different ROS, so the activation of their neutralization systems is an obligatory part of the adaptive response to each of them [47]. In addition, ROS are key signaling molecules that induce a various protective responses and processes, including the perception and transduction of stress signals, activation of protective proteins gene expression etc. Although the main sources of ROS, their compartmentalization and inclusion in signaling systems may differ under the impact of different stressors, it is believed that cross-tolerance formation is due, including, partial overlap between pathways of ROS stress signaling systems [48].

To evaluate the response of the antioxidant protection system of wheat photosynthetic apparatus to the drought impact, we determined the activity of the main antioxidant enzymes of chloroplasts — SOD and APX. One of the main sources of ROS in the photosynthetic cell are chloroplasts, at the functioning of the electron transport chain of which, part of the electrons can be transferred to molecular oxygen with the formation of superoxide anion radicals [13]. The latter are very dangerous ROS, which are detoxified by SOD. As a result of the reaction catalyzed by this enzyme, H_2O_2 is formed, which is reduced by APX to water. Under the effect of stressors, especially such as drought, the generation of superoxide anion radicals increases with a corresponding increase in the formation of H_2O_2. Enzyme systems of chloroplasts antioxidant protection respond to this, as a rule, by increasing their activity to limit the excessive formation of ROS [49].

Determination of the chloroplasts antioxidant enzymes activity in flag leaves showed that SOD activity in transgenic plants under conditions of optimal watering was noticeably higher than in wild type (Table 3). At the same time, on the first day of drought, the changes in this index were opposite: in wild-type plants, its increase was observed, and in transgenic plants — a decrease (as a result, the indices of SOD activity in plants of both genotypes did not differ significantly). On the seventh day of drought, wild type retained a tendency to an excess of SOD activity in the drought-treated plants over control, while in transgenic plants these indices were practically equal.

Differences in APX activity between genotypes and variants on the first day of drought were insignificant with a tendency to decrease in com-

*Table 3. Flag leaves chloroplasts SOD and APX activity in control (70 % FC) and drought-stressed (30 % FC) winter wheat plants of the original line UK065 (wild type) and obtained on its basis transgenic plants with double-stranded RNA suppressor of the proline dehydrogenase gene (transformant) on the first and seventh day of drought (x±SE, n = 4; * – significant difference compared to the control at p < 0.05; Chl. – chlorophyll, AA – ascorbic acid)*

Genotype	Variant	SOD, rel. units/(mg Chl. · h)		APX, μmol AA/(mg Chl. · h)	
		1st day	7th day	1st day	7th day
UK065 wild type	Control	562±29	645±29	204±9	353±12
	Treatment	693±36*	683±18	189±8*	429±18*
UK065 transformant	Control	825±42	864±41	208±10	464±20
	Treatment	674±30*	870±35	223±11	446±19

parison with control in wild-type plants and increase in transgenic plants. On the seventh day of stressor action, the activity of APX in wild-type drought-treated plants significantly exceeded the control, while in transgenic plants, an insignificant decrease was observed.

The genotypic differences in the response of antioxidant enzymes to drought are more clearly demonstrated by calculations of their activity ratio under the treatment and in control. In Fig. 3, it can be seen that after an increase in SOD activity on the first day of drought in wild-type plants compared to control, and a decrease in transgenic plants, on the seventh day of drought this parameter practically returned to the control values. As for APX activity, the treatment/control ratio was slightly higher in transgenic plants as compared to the wild type, and, on the contrary, significantly lower at the end of drought period.

For wild-type plants, the observed dynamics of antioxidant enzymes activities is quite typical. An increase in SOD activity at the onset of

