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ADAPTIVE REACTIONS OF WHEAT PLANTS TO DROUGHT CONDITIONS UNDER THE INFLUENCE OF SALICYLIC ACID

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Background. Drought is one of the most important factors affecting plant growth and productivity. The study addressed the perspectives of usage of salicylic acid, a stress protective compound, to increase the adaptive potential of *Triticum aestivum* L. plants under drought conditions. The effect of salicylic acid on the photosynthetic apparatus of wheat plants under the conditions of drought with a subsequent gradual resumption of watering was investigated.

Materials and methods. Plants of the Podolyanka variety of wheat (*Triticum aestivum* L.) were the objects of our study. Seeds were pre-soaked in a solution of salicylic acid (50 mM) for 3 h. First seeds germinated in an incubator, and on the 3rd day of growth were transplanted into plastic pots (d = 14 cm). Plants were grown on soil substrate, whose humidity was maintained at 60 % of full moisture capacity – the optimal water supply. The model of drought was created by the simultaneous cessation of irrigation (30 % of soil moisture capacity) for 12 days. Upon termination of the drought, soil moisture in the pots was adjusted to 60 % of its full capacity. The control plants were grown from the seeds not treated with salicylic acid under conditions of optimal water supply (60 %). Study samples were taken from the shoots of wheat on the 7th, 9th and 12th days of the drought period and on the first day after the resumption of irrigation (14 days). The concentrations of TBA-active products, chlorophyll, carotenoids, glucose, sucrose and chlorophyllase activity were determined.

Results. The influence of salicylic acid on the content of chlorophyll, carotenoids and TBA-active products along with chlorophyllase activity in shoots of wheat under the drought conditions on the early stages of ontogenesis was determined. It was found that both drought and salicylic acid modify the content of chlorophyll and carotenoids in the shoots of *Triticum aestivum* L., apparently by altering their metabolism. Chlorophyllase plays an important role in the adaptation of photosynthetic apparatus to the drought conditions. Our results suggest that salicylic acid causes an increase in the content of photosynthetic pigments and a decrease in chlorophyllase activity in drought conditions. In addition, salicylate causes the accumulation of glucose and sucrose in the shoot tissues of wheat plants under stressful conditions.

Conclusions. Our research suggests participation of salicylic acid in the plants' adaptation processes under the conditions of moisture deficiency. It was also found that under the effect of salicylic acid the content of TBA-active products decreases in the plant shoots under the conditions of drought. Considering the fact that the content of TBA-active products is an important indicator of lipid peroxidation intensity, the decrease of this index reveals the reduced activity of free radical processes in plant tissues under the effect of salicylic acid. Thus, it can be assumed that SA has a positive effect on plant metabolism under drought conditions.

Keywords: adaptation, drought stress, *Triticum aestivum* L., TBA-active products, photosynthetic pigments, chlorophyllase, carbohydrates

INTRODUCTION

Drought is a major abiotic factor that has a negative influence on the productivity of agricultural crops worldwide (Nxele *et al.*, 2017). Photosynthesis is a crucial factor of plant productivity. Pigments play an important role in the functioning of the photosynthetic apparatus; their content being a very sensitive indicator of plants' physiological condition. Even a short-term drought can cause negative effects on the photosynthetic processes in plants. Adaptation to water stress in plants is a metabolically active process (Moustakas *et al.*, 2022). During the formation of stress response to the action of unfavorable factors the following processes take place in plants: alteration of genome expression, increase in the activity of antioxidant enzymes, accumulation of stress-protective compounds that have osmotic activity, and stress phytohormones production to begin with ethylene and ABA (Ulla *et al.*, 2018). The launching inductor of these metabolic chains is the altering cell environment which takes place under the effect of an adverse factor – an increase in lipid peroxidation (LPO) activity foremost in the biological membranes (Nahar *et al.*, 2022).

Phytohormones are able to reduce the harmful influence of abiotic stress factors on plants. In particular, salicylic acid (SA) is known as a stress-protective compound due to its ability to control directly or mediately the main biochemical pathways in the plant organism (Khan *et al*., 2015). However, the impact of SA on the plant photosynthetic apparatus under the conditions of drought remains insufficiently explored.

MATERIALS AND METHODS

Plant growing conditions. Plants of the Podolyanka variety of wheat (*Triticum aestivum* L.) were the objects of our study. Seeds were pre-soaked in in a solution of SA (50 mM) for 3 h. First seeds germinated in an incubator, and on the 3rd day of growth were transplanted into plastic pots (d = 14 cm). Plants were grown on soil substrate, whose humidity was maintained at 60 % of full moisture capacity – the optimal water supply. The model of drought was created by the simultaneous cessation of irrigation (30 % of soil moisture capacity) for 12 days. Upon termination of the drought, soil moisture in the pots was adjusted to 60 % of its full capacity. The control plants were grown from the seeds not treated with salicylic acid under conditions of optimal water supply (60 %). Study samples were taken from the shoots of wheat on the 7th, 9th and 12th-days of the drought period and on the first day 1 h after the resumption of irrigation (14 days).

