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RESISTANCE OF GENETICALLY MODIFIED WHEAT PLANTS, CONTAINING A DOUBLE-STRANDED RNA SUPPRESSOR OF THE PROLINE DEHYDROGENASE GENE, TO SOIL MOISTURE DEFICIENCY

O. V. Dubrovna, O. O. Stasik, G. O. Priadkina, O. V. Zborivska, O. G. Sokolovska-Sergiienko

Institute of Plant Physiology and Genetics, NAS of Ukraine 31/17, Vasylkivska Str., Kyiv, Ukraine, 03022

E-mail: dubrovny@ukr.net, o_stasik@yahoo.com, galpryadk@gmail.com, masluikivska@ukr.net, sokolovska@ukr.net*

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Aim. Analysis of physiological and biochemical characteristics and grain productivity traits of transgenic common wheat plants (*Triticum aestivum* L.) with a double-stranded RNA suppressor of the proline dehydrogenase gene (*pdh*) compared to non-transgenic genotypes under conditions of soil drought. **Methods.** Biochemical assays: spectrophotometric measurements of antioxidant enzymes (SOD, APX) activity, proline dehydrogenase activity and free L-proline content; biotechnological assays: *Agrobacterium*-mediated transformation *in planta*; physiological: morphometric traits and elements of grain productivity; mathematical statistics. **Results**. The presence of a double-stranded RNA suppressor of the gene *pdh* in transgenic plants leads not only to a decrease in enzyme activity (on average 2 times compared to the non-transgenic plants), but also to an increase in the content of free L-proline both under optimal conditions and under soil drought (2.6–4.1 times). Under soil moisture deficiency, transgenic plants with reduced *pdh* gene activity in terms of yield structure significantly exceeded the corresponding values of grain productivity elements for the non-transformed ones. In terms of the grain weight from the main stem, the biotechnological plants under soil moisture deficiency exceeded the control plants 1.5–1.6 times, while the number of grains did not differ significantly. The grain productivity of the whole plant in the transformed lines under conditions of soil moisture deficiency was somewhat inferior to those in the non-transformed plants grown under optimal conditions. Soil drought caused a significant increase in SOD and APX activity (by 40 and 11 %, respectively) in plants of the original variety. On the contrary, the activity of both antioxidant enzymes under drought conditions in transgenic plants decreased: SOD – down to 73–76 %, APX – down to 82–86 %, compared with the variant of 70 % of field capacity. **Conclusions**. The analysis of physiological and biochemical characteristics, as well as economic and grain productivity elements of transgenic common wheat plants with a double-stranded RNA suppressor of the proline dehydrogenase gene showed their increased tolerance to soil drought, compared with non-transgenic genotypes, which may be associated with higher proline accumulation and an increase in the antioxidant enzymes activity. Under soil moisture deficiency, transgenic wheat plants with reduced *pdh* gene activity significantly exceeded the corresponding values of grain productivity elements for non-transformed plants. The analysis of the antioxidant enzymes activity in the chloroplasts of transgenic plants showed that under physiological conditions, the antioxidant system works more actively in comparison with non-transgenic genotypes, which may be a prerequisite for increasing the tolerance of these plants to the influence of stressors of various origin. It is likely that the positive relationship between the level of free L-proline and the resistance of transgenic wheat plants to osmotic stress is associated either with the effect of L-proline on the expression of other genes of the stress-strain response of plants, or with the positive effect of the increased content of this amino acid on resistance at the early stages of stress development. It has been suggested that an increase in the antioxidant enzymes activity in biotechnological plants can be caused by the expression of heterologous genes.

Key words: transgenic wheat, soil drought, proline, antioxidant enzymes (SOD, APX), grain yield.

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INTRODUCTION

Common wheat is one of the most sensitive cereals in terms of environmental conditions (Shewry P, 2009). It is known that a significant increase in the air temperature, or drought, in any period of plant development, salinization and other stress factors inhibit and impair reproductive processes of ear formation, cause the reduction in spikelet primordia, pollen sterility and grain shriveling, inhibit chlorophyll synthesis and the activity of photosynthesis apparatus, accelerate aging process and reduce the lifetime of leaves (Nyachiro J et al., 2001, Mahajan S and Tuteja N, 2005, Nowsherwan I et al., 2018). The deterioration of metabolism processes and a reduction in the number and weight of grain in the ear, both caused by unfavorable conditions, lead to considerable yield losses. One of the ways to increase the yield of common wheat under unfavorable conditions may be found in creating varieties with increased adaptivity to abiotic stresses.