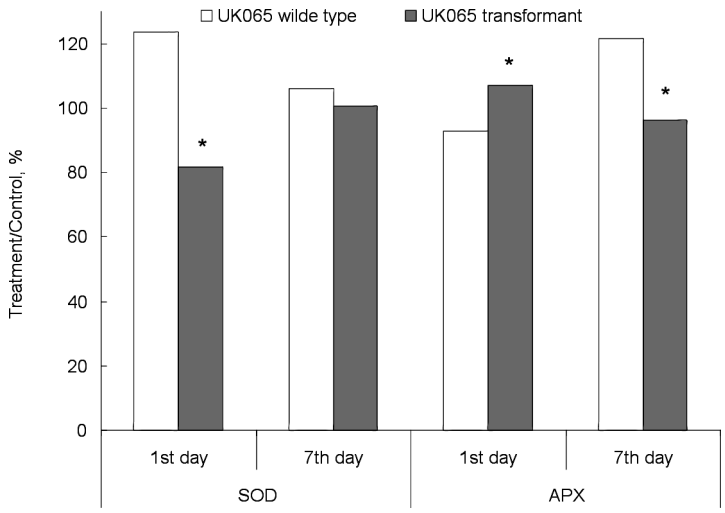

Fig. 3. Changes relative to the corresponding control at 70 % FC in SOD and APX activity in chloroplasts of the flag leaves of drought-stressed (30 % FC) wheat plants of the original line UK065 (wild type) and obtained on its basis transgenic plants with double-stranded RNA suppressor of the proline dehydrogenase gene (transformant) on the first and seventh day of drought ($n = 4$; * — significant difference compared to the wild type at $p < 0.05$)

drought against the background of little changes in APX activity leads to the formation of an increased amount of H_2O_2, which is not only ROS, as already mentioned, but an important signaling molecule that triggers a cascade of defense reactions in the signaling networks of cells [44]. There are known works on the priming of plants by exogenous treatment with H_2O_2 to the impact of stressors of different nature [50]. Then, as the protective mechanisms are triggered with the continuation of the drought effect, the functioning of APX is activated, which detoxifies excess H_2O_2. This is accompanied by the development of an adaptation syndrome, that was observed in our experiments, in particular, for the photosynthetic and transpiration rate.

In transgenic plants containing double-stranded RNA suppressor of the *pdh* gene, the dynamics of antioxidant enzymes activity evidently was modified by their constitutively inherent increased proline level [25]. It is known that in addition to osmotic activity, which increases the water-retention capacity of cells, this substance exhibits antioxidant properties [22]. It can be assumed, that in transgenic plants the photosynthetic apparatus is initially better protected from the impact of such a stressor as drought, and an increase in SOD activity (already increased in comparison to the wild type) is not required, whereas an excess of superoxide anion radicals is neutralized in other ways, including proline. Some increase in APX activity at the beginning of drought can be explained by an increase in the formation of H_2O_2 from other sources, for example, as a result of an increase in the proportion of photorespiration in leaf gas exchange, which is usually observed under drought [48]. On the seventh day of drought, the SOD and APX activities in the chloroplasts of flag leaves of transgenic plants returned almost to the control values as a result of effective acclimation to the drought effect. Obviously, the above-noted phenomenon of cross-adaptation to high temperature, which was more pronounced in transgenic plants, was due to the peculiarities of their defense mechanisms.

So, the physiological parameters we measured, such as water relations, indices of CO_2 and H_2O gas exchange in flag leaves, and antioxidant enzymes activity indicate that transgenic plants are better adapted to the effect of drought than the original line. However, the final and most important criterion for assessing the drought resistance of various genotypes of cultivated plants is their productivity, which we determined when the grain was fully ripe. From Table 4, it can be seen that in terms of productivity, transgenic plants grown at optimal soil moisture were not inferior to the wild type (the differences in most cases were insignificant). The productivity of plants of both genotypes subjected to drought during the heading—anthesis period was lower than in the control, but the degree of its decrease was different. In almost all parameters, both for the main shoot and for the whole plant, the transgenic ones showed a much lower degree of reduction than the wild type. Thus, the decrease in the weight of grain from the main shoot in transgenic plants compared to the control was 21.3 %, and in the wild type — almost 40 %. The same index for the whole plant was 33.8 and 52 %, respectively (because of a decrease in number of productive shoots). Similar differences were observed for other indices of grain productivity — the grain number and weight of 1000 grains. The

*Table 4. Productivity (calculated on dry matter) of control (70 % FC) and drought-stressed (30 % FC at stage of heading-anthesis (GS 59–65)) winter wheat plants of the original line UK065 (wild type) and obtained on its basis transgenic plants with double-stranded RNA suppressor of the proline dehydrogenase gene (transformant) (x±SE; n = 20; * – significant difference compared to the control at p < 0.05; # – significant difference compared to wild type at p ≤ 0.05)*