The **activity of lipid peroxidation** was determined spectrophotometrically by the content of TBA-active products (Voitsekhivska *et al.*, 2010). The weighted sample of plant material (0.5 g) was cut with scissors and homogenized in the porcelain mortar with the addition of 3 mL of distilled water. Before continuing homogenization, 3 mL of 20% trichloroacetic acid (TCA) was added to the homogenate. Then 2 mL of the obtained homogenate was placed into the test tube with the addition of 2 mL of 0.5% dilution of tiobarbituric acid (TBA) in the TCA. The samples were incubated for 30 min on the boiling water bath, cooled and centrifuged at 3000 rpm for 10 min.

The optical density was measured spectrophotometrically at the wavelength of 532 nm on the spectrophotometer ULAB101 (China). Analytical replication was fivefold. The content of MDA (nmol/g of fresh matter) was calculated using the formula:

$$X = \frac{D \cdot 1000000 \cdot V \cdot A}{H \cdot \varepsilon},$$

where D – the optical density of the dilution; V – the volume of reactive compound, mL; A – the correlation of total volume of the extract to the sample volume; ε – the molar extinction coefficient (155000 l/cm·mol); H – the weighted sample of plant material, g.

The **content of photosynthetic pigments** was analyzed in the acetone extract (Voitsekhivska *et al.*, 2010). To get the extract a sample of leaves weighted to 100 mg was grinded and homogenized in the porcelain mortar with 10 mL of acetone and with the addition of a little amount of calcium carbonate. The obtained extract was filtered and read on the spectrophotometer at wavelengths of 662, 644 and 440.5 nm on the spectrophotometer ULAB101 (China). Analytical replication was fivefold. The concentrations of chlorophyll *a, b* and carotenoids were calculated according to the formulas of Holm-Wettstein:

$$C_{\text{chlorophyll a}} = 9.784 \cdot D_{662} - 0.990 \cdot D_{644};$$
$$C_{\text{chlorophyll } b} = 21.426 \cdot D_{644} - 4.650 \cdot D_{662};$$
$$C_{\text{chlorophyll a+}b} = 5.134 \cdot D_{662} + 20.436 \cdot D_{644};$$
$$C_{carotenoids} = 4.695 \cdot D_{440,5} - 0.268 \cdot (C_{\text{chlorophyll a+}b}).$$

The amounts of pigments were expressed in mg/g of fresh matter and calculated applying the formula:

$$A = \frac{C \cdot V}{H \cdot 1000},$$

where C – the concentration of pigments, mg/L; V – the volume of extract, mL; H – the weighted plant material, g.

In order to determine the **chlorophyllase activity** we used two samples with the weight of 0.5 g. One sample was homogenized with 50 mL of 80% acetone (adding the calcium carbonate). Another sample was grinded with 25 mL of 60% acetone and incubated for 1 hour in the dark for the chlorophyllase impact. In an hour, 25 mL of 100% acetone was added to the second sample to disable the chlorophyllase effect. Each sample was split up into two parts. In one part of the samples, the chlorophyll content was determined by spectrophotometric method and then its concentration was calculated using the equation of Vernon:

$$C_{\text{chlorophyll } a+b} = 6.45 \cdot D_{665} + 17.72 \cdot D_{649}.$$

The remaining samples were transferred to the separatory funnels adding 2.5 mL of 0.02 M NH_4OH, 0.2 mL of saturated NaCl solution and 5 mL of light fracture of petroleum ether. The separatory funnels were shaken vigorously a few times, then the lower (water-acetone) fractures that contained phytol-free pigments were subtracted into separate test tubes. The extraction was performed several times. The obtained extract was spectrophotometrized. The content of chlorophyllide in the extract was calculated by Vernon's formula using 0.69 coefficient. The chlorophyllase activity was calculated as a conversion rate of chlorophyll to chlorophyllide in the 60% acetone during 1-hour incubation (Voitsekhivska *et al.*, 2010).

The **glucose content** in plant tissues was determined by the enzymatic method. The plant material was homogenized with 5% $HClO_4$ solution in a ratio of 1:5. The homogenate was centrifuged and the extract was neutralized with $KHNO_3$. After that, 0.5 mL of 0,3 M Tris-HCl (pH = 7.5), 0.15 mL of 0.1 M $MgSO_4$, 0.3 mL of 0.01 M ATP, 1 mL of supernatant, 0.03 mL of hexokinase and 0.02 mL of glucose-6-phosphate dehydrogenase were added to the spectrophotometric cuvette. The contents of the cuvette were mixed and the optical density was determined at 340 nm (E1) on the spectrophotometer ULAB101 (China). Then, 0.03 mL of NADP+ was added and after 5 minutes (reaction time) the second value of E2 was determined. The glucose content was defined as a difference between E2 and E1 (Buysse & Merckx, 1993).

To **determine the content of sucrose**, it was extracted with distilled water and measured spectrophotometrically using the phenol-sulfuric acid method (Sadasivam & Manickam, 2007). Firtsly, 50 mg of plant material was homogenized with 2 mL of distilled water and subsequently extracted for 12 hours. Then it was centrifuged and sucrose content was determined in the supernatant; 0.1 mL of supernatant was collected and adjusted to 1 mL with distilled water. To the obtained extract was added 1 mL of 5% phenol solution and 5 mL of 96% sulfuric acid, then mixed gently. The optical density was measured at a wavelength of 490 nm on the spectrophotometer ULAB101 (China). The sucrose content was calculated using the calibration curve.

Statistical analysis. All experimental data reported are mean values ± standard deviation (SD). Since the design of the experiment provided for the study of the effects of two factors (SA and drought), data comparison was carried out using two-way ANOVA. The statistical significance ($p < 0.05$) of the results was established by comparing the studied mean values of the sample and control mean values.

