UDC 581.132 https://doi.org/10.7124/FEEO.v33.1580

KEDRUK A.S., STASIK O.O.✉, KIRIZIY D.A., SOKOLOVSKA-SERGIIENKO O.G.
Institute of Plant Physiology and Genetics, National Academy of Sciences of Ukraine,
Ukraine, 03022, Kyiv, Vasylkivska str., 31/17, ORCID: 0009-0005-9481-2687, 0000-0001-5023-2529, 0000-0001-6079-893X, 0000-0001-6967-8221
✉ *o_stasik@yahoo.com, (067) 389-71-65*

ACCLIMATION OF PHOTOSYNTHETIC APPARATUS TO MODERATE DROUGHT STRESS IN WHEAT VARIETIES DIFFERING IN TOLERANCE

Aim. Drought is one of the most harmful abiotic stresses limiting crop productivity. We study the ability of photosynthetic apparatus of winter wheat varieties differing in their tolerance to acclimate to moderate drought stress under pot experiment. ***Methods.*** Dynamics of relative water content (RWC), chlorophyll content, CO_2 assimilation rate (Pn), activity of antioxidant enzymes in chloroplasts of flag leaf of drought-tolerant variety Yednist and less drought-tolerant varieties Podilska Nyva and Darunok Podillia during weeklong moderate drought at flowering and one week after resumption of optimal watering were studied. ***Results.*** RWC and chlorophyll content gradually decreased from the onset to the end of drought period. In contrast, Pn inhibition was notably stronger at the onset than at the end of the drought period in Yednist and Podilska Nyva cultivars and remained the same in Darunok Podillia cultivar. On the 7th day of post-drought period, Pn in treated plants of all varieties was restored to the control level despite significantly lower chlorophyll content. ***Conclusions.*** Photosynthetic apparatus of more tolerant variety has a greater ability to acclimate to prolonged moderate drought which was related to higher activity of antioxidant enzymes and resulted in less grain yield losses.

Keywords: *Triticum aestivum* L., varieties, drought, photosynthesis, antioxidant enzymes, yield.

Water deficit is one of the most common abiotic stresses significantly limiting the yield genetic potential of cultivated plants, in particular wheat – one of the staple food crops in the world [1]. This problem is aggravated due to global warming accompanied by adverse climate change, increasing temperature fluctuations, spatial and temporal precipitation unevenness, which threatens the stability of grain production. Thus, the issue of screening wheat genotypes for drought resistance based on physiological characteristics becomes more and more relevant [2].

Soil moisture deficiency at the level of the entire plant organism leads to a decrease in the intensity of photosynthesis and growth processes. The effects of drought on the activity and capacity of photosynthetic apparatus, metabolism and production process of plants have been studied for a long time, however mechanisms of plant drought-tolerance remain insufficiently elucidated [3].

The main cause of the decrease in the activity of photosynthesis at the initial stages or under conditions of short-term drought is the reduction in CO_2 diffusion rate from the atmosphere into the photosynthesizing cells due to stomatal closure, while the major reasons for the effects of long-term water stress are significant metabolic rearrangements and functional and structural damages to photosynthetic apparatus [4, 5]. Inhibiting the activity of the photosynthesis results in a consistent depletion of assimilate abundance in the plant organism, which leads to a decrease in the intensity of vital processes in plant.

However, plants are naturally equipped with complex protective mechanisms to cope with and acclimate to water scarcity [4, 6]. The aptitude of plants to protect the photosynthetic apparatus from damage under drought stress is important for the rapid recovery of the active functioning photosynthetic apparatus, when normal water regime returns, and helps to weaken the negative impact of drought on productivity [7]. Understanding the physiological mechanisms of wheat photosynthetic apparatus acclimation to water deficiency would provide guidance for breeding programs to improve drought tolerance in wheat, as well as facilitate physiological approaches to establish accurate screening methods for traits associated with higher crop yields under drought conditions [2].

The aim of our work was to study the ability of photosynthetic apparatus of winter wheat varieties differing in their tolerance to acclimate to moderate drought stress.