It was determined that the resistance to drought, salinization and temperature stresses are complex traits, and the complete set of genes, defining this phenotype, is unknown (Agarwal S, Grover A, 2006). Many studies relate these traits to the content of free L-proline in the tissues of plants which is actively synthesized in response to different stress factors, acting as an osmoprotector (Szabados L, Savouré A, 2009, Anjum S et al., 2017, Renu K et al., 2019). For instance, it was determined that the content of proline, average for 108 lines of wheat, increased by 39 % under drought, by 32 % – under the action of higher temperatures, by 132 % – under their combined effect, compared to the control (Qaseem M et al., 2019). It was also determined that moisture deficiency, occurring after earing, resulted in almost 5-fold increase in proline content in flag leaves of N84-12 line of durum wheat compared to well-irrigated control variant, although the difference between the control and the variant with moisture deficiency in the shooting period was insignificant (Maralian H et al., 2010). The analysis of the results of a multi-factor experiment in studying the impact of drought and temperature stress on grain productivity of wheat demonstrated that the increase in productivity was related to the increase in proline content due to enhanced regulation of water potential of leaves (Ahmed M et al., 2017). Therefore, obtaining plants with higher content of this aminoacid may promote the selection of varieties with high adaptive potential, including resistance to drought.

A promising trend in creating drought-resistant forms of wheat is the application of biotechnological approaches, including the methods of genetic engineering. Novel molecular biotechnologies involving various strategies, including the ones, aimed at obtaining stable genotypes via integration of recombinant DNA molecules, capable of controlling adaptation/resistance processes on the genetic level, into the genome of cultivated plants, have recently been under intensive development (Hiei Y et al., 2014). Considerable attention is paid to the elaboration of a new approach of metabolic engineering, related to the identification and analysis of structural genes, controlling, in particular, the synthesis and catabolism of proline (Manavalan L et al., 2012, Morgun V et al., 2016). The gene of proline dehydrogenase, related to L-proline catabolism, is of practical significance for genetic engineering, as partial inhibition of its expression may result in the increase in free L-proline content and, as a result, in the level of resistance of plants to abiotic stresses (Ayliffe M et al., 2005, Ibragimova S et al., 2012). In particular, it has been determined that a promising approach for partial suppression of the gene of proline dehydrogenase is the application of vector constructs where a double-stranded RNA-suppressor is located as an inverted repeat (Manavalan L et al., 2012). It is suggested that due to RNA-interference this construct is more efficient to increase the level of L-proline. Due to this fact, a vector construct with a double-stranded RNA-suppressor of proline dehydrogenase gene of Arabidopsis was used by us for the studies.

Scientific literature presents many evidences of direct relationship between the level of proline accumulation and stress-tolerance of genetically modified plants (Verdoy D et al., 2006, Khan M et al., 2015, Tishchenko O et al., 2016). At the same time, the positive impact of the increase in the content of free L-proline on the adaptation of transgenic plants to the stress is still noted not for all the crops and conditions. These studies are usually conducted in simulated environment and are not always confirmed in field conditions (Mansour M and Ali E, 2017).

This study was aimed at analyzing physiological and biochemical characteristics and grain productivity traits of transgenic common wheat plants (*Triticum aestivum* L.) with a double-stranded RNA suppressor of the proline dehydrogenase gene (*pdh*) compared to non-transgenic genotypes under soil drought. The initial hypothesis of the study was the assumption that biotechnological wheat plants would demonstrate higher tolerance towards moisture deficiency due to higher accumulation of proline and higher activity of the antioxidant system.

MATERIALS AND METHODS

The object of the study was three transgenic lines of wheat, Zymoiarka variety, generation T_2 (Zymoiarka 32, Zymoiarka 74 and Zymoiarka 86), obtained by *Agrobacterium*-mediated transformation *in planta*. *Agrobacterium*-mediated transformation was per-

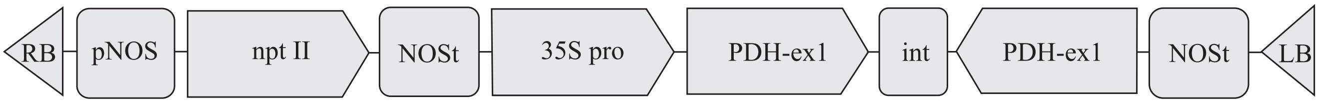

Fig. 1. The schematic presentation of T-DNA of the genetic construct pBi2E: pNOS – promoter of nopaline synthase; 35S pro – promoter 35S of cauliflower mosaic virus (CaMV) RNA; PDH-ex1 – first exon of *pdh* gene (the construct has two fragments, located in the form of an inverted repeat); int – fragment of the first intron of *pdh* gene; *ntp*II – gene of neomycin phosphotransferase II E.*coli*; NOSt – terminator of nopaline synthase gene, polyadenylation signal; LB – left border of T-DNA, RB – right border of T-DNA (Tishchenko O et al., 2014)

formed using AGL0 strain, containing a binary vector construct pBi2E, which included a heterologous double-stranded RNA-suppressor of proline dehydrogenase gene, obtained on the basis of proline dehydrogenase gene of Arabidopsis (ds-RNA suppressor *PDH1*), and a selective gene of neomycin phosphotransferase II (*npt*II) of *E. coli* (Fig. 1). The vector construct was kindly provided by the corresponding member of RAS, Doctor of Science (Biology), O. V. Kochetov, the Institute of Cytology and Genetics of the Siberian Branch of RAS, Novosibirsk.