Genotype	Variant	Total weight, g	Grain weight, g	Number of grains, pcs.	1000 grains weight, g	Harvest index	Number of productive shoots, pcs.
				Main shoot			
UK065 wild type	Control	3.98±0.08	2.17±0.05	47.5±1.2	46.0±1.0	0.55±0.01	–
	Treatment	2.28±0.07	1.30±0.06	41.2±1.4	31.6±1.2	0.45±0.01	–
	% relative to control	57.2*	59.9*	86.7*	68.7*	81.8*	–
UK065 transformant	Control	3.29±0.11	2.02±0.08	47.8±1.6	42.5±1.1	0.61±0.03	–
	Treatment	3.30±0.10	1.59±0.08	48.6±1.4	32.7±1.5	0.48±0.01	–
	% relative to control	100.3#	78.7*,#	101.6#	76.9*	78.7*	–
				Whole plant			
UK065 wild type	Control	10.43±0.75	5.97±0.38	143.7±9.0	41.7±0.7	0.62±0.07	3.57±0.25
	Treatment	6.12±0.34	2.84±0.22	94.4±5.9	30.1±1.0	0.46±0.01	2.58±0.19
	% relative to control	58.6*	47.5*	65.7*	72.3*	74.2*	72.2*
UK065 transformant	Control	9.95±0.55	5.45±0.3	148.8±11.1	38.1±1.0	0.56±0.03	3.48±0.23
	Treatment	7.99±0.46	3.61±0.23	122.6±6.5	29.5±1	0.45±0.01	3.16±0.17
	% relative to control	80.3*,#	66.2*,#	82.4*,#	77.5*	80.3*	90.8*,#

decrease in last parameter indicates that in plants subjected to drought at the period of heading—anthesis, the photosynthetic apparatus did not fully restore its activity during the period of grain filling, when their normal watering was resumed.

Thus, transgenic winter wheat plants of the UK065 line with a double-stranded RNA suppressor of the proline dehydrogenase gene in terms of the water relations, the parameters of gas exchange in flag leaves, the antioxidant enzymes activity, and grain productivity demonstrated significantly higher resistance to soil drought during the heading—anthesis period. In addition, according to the indices of gas exchange in flag leaves on the seventh day of drought, the effect of photosynthetic apparatus cross-adaptation to high temperature was revealed, which was also more pronounced in transgenic plants. All this confirms the prospect of using transgenesis for increasing the drought resistance of wheat plants in general, and applying of genes that regulate proline metabolism, in particular. Such research is especially important in view of the risks to agriculture associated with global climate change.

REFERENCES

1. Kaur, V. & Behl, R.K. (2010). Grain yield in wheat as affected by short periods of high temperature, drought and their interaction during pre- and post-anthesis stages. Cereal Research Communications, 38, No. 4, pp. 514-520.