Materials and methods

The research was carried out on plants of winter bread wheat (*Triticum aestivum* L.) of drought-tolerant variety Yednist and less drought-tolerant varieties Podilska Nyva and Darunok Podillia. The plants grew in pots with 10 kg of fertilized soil at natural light. Fertilizers were added twice in equal quantities ($N_{80}P_{80}K_{80}$ mg per kg of soil) when the pots were filled with soil, and at the middle of the stem elongation stage (BBCH 34). For each variety, 10 pots with 20 plants each were set up.

For the control (well watered, WW) plants, soil moisture was maintained at the level of 70 % of field capacity (FC) during the entire growing season. At the end of earing stage (BBCH 55), watering of plants in the one half of pots (drought-stressed, DS) was stopped; during three days the soil moisture was reduced to the level of 30 % FC, which was maintained for the next seven days. After that, watering stressed plants was resumed to a control level that was maintained until the end of the growing season. The period of limited moisture supply of treated plants covered the flowering stage.

Relative water content (RWC), chlorophyll content, activity of antioxidant enzymes in chloroplasts and CO_2 assimilation rate in flag leaf of control and treated plants were measured on the first day reaching soil moisture of 30 % FC, on the seventh day of cultivation at this moisture level, and a week after resumption of optimal watering.

Relative water content was determined according to the standard method [8]. The content of the chlorophylls was measured after extraction with dimethyl sulfoxide (DMSO) by the spectrophotometric method [8]. Chloroplasts for antioxidant enzymes activity determination were isolated as described previously [5]. The superoxide dismutase (SOD, EC 1.15.1.1) activity was determined spectrophotometrically using nitrotetrazolium blue at a wavelength of 560 nm [10]. 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 and Asada method [11].

The net CO_2 assimilation rate of attached leaf was recorded under controlled conditions (1800 μmol/(m^2·c) PAR and 25°C in air) using infrared gas analyzer GIAM-5M (RF), switched on according to a differential scheme. The components of plant grain productivity were determined by weighing air-dry material after achieving full ripeness of the grains.

The repetition of measuring physiological and biochemical parameters was 3 to 5 times. Data on components of grain productivity were determined as average of measurements of 20 individual plants. The obtained data were processed by generally accepted methods of variation statistics. The figures and the tables show the arithmetic mean and standard error of the mean. The significance of the differences between controls and treatments was evaluated using one-way ANOVA. Differences were considered significant at $p \leq 0.05$.

Results and discussion

Relative water content – the amount of water in a leaf at the time of sampling relative to the maximal water the leaf can retain – in flag leaf of WW plants at the beginning of drought experiment was around 95-97 % (Fig. 1a). It tended to decrease slightly during first 7 days of experiment and stabilized throughout next two weeks what can be explained, in part, by the gradual increase in air temperature and decrease in its humidity at that time [12]. Reduction of soil moisture to 30 % of FC caused insignificant changes in RWC in leaves of DS plants of Yednist and Darunok Podillia on first day of experiment while little statistically significant decline in RWC was observed for Podilska Nyva. On the seventh day of cultivation under drought conditions, the RWC in DS plants decreased largely and differences between varieties became apparent. The highest leaf tissue dehydration was observed in the DS plants of Darunok Podillia (79 % of WW plants) and the lowest in Yednist variety (about 91 % of WW plants). The value of RWC on last day of drought-treatment in varieties Yednist, Podilska Nyva and Darunok Podillia amounted respectively 82, 76, and 69 % which corresponded to mild level of drought stress for first variety and moderate level for two last ones [6]. These differences can be attributed to different capability of water acquisition and osmotic adjustment [3]. On the 14th day of the experiment (a week after restoring the soil moisture to 70 % FC), the RWC in the flag leaves of the DS plants increased in Darunok Podillia, tended to increase in Podilska Nyva and remained the same in Yednist variety. The difference between varieties for DS plants was leveled again, however the values of RWC in DS plants were markedly lower than in control plants indicating that water relations in DS plants were not fully restored.

Drought-induced irreversible damage to flag leaf photosynthetic apparatus was clearly manifested by the decline in chlorophyll content (Fig. 1b).