RESULTS AND DISCUSSION

The effect of drought and SA on the content of TBA-active products. The investigation of drought influence on plants showed a significant increase of TBA-active products content in the organs of *Triticum aestivum* L. The dynamics of TBA-active products formation is an evidence of their accumulation with the increase in the time of drought exposure. Noteworthy, a higher content of these metabolites was detected in the shoots of the investigated plants than in their roots. (**Fig. 1, 2**). Affected by the exogenous growth regulator – SA, the content of TBA-active products did not change significantly relative to the control under normal conditions. However, under the simultaneous action of moisture deficiency and salicylate, its significant decrease was observed with regard to the sample exposed to the stress factor alone. After resumption of watering on the first day of plants' growth, the content of TBA-active products lowered rapidly.

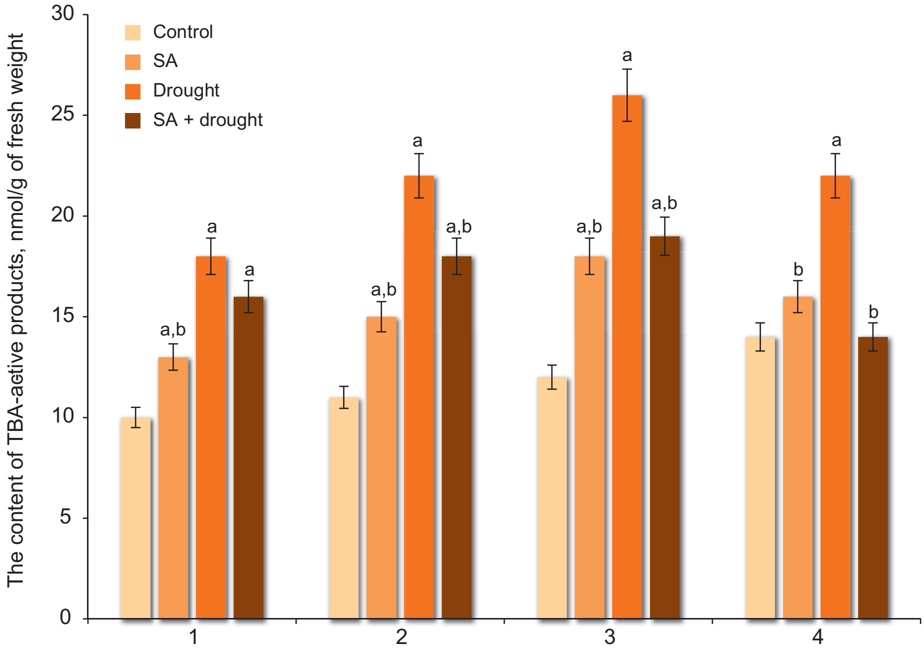

Fig. 1. The influence of salicylic acid (SA) and drought on the content of TBA-active products in the shoots of *Triticum aestivum* L. plants, nmol/g of dry weight: 1–3 – 7th, 9th and 12th days of drought action respectively, 4 – 1st day after watering resumption; a – significant difference compared to control; b – significant difference compared to the sample with drought effect at p≤0.05

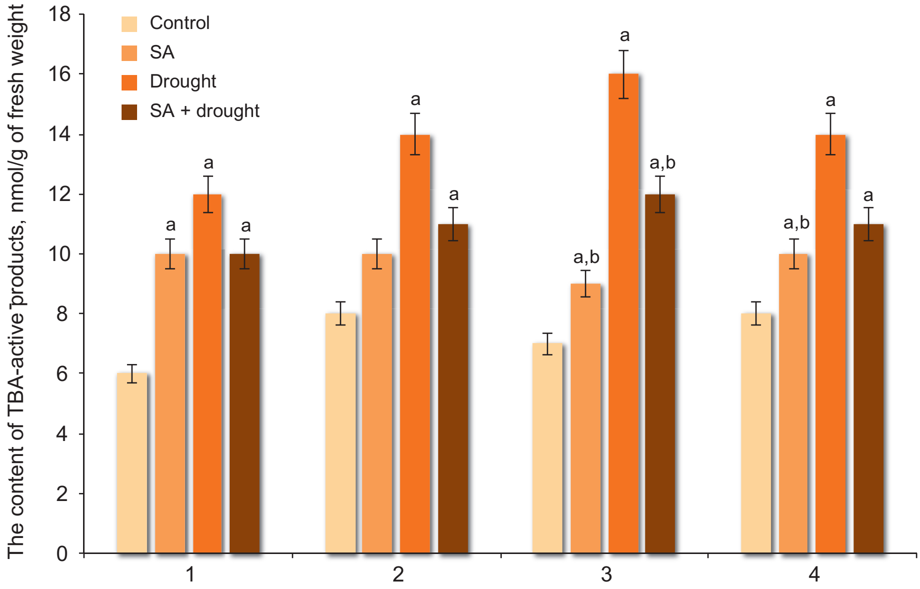

Fig. 2. The influence of salicylic acid (SA) and drought on the content of TBA-active products in the roots of *Triticum aestivum* L. plants, nmol/g of dry weight: 1–3 – 7th, 9th and 12th days of drought action respectively, 4 – 1st day after the resumption of watering. a, b – same as in Fig. 1

Effect of drought and SA on the content of photosynthetic pigments and chlorophyllase activity. At the next stage of our study, we determined the content of photosynthetic pigments as well as the activity of chlorophyllase – the key enzyme of their metabolism in plants under the action of drought (Yang *et al.*, 2021). Table 1 shows the changes in chlorophyll *a* and *b* content, their correlation and carotenoids content in shoots of the *Triticum aestivum* L. plants under the influence of drought and SA. Analysis of these parameters indicates that exposure to drought leads to a decrease in the content of plastid pigments, especially of chlorophyll *a* (Uarrota *et al.*, 2018).