2. Lesk, C., Rowhani, P. & Ramankutty, N. (2016). Influence of extreme weather disasters on global crop production. Nature, 529, No. 7584, pp. 84-87. https://doi.org/10.1038/nature16467
3. Morgun, V.V., Kiriziy, D.A. & Shadchina, T.M. (2010). Ecophysiological and genetic aspects of adaptation of cultivated plants to global climate changes. Physiol. biochem. cult. plants, 42, No. 1, pp. 3-22 [in Russian].
4. Morgun, V.V. (2017). Contribution of plant physiology and genetics to food security of our country. In: Plant Physiology: Achievements and new directions of development, Vol. 1, (pp. 9-13), Logos, Kyiv [in Ukrainian].
5. Senapati, N., Stratonovitch, P., Paul, M.J. & Semenov, M.A. (2019). Drought tolerance during reproductive development is important for increasing wheat yield potential under climate change in Europe. J. Exp. Bot., 70, No. 9, pp. 2549-2560. https://doi.org/10.1093/jxb/ery226
6. Sadras, V.O. & Richards R.A. (2014). Improvement of crop yield in dry environments: benchmarks, levels of organization and the role of nitrogen. J. Exp. Bot., 65, No. 8, pp. 1981-1995. https://doi.org/10.1093/jxb/eru061
7. Abid, M., Ali, S., Qi, L.K., Zahoor, R., Tian, Z., Jiang, D., Snider, J.L. & Dai, T. (2018). Physiological and biochemical changes during drought and recovery periods at tillering and jointing stages in wheat (Triticum aestivum L.). Sci. Rep., 8, pp. 1-15. https://doi.org/10.1038/s41598-018-21441-7
8. Feller, U. (2016). Drought stress and carbon assimilation in a warming climate: Reversible and irreversible impacts. J. Plant Physiol., 203, pp. 84-94. https://doi.org/10.1016/j.jplph.2016.04.002
9. Ghodke, P.H., Ramakrishnan, S., Shirsat, D.V., Vani, G.K. & Arora, A. (2019). Morphological characterization of wheat genotypes for stay green and physiological traits by multivariate analysis under drought stress. Plant Physiol. Rep., 24, No. 3, pp. 305-315. https://doi.org/10.1007/s40502-019-00458-8
10. Pequeno, D.N.L., Hernandez-Ochoa, I.M., Reynolds, M., Sonder, K., MoleroMilan, A., Robertson, R.D., Lopes, M.S., Xiong, W., Kropff, M. & Asseng, S. (2021). Climate impact and adaptation to heat and drought stress of regional and global wheat production. Environ. Res. Lett., 16, 054070. https://doi.org/10.1088/1748-9326/abd970
11. Caverzan, A., Casassola, A. & Brammer, S.P. (2016). Antioxidant responses of wheat plants under stress. Genet. Mol. Biol., 39, No. 1, pp. 1-6. https://doi.org/10.1590/1678-4685-GMB-2015-0109
12. Morales, F., Ancin, M., Fakhet, D., Gonzalez-Torralba, J., Gamez, A.L., Seminario, A., Soba, D., Mariem, S.B., Garriga, M. & Aranjuelo, I. (2020). Photosynthetic metabolism under stressful growth conditions as a bases for crop breeding and yield improvement. Plants, 9, No. 1, p. 88. https://doi.org/10.3390/plants9010088
13. Krieger-Liszkay, A., Krupinska, K. & Shimakawa, G. (2019). The impact of photosynthesis on initiation of leaf senescence. Physiologia Plantarum, 166, pp. 148-164. https://doi.org/10.1111/ppl.12921
14. Moore, C.E., Meacham-Hensold, K., Lemonnier, P., Slattery, R.A., Benjamin, C., Bernacchi, C.J., Lawson, T. & Cavanagh, A.P. (2021). The effect of increasing temperature on crop photosynthesis: from enzymes to ecosystems. J. Exp. Bot., 72, No. 8, pp. 2822-2844. https://doi.org/10.1093/jxb/erab090
15. Riaz, M.W., Yang, L., Yousaf, M.I., Sami, A., Mei, X.D., Shah, L., Rehman, S., Xue, L., Si, H. & Ma, C. (2021). Effects of heat stress on growth, physiology of plants, yield and grain quality of different spring wheat (Triticum aestivum L.) genotypes. Sustainability, 13, p. 2972. https://doi.org/10.3390/su13052972
16. Cohen, I., Zandalinas, S.I., Huck, C., Fritschi, F.B. & Mittler, R. (2021). Meta-analysis of drought and heat stress combination impact on crop yield and yield components. Physiol. Plant., 171, No. 1, pp. 66-76. https://doi.org/10.1111/ppl.13203
17. Abdelhakim, L.O.A., Rosenqvist, E., Wollenweber, B., Spyroglou, I., Ottosen, C.-O. & Panzarova, K. (2021). Investigating combined drought- and heat stress effects in wheat under controlled conditions by dynamic image-based phenotyping. Agronomy, 11, p. 364. https://doi.org/10.3390/agronomy11020364