On the first day of the drought at 30 % of FC, the chlorophyll content in the DS and WW plants of varieties Yednist and Darunok Podillia did not differ but in Podilska Nyva DS plants it decreased compared to control and was about 74 % of control. On the seventh day of the drought, the content of chlorophyll in the flag leaf of DS plants of all varieties fell significantly and was about 72 % of the control values in Yednist, 64 % in Podilska Nyva and only 50 % in the Darunok Podillia. The absolute value in DS plants remained practically the same during the next week after restoring normal watering. Noteworthy, chlorophyll content in flag leaf of WW plants decreased for two weeks of experiment by ca. 35 % due to natural aging. As a result, the content of chlorophyll in the flag leaf of DS plants of Yednist variety was 80 % and of Podilska Nyva and Darunok Podillia about 68 % of the control plants.

Although drought duration enhanced impact on RWC and chlorophyll content, time-course of drought-induced changes in flag leaf photosynthetic activity, as indicated by CO_2 assimilation rates was essentially different. The most dramatic decline in CO_2 assimilation rate in DS plants was detected on the first day of drought at 30 % of FC (Fig. 2). Its relative to WW plants value amounted to 61, 47 and 56 % for Yednist, Podilska Nyva and Darunok Podillia varieties, respectively. However, on the seventh day of drought CO_2 assimilation rate increased in DS plants of Yednist and Podilska Nyva varieties yet remained the same in Darunok Podillia. Relative to WW plants value increased to 81, 70 and 60 % in Yednist, Podilska Nyva and Darunok Podillia varieties, respectively. This indicates important ability of photosynthetic apparatus to acclimate to drought and substantial superiority of Yednist over the other two varieties. It should be noted that the absolute and relative values of the CO_2 assimilation rate in Yednist and Podilska Nyva varieties increased against the background of reductions in the RWC and chlorophyll content in leaf (see Fig. 1). However, such acclimation was minimal, if any, in DS plants of Darunok Podillia variety, which met with the largest damages as indicated by changes in RWC and chlorophyll content.

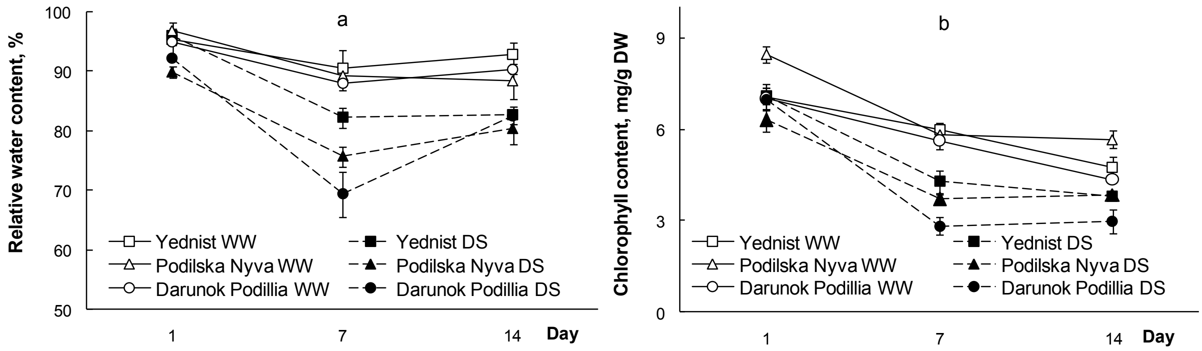

Fig. 1. Relative water content (a) and chlorophyll content (b) in flag leaf of well watered (open symbols) and drought-stressed (closed symbols) plants of winter wheat varieties on 1st and 7th day of drought at 30 % of FC, and 14th day of experiments (7th day after well watering returns).

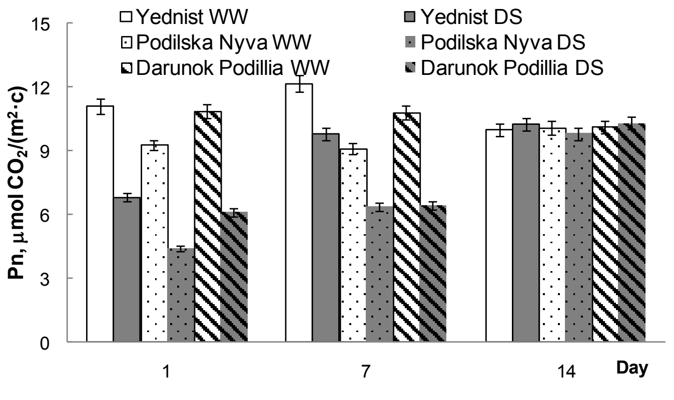

Fig. 2. Net CO_2 assimilation rate (Pn) in flag leaf of well watered (light columns) and drought-stressed (dark columns) plants of winter wheat varieties on 1st and 7th day of drought at 30 % of FC, and 14th day of experiments (7th day after well watering returns).