Under the condition of a decreased chlorophyll content, a slight growth in the content of carotenoids was observed under drought action on the 7th day of plant growth. Meanwhile, such reaction was not detected on the 9th and 12th days. SA partially leveled the drought impact on the content of photosynthetic pigments in shoots of *Triticum aestivum* L. at all stages of plants' growth. The most visible influence of SA was noticed on the 9th and 12th days of plants' growth, when under the conditions of water deficiency, the content of carotenoids and chlorophyll decreased rapidly (see **Table**). The resumption of watering did not cause the growth of photosynthetic pigments on the 1st day, whilst under the effect of SA in the same conditions, their insignificant increase was observed.

The influence of drought and SA on the content of photosynthetic pigments in the shoots of *Triticum aestivum* L. plants, mg/g of dry weight

Sample	Chlorophyll *a*	Chlorophyll *b*	Chl *a*/*b* ratio	Carotenoids
7th day of drought				
Control	1.98 ± 0.03	0.71 ± 0.02	2.79	0.59 ± 0.02
SA	2.11 ±0.08	0.80 ± 0.06	2.64	0.76 ± 0.07[a]
Drought	1.34 ± 0.01[a]	0.65 ± 0.03	2.06	0.68± 0.05[a]
SA + drought	1.76 ± 0.12[ab]	0.89 ± 0.14[ab]	2.07	0.72 ± 0.08[a]
9th day of drought				
Control	2.15 ± 0.06	0.76 ± 0.13	2.83	0.87 ± 0.06
SA	2.24 ± 0.06	0.84 ± 0.05	2.67	0.80 ±0.04
Drought	1.49 ± 0.08[a]	0.69 ± 0.04[a]	2.29	0.69 ± 0.03[a]
SA + drought	1.79 ± 0.15[ab]	0.80 ± 0.07[b]	2.24	0.77 ± 0.03[b]
12th day of drought				
Control	2.40 ± 0.05	0.89 ± 0.10	2.70	0.93 ± 0.06
SA	2.45 ± 0.05	1.03 ± 0.04	2.38	0.86 ± 0.04
Drought	1.32 ± 0.13[a]	0.80 ± 0.01[a]	1.65	0.64 ± 0.03[a]
SA + drought	2.13 ± 0.10[ab]	0.92 ± 0.09[b]	2.32	0.88 ± 0.07[b]
1st day after resumption of watering				
Control	2.76 ± 0.11	1.19 ± 0.06	2.32	0.97 ± 0.10
SA	2.68 ± 0.14	1.14 ± 0.08	2.35	0.94 ± 0.06
Drought	1.25 ± 0.06[a]	0.74 ± 0.03[a]	1.69	0.70 ± 0.04[a]
SA + drought	2.32 ± 0.08[ab]	1.09 ± 0.12[b]	2.13	0.92 ± 0.07[b]

Note: a, b – same as in Figure 1

As it is visible from the data shown in **Figure 3**, the chlorophyllase activity in shoots of the investigated plants was law enough, between 1–2 % of chlorophyll conversion into chlorophyllide, which can be explained by the young age of plants. Simultaneously, the chlorophyllase activity was growing with the increase of drought duration. The high enzyme activity in shoots of the plants under the drought conditions is caused by the significant intensity of chlorophyll catabolism.

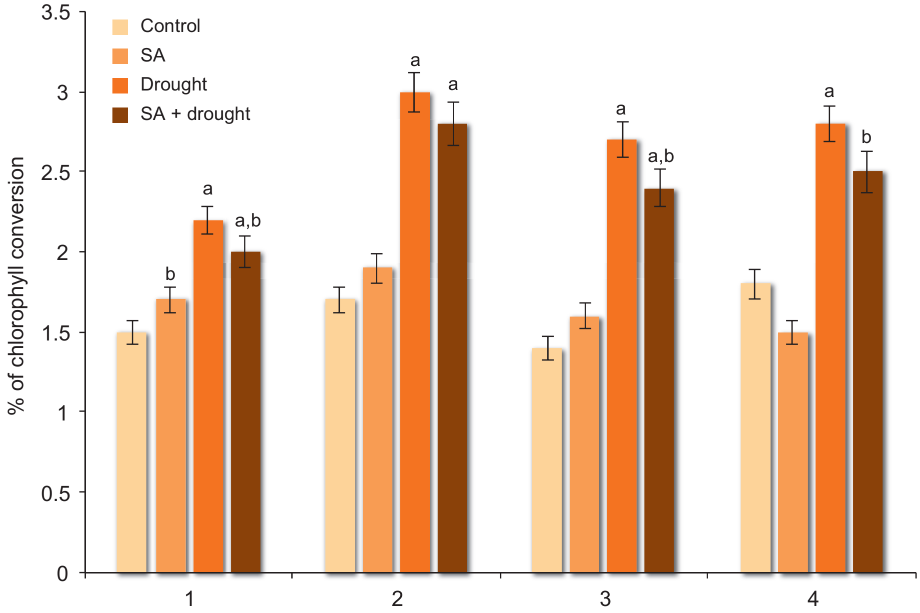

Fig. 3. The chlorophyllase activity under the influence of SA and drought in shoots of *Triticum aestivum* L. plants, % of chlorophyll conversion: 1–3 – 7th, 9th and 12th days of drought action respectively, 4 – 1st day after resumption of watering. a, b – same as in Fig. 1