18. Hiei, Y., Ishida, Y. & Komari, T. (2014). Progress of cereal transformation technology mediated by Agrobacterium tumefaciens. Frontiers in Plant Sci., 5, pp. 1-11.
19. Morgun, V.V., Dubrovna, O.V. & Morgun, B.V. (2016) The modern biotechnologies of producing wheat plants resistant to stresses. Fiziol. rast. genet., 48, No. 3, pp. 196-213. https://doi.org/10.15407/frg2016.03.196 [in Ukrainian].
20. Alsamman, A.M., Bousba, R., Baum, M., Hamwieh, A. & Fouad, N. (2021). Comprehensive analysis of the gene expression profile of wheat at the crossroads of heat, drought and combined stress. Highlights in BioScience, 4. https://doi.org/10.36462/H.BioSci.202104
21. Szabados, L. & Savoure, A. (2010). Proline: a multifunctional amino acid. Trends in Plant Sci., 15, No. 2, pp. 89-97. https://doi.org/10.1016/j.tplants.2009.11.009
22. Kolupaev, Yu. E., Vainer, A.A. & Yastreb, T.O. (2014). Proline: physiological functions and regulation of the content in plants under stress conditions. Visn. Hark. nac. agrar. univ., Ser. Biol., Iss. 2, pp. 6-22 [in Russian].
23. Tischenko, E.N. (2013). Genetic engineering using L-proline metabolism genes to increase plant osmotolerance. Physiology and biochemistry. cult. plants, 45, No. 6, pp. 488-500 [in Russian].
24. Manavalan, L., Chen, H., Clarke, J., Salmeron, J. & Nguyen, H. (2012). RNAi-mediated disruption squalen synthase improves drought tolerance and yield in rice. J. Exp. Bot., 63, pp. 163-175.
25. Dubrovna, O.V., Stasik, O.O., Priadkina, G.O., Zborivska, O.V. & Sokolovska-Sergienko, O.G. (2020). Resistance of genetically modified wheat plants, containing a double-stranded RNA suppressor of the proline dehydrogenase gene, to soil moisture deficiency. Agricultural Science and Practice, 7, No. 2, pp. 24-34.
26. Dubrovna, O.V. & Slivka, L.V. (2021). Optimization of Agrobacterium-mediated transformation conditions of prospective genotypes of winter bread wheat by in planta method. Factors in experimental evolution of organisms, 28, pp. 66-71 [in Ukrainian].
27. Laysk, A.H. (1977). Kinetics of photosynthesis and photorespiration of C_3 plants. M.: Nauka [in Russian]
28. Mokronosov, A.T. & Kovalev, A.G. (Eds.). (1989). Photosynthesis and Bioproductivity: Methods of Determination. Agropromizdat, Moskow [in Russian].
29. Kedruk, A.C., Kiriziy, D.A., Sokolovska-Sergienko, O.G. & Stasik, O.O. (2021). Response of the photosynthetic apparatus of winter wheat varieties to the combined action of drought and high temperature. Fiziol. rast. genet., 53, No. 5, pp. 387-405. https://doi.org/10.15407/frg2021.05.387 [in Ukrainian].
30. Shmatko, I.G., Grigoryuk, I.A., Shvedova, O.E. & Petrenko, N.I. (1985). Determination of the physiological reaction of cereals to deterioration of water availability and temperature increase. IPPG, Kiev [in Russian].
31. Wellburn, A.R. (1994). The spectral determination of chlorophylls a and b, as well as total carotenoids, using various solvents with spectrophotometers of different resolution. J. Plant Physiol., 144, pp. 307-313. https://doi.org/10.1016/S0176-1617(11)81192-2
32. Chen, G.-X. & Asada, K. (1989). Ascorbate peroxidase in tea leaves: occurrence of two isozymes and the differences in their enzymatic and molecular properties. Plant Cell Physiol., 30, No. 7, pp. 987-998. https://doi.org/10.1093/oxfordjournals.pcp.a077844
33. Giannopolitis, C.N. & Ries, S.K. (1977). Superoxide dismutase. Occurrence in higher plants. Plant Physiol., 59, No. 2, pp. 309-314. https://doi.org/10.1104/pp.59.2.309
34. Arnon, D.I. (1949). Copper enzyme in isolated chloroplasts. Polyphenolooxidase in Beta vulgaris. Plant. Physiol., 24, No. 1, pp. 1-5. https://doi.org/10.1104/pp.24.1.1
35. Lopes, M.S., Rebetzke, G.J. & Reynolds, M. (2014). Integration of phenotyping and genetic platforms for a better understanding of wheat performance under drought. J. Exp. Bot., 65, No. 21, pp. 6167-6177. https://doi.org/10.1093/jxb/eru384
36. Zandalinas, S.I., Mittler, R., Balfagon, D., Arbona, V. & Gomez-Cadenas, A. (2018). Plant adaptations to the combination of drought and high temperatures. Physiol. Plant., 162, pp. 2-12. https://doi.org/10.1111/ppl.12540
37. Zandalinas, S.I., Sengupta, S., Fritschi, F.B., Azad, R.K., Nechushtai, R. Mittler, R. (2021). The impact of multifactorial stress combination on plant growth and survival. New Phytologist, 230, pp. 1034-1048. https://doi.org/10.1111/nph.17232
38. Lawson, T. & Matthews, J. (2020). Guard cell metabolism and stomatal function. Annu. Rev. Plant Biol., 71, pp. 273-302. https://doi.org/10.1146/annurev-arplant-050718-100251