A week after the end of the drought, the CO_2 assimilation rate of DS plants increased, particularly for Podilska Nyva and Darunok Podillia, and its values in DS and WW plants of all varieties were practically equal to the contrary to RWC and chlorophyll content.

The acclimation of the photosynthetic apparatus to drought includes hydraulic readjustment and significant physiological and metabolic changes, in particular, the accumulation of osmotically active compounds (proline, glycine betaine, soluble carbohydrates), an increase in the proportion of unsaturated fatty acids in the composition of membranes, activation of the synthesis of chaperone proteins, notably Rubisco activase, and other protective proteins, as well as systems of reactive oxygen species control [3, 4, 13].

To assess the response of the antioxidant defense system of wheat photosynthetic apparatus to the drought, we determined the activity of the main antioxidant enzymes of chloroplasts – SOD and APX. It was found that SOD activity of flag leaf chloroplasts on the first day of drought slightly decreased in plants of Yednist and Podilska Nyva varieties and showed no changes in Darunok Podillia, however on seventh day it increased significantly compared to the control in Yednist variety and only tended to increase in two other varieties (Table 1). A week after normal watering resumed, the activity of SOD in the DS plants of Yednist variety remained higher than control while it did not significantly differ in DS and WW plants of Darunok Podillia and Podilska Nyva.

APX activity in DS plants of all varieties on the first day of drought practically did not differ from the control. Similarly to SOD, activity of APX in DS plants relative to WW plants on seventh day increased significantly in Yednist variety and was the same in Podilska Nyva and Darunok Podillia. A week after drought-treatment termination, APX activity in the DS plants of Yednist and Podilska Nyva varieties exceeded control while Darunok Podillia variety had practically equal APX activity in the DS and WW plants.

It should be also noted, that activity of APX in the chloroplasts of the flag leaves of the DS plants under prolonged drought increased more than SOD in Yednist and Podilska Nyva varieties, which showed a higher stability of the photosynthetic apparatus. This may be related to adaptive changes aimed a stronger control of the level of H_2O_2 in chloroplasts, since it was shown that under conditions of stress and during the recovery period, the content of H_2O_2 positively correlated with the expression of genes involved in programmed cell death, and their excessive activation was harmful to the functioning of the leaf [13].

Table 1. Activities of antioxidant enzymes of chloroplasts in flag leaf of well watered and drought-stressed plants of winter wheat varieties on 1st and 7th day of drought at 30 % of FC, and 14th day of experiments (7th day after well watering resumed) (AA – ascorbic acid)

Variety	Treatment	1	7	14
SOD, rel. units / (g f.w.·h)				
Yednist	WW plants	2290±27	1730±46	1898±116
	DS plants	1780±210	2060±50*	2303±194
	% of control	77.7	119.1	121.3
Podilska Nyva	WW plants	2067±47	2214±95	1764±130
	DS plants	1704±102*	2406±58	1883±125
	% of control	82.4	108.6	106.7
Darunok Podillia	WW plants	2068±85	1900±145	1954±70
	DS plants	2115±149	2120±200	2031±123
	% of control	102.3	111.6	103.9
APX, μmol AA/(g f.w.·h)				
Yednist	WW plants	218±15	212±13	336±7
	DS plants	216±13	330±2*	442±2*
	% of control	99.1	155.6	131.5
Podilska Nyva	WW plants	236±13	231±12	331±4
	DS plants	263±3	257±5	397±7*
	% of control	111.0	111.2	119.9
Darunok Podillia	WW plants	229±5	233±10	305±15
	DS plants	244±23	231±15	309±8
	% of control	106.5	99.1	101.3

Note. * – differences between WW and DS plants are significant at $p \leq 0.05$.