Comparing the content of chlorophyll *a* and *b* and enzyme activity that catalyzes the first stage of chlorophyll degradation (Yang *et al.*, 2021), the correlation of these indicators is visible. It is obvious, that the increase in chlorophyllase activity induced the decrease in the content of photosynthetic pigments under drought conditions. The SA influence caused a significant decrease in chlorophyllase activity. Consequently, the chlorophyll content affected by salicylate was slightly higher under the stress conditions than under the influence of drought alone. At the same time, it should be noted that this dependency had a non-linear character.

The influence of drought and SC on the content of glucose and sucrose. The preliminary role of glucose in plant metabolism is to serve as a main source of energy that is used in various chemical syntheses (Barnaby *et al.*, 2013). Despite the fact that glucose, like fructose, is widely distributed in plants, they almost never occur in the free state and are involved in metabolism in the form of phosphoric acid ethers. Moreover, these sugars are required for the synthesis of nucleic acids, enzymes, coenzymes, glycosides and polysaccharides. Due to the influence of moisture deficiency, the content of glucose in the aboveground part of the studied plants increased significantly. **Figure 4** illustrates the effect of SA on the glucose content in the shoots of *Triticum aestivum* L. under drought conditions.

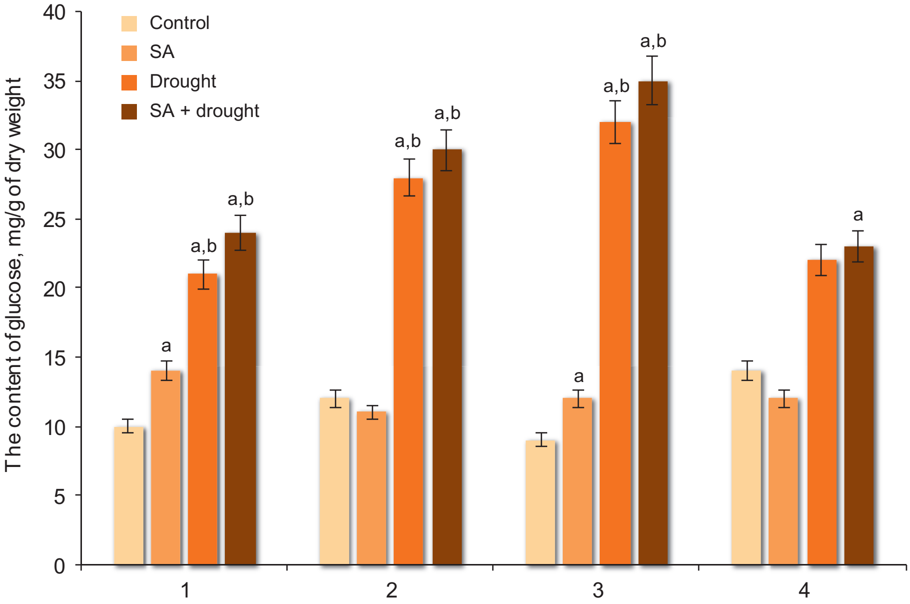

Fig. 4. The influence of salicylic acid (SA) and drought on the content of glucose in the shoots of *Triticum aestivum* L. plants, mg/g of dry weight: 1–3 – 7th, 9th and 12th days of drought action respectively, 4 – 1st day after watering resumption; a, b – same as in Fig. 1

The results of our research showed that under the influence of drought there is a sharp increase in the content of glucose. At the same time, under the combined influence of SA and drought, the amount of this monosaccharide was almost the same as under the action of a stress factor solely.

Considering the fact that under the combined effect of SA and drought, the total soluble carbohydrate content, as well as fructose content, increased significantly, and the glucose content was almost the same as under the influence of the stress factor alone, it can be assumed that the osmotic potential of cell juice under the effect of salicylate increased as a consequence of the increase in the accumulation of fructose or other water-soluble carbohydrates, while glucose did not play an important role in this process.

Apart from fructose and glucose, sucrose is an important soluble metabolite that affects the osmotic potential of plants (Hatanaka & Sugavara, 2010). Therefore, the next step in our work was to determine the concentration of this disaccharide in the tissues of the studied plants. The obtained data showed that the sucrose content in plant tissues increased significantly under drought conditions (**Fig. 5**).

Under the combined effect of stress factor and salicylate, the sucrose content was considerably higher than in plants grown from untreated seeds. After the resumption of watering, the concentration of sucrose in the studied plants sharply decreased and reached the level of control. At the same time, in the absence of stress factor, salicylate did not affect the content of sucrose, as well as the other studied components of carbohydrate metabolism.