Agrobacterium-mediated transformation *in planta* was performed in conditions of a vegetational experiment (Voronova S et al., 2015) and the seeds were gathered at the end of vegetation period. The plants of the seed generation T_0 were used to obtain seed generation T_2 via self-pollination. The seeds were germinated in the cups with soil mixture and the presence of vector construct elements was checked by PCR. After the analysis, the plants were planted into 10 liter vegetative pots, filled with soil mixture. In half of the pots, control and transgenic forms of plants were cultivated under normal irrigation – 70 % from full moisture capacity (FMC). Soil humidity in the remaining pots was decreased down to 30 % FMC by stopping the irrigation during the stem elongation phase and keeping it at this level for 7 days. Soil humidity was controlled by gravimetric method. Biochemical indices (the activity of proline dehydrogenase and antioxidants, and proline content) were measured in the flag leaves of the plants on the 7th day since the beginning of drought. After drought, the plants of the experimental variant were still watered as well as the control ones.

Non-transformed plants of the initial variety, growing under sufficient moisture provision (70 % FMC), served as the negative control, and the same plants, growing under drought (30 % FMC) were the positive control. The elements of yield structure were analyzed in the phase of full grain ripeness. Biochemical and morphometric indices were determined in three repeats.

The concentration of free proline in the tissues was determined by the method (Bates L et al., 1973). The activity of proline dehydrogenase (PDH) was evaluated by the method described in (Mattioni C et al., 1997) by the rate of using NAD$^+$ for proline oxidation, measuring NADH concentration, formed per a time unit.

The antioxidant enzymes activity was determined in chloroplasts of leaves, which were isolated mechanically at 0–4 °C. The average weighed quantity (2 g) was homogenized in 7-fold volume of buffer solution (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 and centrifuged at K-24D centrifuge at 80 g and 0–4 °C for 5 min to precipitate heavy particles. The supernatant liquid was removed and centrifuged at 2,000 g for 10 min to obtain the fraction of chloroplasts. The precipitate of chloroplasts was resuspended in the isotonic medium with 4 mM ascorbic acid, 2 ml of 50 mM tris-HCl (pH 7.5), and further used to determine the activity of superoxide dismutase (SOD, EC 1.15.1.1) and ascorbate peroxidase (APO, EC 1.11.1.11). The determination of SOD activity was conducted by photochemical oxidation of nitrotetrazolium blue (NTB) and expressed in unit/mg of chlorophyll (Beyer W and Fridovich I, 1987). The content of chlorophyll in the suspension of chloroplasts was determined by the method described in (Arnon D, 1949). The activity of APX was evaluated in the ultraviolet range at 290 nm by the method described in (Chen G and Asada K, 1989), using ascorbate as a donor for electrons. The calculations for the activity of this enzyme was conducted by the decrease in the optic density of the reaction mixture per a time unit.

The evaluation of grain productivity components involved the following indices: plant height (PH), main spikelet length (MSL), number of grains per main spikelet (NGMS), number of grains per plant (NGP), grain weight per main spikelet (GWMS), grain weight per plant (GWP), and thousand grain weight (TGW).

The statistical processing of the obtained data was conducted using ANOVA and the Tukey HSD Test with the average values. The results were presented in the form of mean values and standard error (m ± SE). The difference between the data was considered significant, if $p \leq 0.05$.

The authors declare that this study complies with the standards of current laws of Ukraine.

RESULTS

To the direct estimation of the degree, to which the introduced construct inhibits the expression of pdh gene, the activity of proline dehydrogenase in transformed and control plants of Zymoiarka variety was measured on the 7th day of drought. After drought, to launch the mechanisms of restoring after stress, the plants were watered abundantly, and the activity of the enzyme was measured 24 h later. It was determined (Fig. 2) that under normal irrigation, transgenic plants had somewhat lower level of enzyme activity compared to the control (control − 2.8 ± ± 0.4 nmol NADH/min × mg of protein, and transgenic forms – from 1.8 ± 0.3 to 2.3 ± 0.4 nmol NADH/min × × mg of protein). Under stress, gene expression is considerably inhibited both in the control and in the transgenic forms: control − 1.3 ± 0.2 nmol NADH/min × mg of protein, while in the transgenic forms it was 0.6 ± ± 0.1 nmol NADH/min × mg of protein on average. However, under conditions of restoring from stress, PDH activity in the control plants increased considerably (4.8 ± 1.1 nmol NADH/min × mg of protein), while the enzyme activity in the transformed plants did not increase, it decreased instead, on average, 1.0 ± ± 0.1 nmol NADH/min × mg of protein compared to normal irrigation. Thus, the transformed plants are characterized by the decrease in the activity of proline dehydrogenase enzyme, which is manifested during the change in conditions “norm-stress-norm”.