Table 2. Effects of drought during the flowering stage on elements of main shoot grain productivity of bread wheat varieties differing in their tolerance

Variety	Treatment	Total weight, g	Grain weight, g	Grain number, pc.	1000 grain weight, g
Yednist	WW plants	4.56±0.07	2.00±0.03	44.7±1.7	45.7±1.8
	DS plants	3.17±0.07*	1.31±0.04*	35.9±1.0*	36.9±1.6*
	% of control	69.6	65.4	80.3	80.6
Podilska Nyva	WW plants	4.74±0.15	2.22±0.08	48.4±1.9	46.2±1.2
	DS plants	3.10±0.18*	1.28±0.12*	37.3±1.6*	33.6±2.0*
	% of control	65.4	57.6	76.9	72.7
Darunok Podillia	WW plants	4.56±0.15	2.14±0.08	46.5±1.8	46.1±0.7
	DS plants	3.05±0.12*	1.19±0.06*	35.0±1.4*	34.3±1.4*
	% of control	66.9	55.8	75.3	74.4

Note. * – differences between WW (well watered) and DS (drought-stressed) plants are significant at $p \leq 0.05$.

Drought during the flowering period resulted in a significant reduction in the total weight of the above-ground part and elements of grain productivity of the main shoot of all studied varieties, although to varying degrees (Table 2). The smallest decline in grain weight per plant was observed in the Yednist variety, and the largest in the Darunok Podillia. It should be noted that this grain yield loss resulted from decreases both in the number of grains and in the mass of 1000 grains.

Drought stress during flowering period reduced the grain number in the ear of wheat due to premature flower abortion, which resulted in a reduced number of grains in the ear [14]. Thousand grain weight reduction in this experiment with drought-treatment terminated before grain filling resulted from insufficient provision of assimilates due to the lessening its reserves in the stem, which are formed for the most part during the earing-flowering period [15]. Another reason for the decrease in the availability of assimilates for grain filling processes could be the acceleration of the aging of plants that were affected by drought, as evidenced by the irreversible decrease in the chlorophyll content in their leaves (Fig. 1b) and the general shortening of the vegetation period.

Conclusions

Thus, obtained data show that more tolerant variety has greater ability to acclimate to prolonged moderate drought compared with less tolerant. This competence is related to higher activity of antioxidant enzymes in chloroplasts and promote superior activity of photosynthesis during the drought and post-drought period that resulted in less grain yield losses.

References

1. Leng G., Hall J. Crop yield sensitivity of global major agricultural countries to droughts and the projected changes in the future. *Science of the Total Environment*. 2019. Vol. 654. P. 811–821.doi: 10.1016/j.scitotenv.2018.10.434.
2. 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. Climate impact and adaptation to heat and drought stress of regional and global wheat production. *Environ. Res. Lett*. 2021. Vol. 16. 054070. doi: 10.1088/1748-9326/abd970.
3. Ahanger M. A., Siddique K. H. M., Ahmad P. Understanding drought tolerance in plants. *Physiologia Plantarum*. 2021. Vol. 172(2). P. 286–288. doi: 10.1111/ppl.13442.
4. Feller U. Drought stress and carbon assimilation in a warming climate: Reversible and irreversible impacts. *J. Plant Physiol*. 2016. Vol. 203. P. 84–94. doi: 10.1016/j.jplph.2016.04.002.
5. Stasik O. O., Kiriziy D. A., Sokolovska-Sergiienko O. G., Bondarenko O. Yu. Influence of drought on the photosynthetic apparatus activity, senescence rate, and productivity in wheat plants. *Fiziol. rast. genet*. 2020. Vol. 52(5). P. 371–387. doi: 10.15407/frg2020.05.371.
6. Stasik O. O. The response of photosynthetic apparatus of C_3 plants to water deficits. *Fiziologiia i biokhimiia kul'turnykh rastenii*. 2007. Vol. 39(1). P. 14–27. [in Ukrainian]
7. Morgun V. V., Stasik O. O., Kiriziy D. A., Pryadkina G. O. Relations between reactions of photosynthetic traits and grain productivity on soil drought in winter wheat varieties contrasting in their tolerance. *Fiziol. rast. genet*. 2016. Vol. 48, No. 5. P. 371–381. doi: 10.15407/frg2016.05.371. [in Ukrainian]
8. Arndt S. K., Irawan A., Sanders G. J. Apoplastic water fraction and rehydration techniques introduce significant errors in measurements of relative water content and osmotic potential in plant leaves. *Physiologia Plantarum*. 2015. Vol. 155. P. 355–368. doi: 10.1111/ppl.12380.
9. Wellburn A. R. The spectral determination of chlorophylls a and b, as well as total carotenoids, using various solvents with spectrophotometers of different resolution. *J. Plant Physiol*. 1994. Vol. 144. P. 307–313. doi: 10.1016/S0176-1617(11)81192-2.