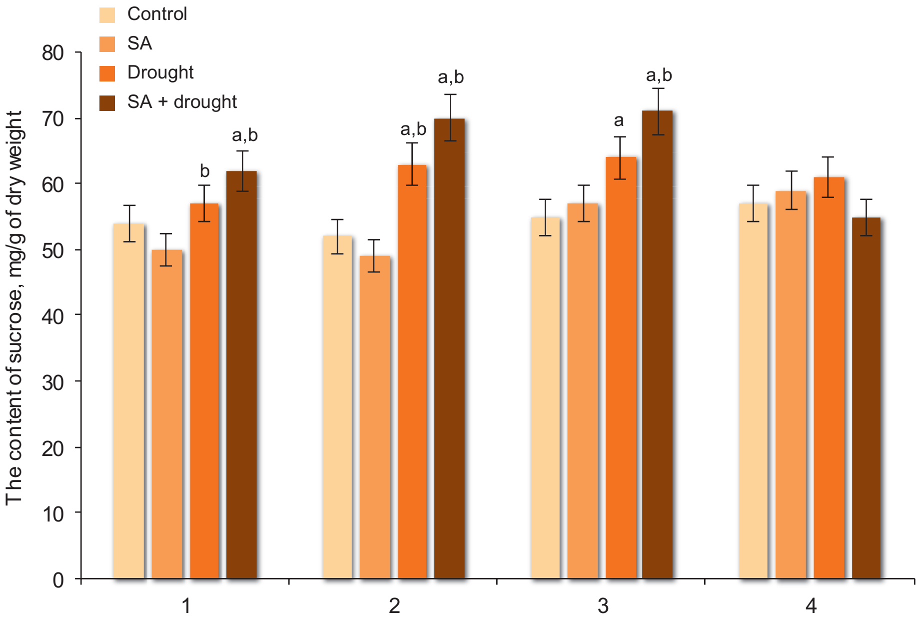

Fig. 5. The influence of salicylic acid (SA) and drought on the content of sucrose in the shoots of *Triticum aestivum* L. plants, mg/g of dry weight: 1 – 3 – 7th, 9th and 12th days of drought action respectively, 4 – 1st day after watering resumption; a, b – same as in Fig. 1

DISCUSSION

The investigation of drought influence on plants showed a significant increase in the content of TBA-active products in the organs of *Triticum aestivum* L. The reason for that is the growth in content of reactive oxygen species (ROS) under stress conditions. ROS perform the function of signalling molecules and launch a range of reactions that are involved in the formation of plant resistance to the stress factor (Gupta *et al.*, 2015).

It is known that ROS can potentially be formed in almost any cellular compartment. However, due to a high oxygen concentration, chloroplasts, that are localized in green parts of a plant, are a special place. Under the effect of unfavourable environmental factors such as drought, hypothermia, HM, salinization the decrease of CO_2-fixation and use of NADPH in the dark phase of photosynthesis take place.

Therefore, the highly active oxygen intermediates are mainly formed in chloroplasts owing to oxygen overproduction and high-energy reactions of electrons transport that are associated with thylakoid membranes. In particular, the production of superoxide anion occurs in both photosystem I (mostly with the participation of ferredoxin) and photosystem II (during the photolysis of water). The occurrence of superoxide radical in chloroplasts may be induced by the main enzyme of CO_2-fixation – ribulose-1,5-bisphosphate carboxylase. As a consequence of ROS overproduction, part of chlorophyll molecules can be damaged by these components. The increased intensity of lipid peroxidation processes under the stress conditions causes changes in the protective antioxidant system, in particular the activity redistribution of antioxidant enzymes and components of low molecular antioxidants pool takes place (Li & Kim, 2022).

The most sensitive to water stress plant processes are growth and photosynthesis. It was proven that the intensity of leaves photosynthesis drops with decreasing air or soil humidity, sometimes even with slight or invisible shifts of water deficiency in leaf. The latest studies indicate that the decrease in photosynthesis intensity under these circumstances is conditioned by the decreased leaf conductance due to stomatal closure. This process is very fast and plays an important role in the resumption of normal functioning of photosynthetic apparatus after water withdrawal. Nevertheless, lasting and severe droughts cause the inhibition of photosynthetic apparatus activity, changes in metabolism, and as a consequence, a change in plant organism structure. The reaction of photosynthetic apparatus to water stress in different plants species is not the same (Wang *et al.*, 2018).

Pigments play the key role in the functioning of photosynthetic apparatus. Photosynthetic pigments, in particular chlorophyll, are very sensitive indicators of plant leaves state. Even a short-term water stress can cause acceleration of chlorophyll degradation, however this effect differs in deferent ecological groups (Guidi *et al.*, 2019).

Research suggests that chlorophyll a/b correlation decreases significantly under these conditions. The reason is a considerable reduction of chlorophyll *a* content as a consequence of several processes. The most probable cause is the slowdown of protochlorophyll formation and the overall speed of chlorophyll synthesis, which is common under the conditions of moisture deficiency. On the other hand, the reduced amounts of pigments can be conditioned by chlorophyllase activation that results in chlorophyll degradation (Yang *et al.*, 2021).

Obviously, the growth of total carotenoids content was caused by their protective function under the stress conditions. These pigments not only absorb and supply additional light energy to the reaction centers of photosystems I and II, but also protect them from the destructive action of light and ROS. Carotenoids are usually less sensitive to the influence of water stress than chlorophyll (Uarrota *et al.*, 2018). However, the following durable growth of water deficiency (9 and 12 days) caused the reduction of the amounts of these pigments, most probably, as a consequence of their enzymatic or non-enzymatic (photochemical) oxidation (Nisar *et al.*, 2015).