The content of free L-proline in the leaves of the control plants under optimal irrigation was 21 ± 3 mg% of fresh weight, and it was 1.5–2 times higher in the transgenic plants: from 33 to 48 mg% of fresh weight (Fig. 3). Under drought, the content of this aminoacid increased both in the control and transgenic forms: in the transgenic wheat plants, containing the suppressor of *pdh* gene, it was in the range from 152 to 237 mg% of fresh weight, and exceeded this value in the control plants 2.6–4.1 times (58 ± 3 mg% of fresh weight). In the variant with drought, the content of proline in the leaves of plants of the original genotype increased 2.8 times compared to the control, while in the genetically modified forms it increased 4.6–4.9 times. Therefore, the transgenic plants differed by the increased content of L-proline both at norm and stress. Thus, the presence of a double-stranded RNA suppressor of *pdh* gene in the transgenic plants leads not only to a decrease in enzyme activity, but also to an increase in the level of accumulating free L-proline both under conditions, approximated to optimum, and under soil drought.

To estimate the resistance of genetically modified wheat lines to drought, we analyzed its impact on morphometric indices and yield structure elements for T_2 generation plants. Higher resistance of T_2 plants to moisture deficiency compared to the initial plants was vividly reflected in the character of their growth. Under normal moisture provision, the average height of plants of the original variety and the transformed ones in the stem elongation phase was close, 55 cm on average. Under moisture stress, it was 45 ± 2 cm for the initial plants and 10 cm higher on average for transformed lines (Fig. 4). During the phase of full grain ripeness under drought, the height of the transgenic plants exceeded its values for the initial plants by 13–25 cm (Table 1).

According to the data obtained, there were some differences for PH, MSL, NGMS, NGP, GWMS, GWP, TGW indices between the transgenic plants and the positive control – the plants of the original variety, cultivated under moisture deficiency. The transgenic wheat plants with reduced *pdh* gene activity in terms of yield structure indices significantly exceeded the corresponding values for the non-transformed plants under moisture deficiency (Table 2). Their weight of grain was also considerably higher compared to the control plants, while in terms of the number of grains they were close.

It is also noteworthy that under moisture deficiency in soil, the transformed plants of all three lines were somewhat inferior to the control ones, grown under optimal conditions, in terms of grain productivity. However, when the decrease in grain productivity of the main tiller and the whole non-transformed plant under drought was 45 and 28 % respectively, in the transgenic plants these values were 15 and 8 % respectively. Such a considerable decrease in the damaging impact of soil drought on grain productivity demonstrated high adaptive potential of the transformed plants.

It is known that drought causes a decrease in airway permeability which results in considerable reduction in CO_2 inside leaves (Chaves M et al., 2008). In its turn, it promotes enhanced generation of reactive oxygen species (ROS) in chloroplasts. The detoxication of such oxygen forms (first of all, superoxide radical and

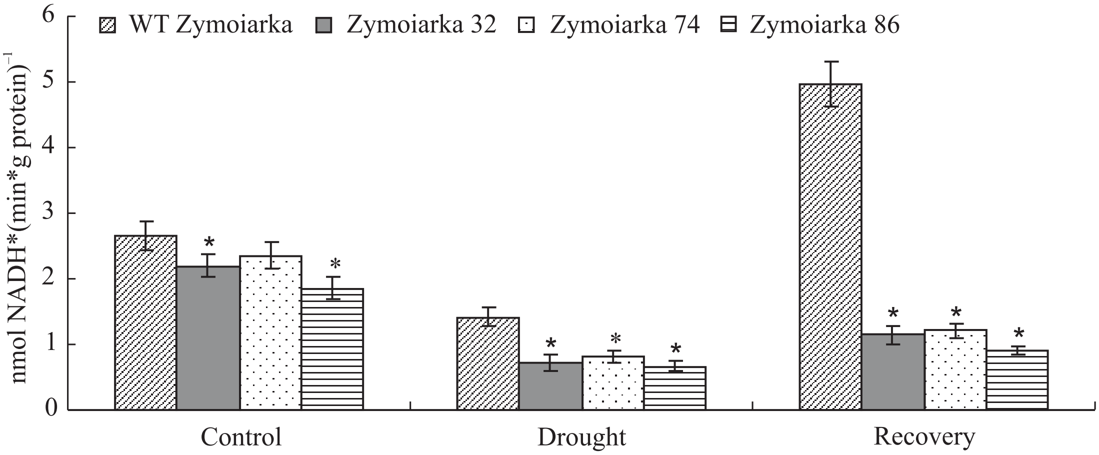

Fig. 2. The activity of proline dehydrogenase enzyme in the leaves of the original variety and the transformed plants of common wheat under different conditions of moisture provision in soil. Note: m ± SE, *– difference from the plants of original variety is considerable at $p \leq 0.05$