10. Giannopolitis C. N., Ries S. K. Superoxide dismutase. Occurrence in higher plants. *Plant Physiol*. 1977. Vol. 59(2). P. 309–314. doi: 10.1104/pp.59.2.309.
11. Chen G.-X., Asada K. Ascorbate peroxidase in tea leaves: occurrence of two isozymes and the differences in their enzymatic and molecular properties. *Plant and Cell Physiol.* 1989. Vol. 30(7). P. 987–998. doi: 10.1093/oxfordjournals.pcp.a077844.
12. Schönbeck L. C., Schuler P., Lehmann M. M., Mas E., Mekarni L., Pivovaroff A. L., Turberg P., Groossiord C. Increasing temperature and vapour pressure deficit lead to hydraulic damages in the absence of soil drought. *Plant, Cell & Environment*. 2022. Vol. 45(11). P. 3275–3289. doi: 10.1111/pce.14425.
13. Sun M., Jiang F., Cen B., Wen J., Zhou Y., Wu Z. Respiratory burst oxidase homologue-dependent H_2O_2 and chloroplast H_2O_2 are essential for the maintenance of acquired thermotolerance during recovery after acclimation. *Plant, Cell & Environment*. 2018. 41(10). P. 2373–2389. doi: 10.1111/pce.13351.
14. Farooq M., Hussain M., Siddique K. H. M. Drought stress in wheat during flowering and grain-filling periods. *Crit. Rev. Plant Sci.* 2014. Vol. 33. P. 331–349. doi: 10.1080/07352689.2014.87529.
15. Tarasiuk M. V., Stasik O. O. The effect of drought at flowering stage on the dynamics of accumulation and remobilization of reserve water-soluble carbohydrates in stem segments of winter wheat varieties contrasting in drought resistance. *Fiziol. rast. genet*. 2022. Vol. 54(5). P. 429–449. doi: 10.15407/frg2022.05.429.

КЕДРУК А. С., СТАСИК О. О., КІРІЗІЙ Д. А., СОКОЛОВСЬКА-СЕРГІЄНКО О. Г.
Інститут фізіології рослин і генетики НАН України,
Україна, 03022, м. Київ, вул. Васильківська, 31/17

АКЛІМАЦІЯ ФОТОСИНТЕТИЧНОГО АПАРАТУ ДО ПОМІРНОЇ ПОСУХИ У РІЗНИХ ЗА ТОЛЕРАНТНІСТЮ СОРТІВ ПШЕНИЦІ

Мета. Посуха є одним із найшкідливіших абіотичних стресорів, що знижує продуктивність сільськогосподарських культур. Ми досліджували здатність фотосинтетичного апарату сортів озимої пшениці, які різняться толерантністю, адаптуватися до помірної посухи в умовах вегетаційного досліду. ***Методи***. Визначали динаміку відносного вмісту води (ВВВ), вмісту хлорофілу, інтенсивності асиміляції CO_2 (Pn) і активності антиоксидантних ферментів у хлоропластах прапорцевого листка посухостійкого сорту Єдність та менш посухостійких сортів Подільська Нива і Дарунок Поділля протягом тижневої помірної посухи в період цвітіння та тиждень після відновлення оптимального поливу. ***Результати.*** ВВВ і вміст хлорофілу послідовно знижувався від початку і до кінця періоду посухи. Натомість інгібування Pn було значно сильнішим на початку, ніж у кінці періоду посухи, в сортів Єдність і Подільська Нива і залишалося однаковим у сорту Дарунок Поділля. На 7-му добу відновлення поливу Pn в дослідних рослин усіх сортів повертався до рівня контролю, незважаючи на значно нижчий вміст хлорофілу. ***Висновки.*** Фотосинтетичний апарат стійкішого сорту має ліпшу здатність адаптуватися до тривалої помірної посухи, що пов’язано з вищою активністю антиоксидантних ферментів і сприяє меншим втратам зернової продуктивності.
Ключові слова: *Triticum aestivum* L., сорти, посуха, фотосинтез, антиоксидантні ферменти, урожайність.