It is known that carotenoids can be situated in different parts of chloroplasts and depending on that perform different functions. In particular, pigments that are localized in thylakoid membranes participate in light reactions of photosynthesis and provide the structure stability of pigment-protein light-harvesting complexes. At the same time, carotenoids found on the outer or inner membranes of plastids are important components of ABA biosynthesis and in fact are its forerunner. Thus, the decrease in the amount of carotenoids in plant tissues under the effect of water deficiency that was observed in our study might be caused by the increased ABA content. At times, the concentration of this phytohormone can be increased under the influence of unfavorable factors of biotic or abiotic nature (Skubacz *et al.*, 2016).

Chlorophyllase activity changes both depending on the plant's ontogenetic stage and under the influence of various exogenous factors, in particular salinization, hyperthermia, moisture deficiency. At the same time, chlorophyllase activity grows with the increase in drought duration. The high enzyme activity in the shoots of plants under drought condition is caused by a significant intensity of chlorophyll catabolism. According to other authors, an increase in chlorophyllase activity occurs mainly due to transformation of the enzyme's form from membrane-bound into weakly bound which is much more active (Jo *et al.*, 2023). However, under the combined influence of drought and

SA, the activity of this enzyme was significantly lower than under the effect of drought alone. SA caused a certain decrease in chlorophyllase activity under stress conditions.

Some authors admit that there is no direct correlation between chlorophyll content and chlorophyllase activity. First of all, not all aspects of this enzyme's functional activity have been explored. Another reason is the ability of different enzymes, except chlorophyllase, to participate in the process of chlorophyll catabolism (Yilmaz & Gökmen, 2016).

Reducing sugars in the assimilation organs of the studied objects are represented by glucose and fructose. They are the main substrates of oxidation for the process of cellular respiration. These monosaccharides play a protective role against the effects of adverse environmental factors, such as droughts, high temperatures, X-rays, pollutants, etc. (Iusypiva & Borisova, 2015). Thus changes in the content and correlation of different forms of carbohydrates are important indicators of changes in plant metabolism under the conditions of adaptation to drought. Another important function of mono- and disaccharides that are accumulated under the stress conditions is their effect on the protein-lipid components of cells that undergo dehydration under the conditions of water deficiency (Kobyletska, 2020).

One of the mechanisms of plant adaptation to conditions of water deficiency is the regulation of osmotic pressure by increasing the number of soluble compounds capable of maintaining the osmotic balance between the cytoplasm and vacuoles. Therefore, the ability to accumulate osmotically active substances, such as proline, sucrose, monosaccharides, raffinose, or stachyose indirectly indicates the formation of plant resistance to stress.

Sucrose is the most common oligosaccharide, which can be accumulated in large quantities in the vacuoles of plant cells. In addition to being the main transport carbohydrate of plants, it plays a special role in shaping the plants' resistance to stress factors. Under these conditions, the function of sucrose is not limited to osmoprotective properties. It is known that sucrose can replace water in formation of the phospholipid structure under conditions of moisture deficiency. In particular, in the resistant moss *Syntrichia ruralis* (Hedw.) F. Weber & D. Mohr, the content of sucrose, which is an important component of the biological „glass" formed by drying due to vitrification, can reach 10% of dry weight of plants (Hatanaka & Sugavara, 2010). Oligosaccharides, in particular sucrose, are known to have the ability to stabilize phospholipid bilayers thanks to the hydrogen bonds to polar groups. Thuswise, the distance between the phospholipid bilayers of the membranes is maintained and damage to the phase transitions can be minimized.

The accumulation of soluble sugars, in particular glucose and fructose, in the assimilation part of the shoots of the studied plants is caused by their high metabolic and transport activity, which affects their growth and development. Besides, they are osmotically active compounds that play an important role in the cell's structural and functional stability under conditions of water deficit.

An important element of plant adaptation to drought is the alteration in carbohydrate metabolism that is directly connected to the process of photosynthesis and transport of assimilates. As it can be seen from the results of our research, this process is very sensitive to changes in water status. The increased concentration of soluble carbohydrates at the initial stages of drought is one of the rounds of plants' physiological response and plays an essential role in the osmotic adaptation. Glucose is an important metabolite of plants as it is the main precursor in the biosynthetic reactions of carotenoids, ascorbate and amino acids which are required for the glutathione formation (Barnaby *et al.*, 2013). All these compounds are important components of the stress-adaptive response of plants, so the growth of their content under the influence of SA can be considered a positive effect.

It is known, that under the conditions of moisture deficiency, the protective functions in plants are performed by a range of compounds: soluble carbohydrates, free amino acids, antioxidant systems that minimize the negative effects of dehydration, and proteins, in particular LEA-proteins (late embryogenesis abundant proteins), which are formed and accumulated in plant cells as a response to moisture loss by the plant organism due to the action of water, osmotic and low temperature stresses (Magwanga *et al.*, 2018).