39. Zhang, X. & Huang, B. (2020). Drought priming-induced heat tolerance: Metabolic pathways and molecular mechanisms. In: Priming-Mediated Stress and Cross-Stress Tolerance in Crop Plants (pp. 149-160), Elsevier Inc. https://doi.org/10.1016/B978-0-12-817892-8.00009-X
40. Wang, X., Liu, F. & Jiang, D. (2017). Priming: a promising strategy for crop production in response to future climate. J. Integr. Agric., 16, No. 12, pp. 2709-2716. https://doi.org/10.1016/S2095-3119(17)61786-6
41. Llorens, E., Gonzalez-Hernandez, A.I., Scalschi, L., Fernandez-Crespo, E., Camanes, G., Vicedo, B. & Garcia-Agustin, P. (2020). Priming mediated stress and cross-stress tolerance in plants: concepts and opportunities. In: Priming-Mediated Stress and Cross-Stress Tolerance in Crop Plants (pp. 1-20), New York: Acad. Press. https://doi.org/10.1016/B978-0-12-817892-8.00001-5
42. Zhang, X., Xu, Y. & Huang, B. (2019). Lipidomic reprogramming associated with drought stress priming-enhanced heat tolerance in tall fescue (Festuca rundinacea). Plant, Cell & Environ., 42, Iss. 3, pp. 947-958. https://doi.org/10.1111/pce.13405
43. Wani, S.H., Kumar, V., Shriram, V. & Sah, S.K. (2016). Phytohormones and their metabolic engineering for abiotic stress tolerance in crop plants. Crop J., 4, No. 3, pp. 162-176. https://doi.org/10.1016/j.cj.2016.01.010
44. Kolupaev, Yu.E. & Oboznyi, A.I. (2013). Reactive oxygen species and antioxidative system at cross adaptation of plants to activity of abiotic stressors. Visn. Hark. nac. agrar. univ., Ser. Biol., Iss. 3, pp. 18-31 [in Ukrainian].
45. Perez, I.B. & Brown, P.J. (2014). The role of ROS signaling in cross-tolerance: from model to crop. Frontiers in Plant Science, 5, p. 754. https://doi.org/10.3389/fpls.2014.00754
46. Radchenko, M., Sychuk, A. & Morderer, Ye. (2014). Decrease of the herbicide fenoxaprop phytotoxicity in the drought condition: the role of antioxidant enzymatic system. Journal of Plant Protection Research, 54, No. 4, pp. 390-394. https://doi.org/10.2478/jppr-2014-0058
47. Taran, N.Yu., Okanenko, O.A., Batsmanova, L.M. & Musienko, M.M. (2004). Secondary oxidative stress as an element of the general adaptive response to unfavourable environment action. Physiol. biochem. cult. plants, 36, No. 1, pp. 3-14 [in Ukrainian].
48. Zandalinas, S.I., Fichman, Y., Devireddy, A.R., Sengupta, S., Azad, R.K. & Mittler, R. (2020). Systemic signaling during abiotic stress combination in plants. Proc. Natl. Acad. Sci., 117, No. 24, pp. 13810-13820. https://doi.org/10.1073/pnas.2005077117
49. Morgun, V.V., Stasik, O.O., Kiriziy, D.A. & Sokolovska-Sergiienko, O.G. (2019). Effect of drought on photosynthetic apparatus, activity of antioxidant enzymes, and productivity of modern winter wheat varieties. Regulatory Mechanisms in Biosystems, 10, No. 1, pp. 16-25. https://doi.org/10.15421/021903
50. Kerchev, P., van der Meer, T., Sujeeth, N., Verlee, A., Stevens, Ch.V., Van Breusegem, F. & Gechev, T. (2020). Molecular priming as an approach to induce tolerance against abiotic and oxidative stresses in crop plants. Biotechnology Advances, 40, 107503. https://doi.org/10.1016/j.biotechadv.2019.107503.