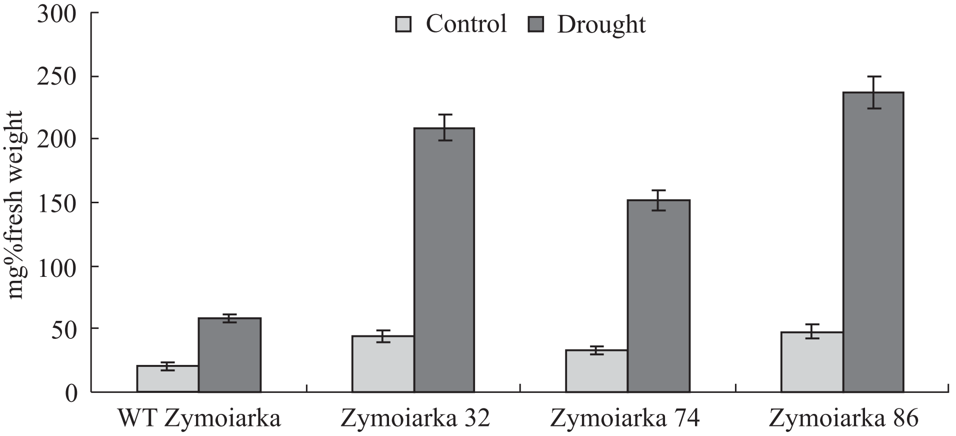

Fig. 3. The content of free L-proline in the leaves of the initial genotype and transformed common wheat plants under conditions of optimal moisture capacity (70 % OMC) and drought (30 % OMC). Note: m ± SE, difference from the plants of original variety is considerable at $p < 0.05$ for all the experiment variants

peroxides) is catalyzed by antioxidant enzymes (Kolupayev Yu et al., 2019, Laxa M et al., 2019). Therefore, we have compared the activity of superoxide dismutase and ascorbate peroxidase of chloroplasts in the flag leaf of both control and transgenic plants.

Under sufficient moisture provision, SOD activity in the leaves of the control was 1385 ± 57 relative units/(mg of chlor. per h), whereas in the transgenic forms it was 23–35 % higher (Table 3). Under these conditions, APX activity in the transgenic lines exceeded this index for the non-transgenic plants considerably as well (by 11–23 %). It demonstrated that the antioxidant system in the transgenic plants worked more actively compared to the non-transgenic genotypes. Thus, the presence of a double-stranded RNA-suppressor of proline dehydrogenase gene in the transgenic plants led both to the increase in the level of accumulating L-proline and activated antioxidant enzymes. In the plants of the initial variety, soil drought caused a considerable increase in the activity of both the first and second enzymes by 40 and 11 % respectively. On the contrary, under drought SOD activity in the transgenic plants decreased and was 73–76 % from the activity of this enzyme in the plants under sufficient moisturization. APX activity in the transgenic forms decreased down to 82–96 too, compared to the variant of 70 % FMC. Therefore, due to the stress factor in the non-transgenic plants, the antioxidant enzymes activity was increased, contrary to the genetically modified forms, where their activity was lower compared to optimal irrigation conditions. In our opinion, an opposite response of transgenic plants under such conditions may be related to the fact that due to higher activity of antioxidants, the biotechnological genotypes (Table 3) had better pre-adaptation for stress conditions. In addition, under water deficiency, transgenic plants had a considerable (4.6–4.9-fold) increase in the level of free L-proline (Fig. 2). In its turn, it also decreased the need for higher antioxidant enzymes activity.

DISCUSSION

The analysis of physiological and biochemical characteristics and productivity traits of the transgenic common wheat plants with a double-stranded RNA-suppressor of proline dehydrogenase gene (*pdh)* compared to non-transgenic genotypes demonstrated enhanced tolerance of biotechnological plants to moisture deficiency. We have determined a positive relationship between the level of free L-proline and the resistance of transgenic wheat plants to osmotic stress which may be associated either with the effect of L-proline on the expression of other genes of the stress-strain response of plants, or with the positive effect of the increase content of this amino acid on resistance at the early stages of stress development.

The analysis of the activity of antioxidant enzymes in the chloroplasts of transgenic plants demonstrated that under physiological conditions, the antioxidant system worked more actively in comparison with the non-transgenic genotypes, which may be a prerequisite for increasing the tolerance of these plants to the influence of stressors of various origin. It was determined that under moisture deficiency the activity of antioxidants in the biotechnological plants was comparatively lower than in the control ones, which may be explained by the fact that the transgenic genotypes were pre-adapted to stress conditions. Under moisture deficiency, the biotechnological wheat plants with the decreased activity of proline dehydrogenase had a sharp increase in the level of free L-proline, thus, there was no need for higher activity of antioxidants. The maintenance of a sufficient pool of antioxidants may serve as a mechanism for fast counteraction to the rise in osmotic disbalance which was observed under drought.