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РЕАКЦІЯ ФОТОСИНТЕТИЧНОГО АПАРАТУ ГЕНЕТИЧНО МОДИФІКОВАНИХ РОСЛИН ПШЕНИЦІ, ЩО МІСТЯТЬ ДВОЛАНЦЮГОВИЙ РНК-СУПРЕСОР ГЕНА ПРОЛІНДЕГІДРОГЕНАЗИ, НА ПОСУХУ І ВИСОКУ ТЕМПЕРАТУРУ

Д.А. Кірізій, А.С. Кедрук, О.Г. Соколовська-Сергієнко, О.В. Дубровна, О.О. Стасик

Інститут фізіології рослин і генетики Національної академії наук України
03022 Київ, вул. Васильківська, 31/17, Україна
e-mail: o_stasik@yahoo.com

В умовах вегетаційного досліду вивчали особливості впливу посухи на водний режим, вміст хлорофілу, активність антиоксидантних ферментів хлоропластів, асиміляцію CO_2 та транспірацію за оптимальних і підвищених температур, а також зернову про-

дуктивність трансгенних рослин селекційної лінії УК065 озимої м'якої пшениці, що містять дволанцюговий РНК-супресор гена проліндегідрогенази (*pdh*), порівняно з вихідним генотипом. Посуху для рослин обох генотипів створювали, знижуючи вологість ґрунту в дослідному варіанті до рівня 30 % повної вологоємності (ПВ), який підтримували впродовж семи діб в період колосіння-цвітіння. Вологість ґрунту в рослин контрольного варіанта, а також дослідних рослин за винятком періоду посухи підтримували на оптимальному рівні 60—70 % ПВ. Вимірювали фізіолого-біохімічні показники на прапорцевому листку на першу добу зниження вологості ґрунту до 30 % ПВ та на сьому добу вегетації за цього рівня вологості. Встановлено, що за семидобової посухи відносний вміст води в листках трансгенних рослин знижувався порівняно з контролем менше, ніж у рослин вихідної лінії. Водночас посуха сильніше зменшувала вміст хлорофілу в трансгенних рослин, ніж у нетрансгенних. Нестача вологи в ґрунті інгібувала інтенсивність асиміляції CO_2 і транспірації, проте в трансгенних рослин, на відміну від нетрансгенних, ступінь зниження в кінці періоду посухи був істотно меншим, ніж на початку, що свідчить про поліпшену адаптивну здатність фотосинтетичного апарату в трансгенних рослин зі зниженою експресією гена проліндегідрогенази. Це підтверджує також формування крос-толерантності — ослаблення інгібувального впливу високої температури (прогрів при 42 °С) на інтенсивність асиміляції CO_2 підданих дії водного стресу рослин після семидобової посухи, порівняно з її початком. Зниження негативного впливу високої температури на фотосинтез за тривалої посухи було сильнішим у трансгенних рослин. Наявність дволанцюгового РНК-супресора гена *pdh* у трансгенних рослин стабільно підвищувала активність хлоропластної супероксиддисмутази на відміну від активності хлоропластної аскорбатпероксидази за оптимального режиму поливу і модифікувала зміни активностей цих ферментів за дії посухи, що може бути однією з причин відмінностей трансгенної і нетрансгенної ліній за адаптивною здатністю. За оптимального поливу досліджені лінії не відрізнялися за зерновою продуктивністю рослин, проте посуха значно менше знижувала масу зерна з колоса головного пагона (на 21 %) і цілої рослини (на 33 %) в трансгенних рослин, ніж у рослин вихідної лінії (на 40 і 52 % відповідно). Отже, включення гена супресора проліндегідрогенази в геном рослин озимої пшениці поліпшує адаптивні властивості фотосинтетичного апарату і зменшує втрати зернової продуктивності за умов посухи.

Ключові слова: *Triticum aestivum* L., трансгенні рослини, пролін, посуха, високотемпературний стрес, фотосинтез, крос-адаптація, антиоксидантні ферменти (супероксиддисмутаза, аскорбатпероксидаза), продуктивність.