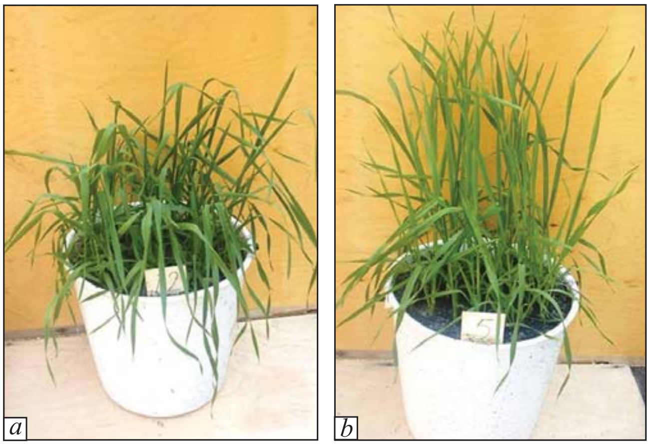

Fig. 4. The plants of the non-transgenic (*a*) and transgenic (*b*) forms during the stem elongation phase under drought

Table 1. The impact of drought on the height and length of the main tiller spikelet in T_2 plants and the original plants

Genotype	Plant height, cm	Main tiller spikelet length, cm
Control (–), 70 % FW	92.2 ± 2.1	9.8 ± 0.7
Control (+), 30 % FW	59.2 ± 6.0	6.6 ± 1.2
Zymoiarka 32	84.6 ± 3.2 *	9.7 ± 0.9 *
Zymoiarka 74	72.0 ± 5.6 *	8.2 ± 0.2 *
Zymoiarka 86	85.2 ± 5.2 *	9.7 ± 0.9 *

Note. Here and in Table 2: m ± SE, * – difference between the control (+) and experiment is significant at $p \leq 0.05$

Table 2. The indices of yield structure for T_2 plants and control plants under osmotic stress

Genotype	Main tiller		Plant		
	number of grains, it.	weight of grain, g	number of grains, it.	weight of grain, g	thousand grain weight, g
Control, 70 % FMC	45.2 ± 4.4	1.41 ± 0.23	167.2 ± 8.1	2.38 ± 0.30	29.6 ± 0.3
Control, 30 % FMC	40.0 ± 4.1	0.78 ± 0.09	88.8 ± 5.6	1.72 ± 0.19	23.4 ± 1.8
Zymoiarka 32	43.8 ± 5.5	1.24 ± 0.11 *	142.8 ± 9.8 *	2.31 ± 0.22 *	27.0 ± 0.6 *
Zymoiarka 74	39.2 ± 6.6	1.15 ± 0.11 *	128.4 ± 8.7 *	2.04 ± 0.28	26.3 ± 0.5 *
Zymoiarka 86	41.2 ± 5.4	1.22 ± 0.09 *	142.4 ± 12.0 *	2.19 ± 0.21 *	26.9 ± 0.7 *

Other researchers also noted different responses in the activity of antioxidants of drought-resistant and drought-susceptible genotypes to drought. For instance, under moderate drought conditions, a drought-tolerant genotype of *Amaranthus tricolor* had a lesser decrease in the activity of SOD, catalase, and guaiacol peroxidase compared to the control (by 53, 28 and 9 %), than the susceptible one (64, 48 and 18 %) (Sarker U and Shinya O, 2018). Under moderate drought, the increase in proline content in the former compared to the control was 72 %, under severe conditions – 176 %, while in the latter, it was higher – 248 and 566 % respectively. It was also determined that the activity of catalase, SOD and ascorbate peroxidase was higher under conditions of severe stress compared to moderate conditions both in resistant and susceptible varieties in terms of this factor (Abid M et al, 2018). Their activity in the tolerant variety was higher compared to the susceptible one. The analysis of the response of the variety and three recombinant inbred wheat lines to osmotic stress demonstrated that the lines with higher tolerance (PL 337 and PL 371) had higher proline content and activity of SOD, catalase, peroxidase and ascorbate peroxidase both under one stress (during the phase of the second leaf), and two osmotic stresses (Amoah J et al, 2019). The authors also note that the non-acclimatized plants had higher water loss, accumulation of H_2O_2 and osmolytes, damaged membranes, higher system of antioxidant protection compared to the acclimatized ones. Similar to our results, this article demonstrated that the increase in the activity of antioxidants in plants with acclimatization to stress (i.e. pre-adapted by the first stress) in all genotypes was smaller compared to the ones, not adapted to stress. The exception was found only for the activity of peroxidase in two drought-resistant inbred lines, where it did not differ much for adapted and non-adapted plants. At the same time, it was determined that under drought the activity of peroxidase and ascorbate peroxidase in wheat varieties, contrast in their drought resistance, which were grown under preliminary acclimatization to water deficiency and without it, was not the same (Khanna-Chopra R and Selote D, 2007). Pre-adaptation of drought-susceptible variety Moti to stress did not impact the activity of these enzymes, whereas in drought-resistant wheat variety C306 their activity under acclimatization conditions was smaller compared to non-adapted plants.

It is known that the increase in SOD activity in transgenic plants may be caused by the expression of both heterologous genes of SOD and other genes. For instance, tobacco plants with transgene *SgNCED1*, (9-cis-epoxycarotenoid dioxygenase, (NCED) with *Stylosanthes guianensis* – a key enzyme of abscisic acid biosynthesis had a 22–27 % increase in SOD activity (Zhang Y et al., 2008). It was accompanied with the decrease in the stomatal conductance, transpiration rate, membrane permeability and resulted in growth enhancement under stresses, caused by mannitol and salinization. The expression of oxalate oxidase (*OxO*) in tobacco plants also increased the activity of antioxidants under normal conditions and under stresses, induced by methylviologen (paraquat) and increased light intensity (Wan X et al., 2009). Under drought, SOD activity increased by 62 % in non-transformed rice plants, but only by 13–31 % in the transgenic plants with decreased expression of gene *OsSAMDC* (S-adenosylmethionine decarboxylase) (Chen M et al., 2014). Our results also allow for the assumption that enhanced activity of antioxidants in transgenic wheat

Table 3. The impact of soil drought on the activity of superoxide dismutase (SOD) and ascorbate peroxidase (APX) in the chloroplasts of the flag leaf in control and transgenic wheat plants

Variant	Content			
	SOD, rel. units/ (mg of chlor. per h)		APX, μmol AA/(mg of chlor. per h)	
	70 % FMC	30 % FMC	70 % FMC	30 % FMC
Control	1385 ± 57	1935 ± 100b	338 ± 9	374 ± 22b
Zymoiarka 32	1870 ± 77a	1406 ± 78ab	417 ± 23a	342 ± 9ab
Zymoiarka 74	1709 ± 64a	1251 ± 83ab	375 ± 19a	321 ± 11ab
Zymoiarka 86	1833 ± 82a	1389 ± 85ab	398 ± 21a	330 ± 8ab

Note: m ± SE, AA – ascorbic acid; a – difference between the transgenic and non-transgenic plants; b – difference between irrigation variants is reliable at $p \leq 0.05$.

plants with a double-stranded RNA-suppressor of *pdh* gene may be caused by the expression of heterologous genes.

There are literature data, proving that the expression of genes is different not only between plants under drought and sufficient moisture provision, but also between the acclimatized and non-acclimatized plants. For instance, it was determined that the expression of genes *TaWRKY2*, *TaNAC1, TabHLH1*, *TabZIP1* and *TaMYB2* in non-acclimatized plants was considerably higher compared to the ones, which were previously subject to drought during the phase of the second leaf, then were well-watered and subjected to the second drought (Amoah J et al., 2019). The highest expression of all five genes was noted in genotype PL 337, the most tolerant to drought. Thus, the authors believe that genotype differences in terms of drought resistance may be at least partially related to the ability of plants for acclimatization and induction of antioxidant protection systems (Amoah J et al., 2019).

It would be relevant to determine metabolic strategies, ensuring yield stability under unfavorable factors, as these would promote stable genotypes via integration of recombinant DNA molecules, capable of controlling processes on the genetic level, into the genome of cultivated plants. The results of these studies will be useful for selection programs, creating wheat varieties with enhanced drought tolerance.

Therefore, complex analysis of the transgenic common wheat plants demonstrated their increased tolerance to moisture deficiency compared to the non-transgenic genotypes. The decrease in the activity of proline dehydrogenase under norm-strain-norm conditions was determined in the biotechnological wheat plants. The analysis of the antioxidant enzymes activity in the chloroplasts of genetically modified wheat plants with a double-stranded RNA-suppressor of proline dehydrogenase gene showed that under physiological conditions, the antioxidant system works more actively in comparison with the non-transgenic genotypes, which may be a prerequisite for increasing the tolerance of these plants to the influence of stressors of various origin. It was determined that under moisture deficiency the antioxidant enzymes activity in the biotechnological plants was comparatively lower than in the control ones, which may be explained by the fact that transgenic genotypes had been pre-adapted to stress conditions. Under moisture deficiency, the biotechnological wheat plants with decreased activity of proline dehydrogenase had a sharp increase in the level of free L-proline, thus, there was no need for higher activity of of antioxidants enzymes. The maintenance of a sufficient pool of antioxidants may serve as a mechanism for fast counteraction to the rise in osmotic disbalance which is observed under drought. Along with the accumulation of proline, soluble sugars, free amino acids and the increase in non-enzymatic antioxidant activity, the increase in the activity of antioxidant enzymes may serve as an efficient mechanism of fast counteraction to the increase in ROS under osmotic and energetic disbalance, caused by stresses.

CONCLUSIONS

The analysis of physiological and biochemical characteristics, as well as economic and grain productivity elements of transgenic common wheat plants with a double-stranded RNA suppressor of the proline dehydrogenase gene showed their increased tolerance under conditions of soil drought, compared with non-transgenic genotypes, which may be associated with higher proline accumulation and an increase in the activity of antioxidant enzymes. Under soil moisture deficiency, the transgenic wheat plants with reduced *pdh* gene activity significantly exceeded the corresponding values of grain productivity elements for the non-transformed plants.

We have determined a positive relationship between the level of free L-proline and the resistance of the transgenic wheat plants to osmotic stress which may be associated either with the effect of L-proline on the expression of other genes of the stress-strain response of plants, or with the positive effect of the increased content of this amino acid on resistance at the early stages of stress development.

It was determined that the activity of antioxidant enzymes in chloroplasts of the transgenic wheat plants with a double-stranded RNA-suppressor of proline dehydrogenase gene under physiological conditions was higher compared to the non-transgenic plants. Soil drought caused a considerable increase in the activity in plants of the initial variety compared to optimal irrigation conditions whereas in the genetically modified forms it decreased. It may be explained by the fact that transgenic genotypes had already been pre-adapted to unfavorable conditions. It has been suggested that an increase in the antioxidant enzymes activity in the biotechnological plants can be caused by the expression of heterologous genes.

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Стійкість генетично-модифікованих рослин пшениці, що містять дволанцюговий РНК-супресор гена проліндегідрогенази, до дефіціту вологи

О. В. Дубровна, О. О. Стасик, Г. О. Прядкіна,
О. В. Зборівська, О. Г. Соколовська-Сергієнко

Інститут фізіології рослин і генетики НАН України,
31/17, вул. Васильківська, м. Київ, Україна, 03022

e-mail: dubrovny@ukr.net*, o_stasik@yahoo.com, galpryadk@gmail.com, masluikivska@ukr.net, sokolovska@ukr.net

Мета. Аналіз фізіолого-біохімічних характеристик та господарських ознак трансгенних рослин м'якої пшениці (*Triticum aestivum* L.) з дволанцюговим РНК-супресором гена проліндегідрогенази (*pdh)* порівняно з нетрансгенними генотипами за дії грунтової посухи. **Методи.** Біохімічні: спектрофотометричне вимірювання активності антиоксидантних ферментів (СОД, АПО), активності проліндегідрогенази та вмісту вільного L-проліну; біотехнологічний: *Agrobacterium*-опосередкована трансформація *in planta*; фізіологічні: морфометричні показники та елементи структури врожаю; математичної статистики. **Результати**. Показано, що наявність у трансгенних рослин дволанцюгового РНК-супресора гена *pdh* призводить не тільки до зниження активності ферменту (в середньому в 2 рази, порівняно з вихідними рослинами), а й до підвищення рівня накопичення вільного L-проліну як за оптимальних умов, так і за умов ґрунтової посухи (у 2,6–4,1 рази). За дефіциту вологі у ґрунті трансгенні рослини пшениці зі зниженою активністю гена *pdh* за показниками структури врожаю значно перевищували відповідні значення у нетрансформованих рослин. Біотехнологічні рослини в умовах дефіциту ґрунтової вологи за масою зерна з головного пагону перевищували контрольні рослини у 1,5–1,6 рази, тоді як за кількістю зерен – істотно не відрізнялися. Показники зернової продуктивності цілої рослини у трансформованих ліній в умовах дефіциту ґрунтової вологи дещо поступались відповідним показникам у вихідних рослин, вирощених за оптимальних умов. Ґрунтова посуха спричиняла істотне підвищення активності СОД та АПО (відповідно на 40 та 11 %) у рослин вихідного сорту. Активність обох антиоксидантних ферментів за умов посухи у трансгенних рослин, навпаки, зменшувалася: СОД – до 73–76 %, АПО – до 82–86 %, порівняно з варіантом 70 % ПВ. **Висновки**. Аналіз фізіолого-біохімічних характеристик та господарських ознак трансгенних рослин м'якої пшениці з дволанцюговим РНК-супресором гена проліндегідрогенази засвідчив їх підвищену толерантність за дії грунтової посухи порівняно з нетрансгенними генотипами, що може бути обумовленим більшим накопичення проліну та більшою активністю антиоксидантної системи. За дефіциту ґрунтової вологи трансгенні рослини пшениці зі зниженою активністю гена *pdh* за показниками структури зернової продуктивності рослини значно перевищували відповідні значення у нетрансформованих рослин. Аналіз активності антиоксидантних ферментів у хлоропластах трансгенних рослин показав, що за фізіологічних умов антиоксидантна система у них працює більш активно порівняно з нетрансгенними генотипами, що може бути передумовою для збільшення толерантності цих рослин до стресорів різного походження. Ймовірно, що позитивна залежність між рівнем вільного L-проліну та стійкістю трансгенних рослин пшениці до осмотичного стресу пов'язана або з впливом L-проліну на експресію інших генів стресової відповіді рослин, або з позитивним впливом підвищеного вмісту цієї амінокислоти на стійкість на ранніх етапах розвитку стресу. Зроблено припущення, що підвищення активності антиоксидантних ферментів у біотехнологічних рослин може викликатися експресією гетерологічних генів.

Ключові слова: пшениця, трансгенні рослини, ґрунтова посуха, пролін, супероксиддисмутаза, аскорбат пероксидаза, зернова продуктивність.

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