The accumulation of carbohydrates might be caused by starch catabolism. This might be one of the reasons for glucose and fructose accumulation in the tissues of the studied plants under the conditions of moisture deficiency, which was determined in our studies. The increase in sugars content contributes to membrane and protein stabilization, maintenance of cell water status. Stress-activated accumulation of amino acids such as glycine, serine and glutamate regulates and integrates metabolism in photosynthetic tissues. The vast majority of these osmolytes are localized in chloroplasts and cytoplasmic compartments of the plant cell and occupy about 20% of its volume. The natural concentration of osmolytes reaches more than 200 mM; such concentrations support cell turgor and create a gradient of water potential for water absorption in drought conditions (Anesheh *et al.*, 2012).

Therefore, an increase in the content of soluble carbohydrates, as well as glucose and sucrose in plant shoots at the prior treatment with SA in drought conditions should be considered as a manifestation of adaptive reactions and the basis for the formation of plant resistance to stress factor.

Our research results showed that under the influence of moisture deficiency, there was an increase in the content of soluble carbohydrates, as well as glucose and sucrose in *Triticum aestivum* L. (C3-plants), which was enhanced by salicylate.

Conclusion. Drought and SA affect the content of chlorophyll and carotenoids in the shoots of *Triticum aestivum* L., apparently by changing their metabolism. Chlorophyllase plays an important role in the process of adaptation of photosynthetic apparatus to the conditions of drought. Participating in the plants' adaptive processes under the conditions of moisture deficiency, SA causes an increase in photosynthetic pigments content and a decrease in enzyme activity. Under the influence of SA combined with moisture deficiency, an increase in the content of soluble carbohydrates glucose and sucrose in the shoots of wheat plants was observed. The reason for the increase in the content of soluble carbohydrates is their important function in plants to overcome the osmotic stress under drought conditions. So, the obtained data on the adaptive reactions of wheat plants under drought conditions prove the prospects of using salicylic acid for increasing the drought resistance of plants.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Human Rights: This article does not contain any studies with human subjects performed by any of the authors.

Animal Studies: This article does not include animal studies.

AUTHOR CONTRIBUTIONS

Conceptualization, [M.K.]; methodology, [M.K; O.M.]; validation, [M.K.]; formal analysis, [M.K; O.V.].; investigation, [M.K; O.M.]; resources, [M.K.]; data curation, [M.S.K.]; writing – original draft preparation, [M.S.K; O.I.V.]; writing – review and editing, [M.K; O.V; O.M]; visualization, [M.S.K; O.M.]; supervision, [M.K.]; project administration, [M.K.].

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REFERENCES

Anesheh, H. P., Emam, Y., Ashraf, M., & Foolad, M. R. (2012). Exogenous application of salicyclic acid and chlomequat chloride alleviate negative effects of drought stress in wheat. *Advanced Studies in Biology*, 4(11), 501–520.
Google Scholar

Barnaby, J. Y., Kim, M., Bauchan, G., Bunce, J., Reddy, V., & Sicher, R. C. (2013). Drought responses of foliar metabolites in three maize hybrids differing in water stress tolerance. *PLoS One*, 8(10), e77145. doi:10.1371/journal.pone.0077145
Crossref ● PubMed ● PMC ● Google Scholar

Buysse, J., & Merckx, R. (1993). An improved colorimetric method to quantify sugar content of plant tissue. *Journal of Experimental Botany*, 44(10), 1627–1629. doi:10.1093/jxb/44.10.1627
Crossref ● Google Scholar

Guidi, L., Lo Piccolo, E., & Landi, M. (2019). Chlorophyll fluorescence, photoinhibition and abiotic stress: does it make any difference the fact to be a C3 or C4 species? *Frontiers in Plant Science*, 10, 174. doi:10.3389/fpls.2019.00174
Crossref ● PubMed ● PMC ● Google Scholar

Gupta, D. K., Palma, J. M., & Corpas, F. J. (Eds.). (2015). *Reactive oxygen species and oxidative damage in plants under stress*. Springer International Publishing. doi:10.1007/978-3-319-20421-5
Crossref ● Google Scholar

Hatanaka, R., & Sugawara, Y. (2010). Development of desiccation tolerance and vitrification by preculture treatment in suspension-cultured cells of the liverwort *Marchantia polymorpha. Planta*, 231(4), 965–976. doi: 10.1007/s00425-010-1101-5
Crossref ● PubMed ● Google Scholar

Iusypiva, T., & Borisova, O. (2015). Man-caused impact on soluble carbohydrate form concentration dynamics in *Acer* L. representatives leaves. *Visnyk of the Lviv University. Series Biology*, 69, 174–182.
Google Scholar

Jo, M., Knapp, M., Boggs, D. G., Brimberry, M., Donnan, P. H., & Bridwell-Rabb, J. (2023). A structure-function analysis of chlorophyllase reveals a mechanism for activity regulation dependent on disulfide bonds. *Journal of Biological Chemistry*, 299(3), 102958. doi:10.1016/j.jbc.2023.102958
Crossref ● PubMed ● PMC ● Google Scholar

Khan, M. I. R., Fatma, M., Per, T. S., Anjum, N. A., & Khan, N. A. (2015). Salicylic acid-induced abiotic stress tolerance and underlying mechanisms in plants. *Frontiers in Plant Science*, 6. doi:10.3389/fpls.2015.00462
Crossref ● Google Scholar

Kobyletska, M. S. (2020). Fatty acid composition of corn and wheat plant shoots under the action of salicylate in drought conditions. *Studia Biologica*, 14(3), 91–104. doi.org/10.30970/sbi.1403.629
Crossref ● Google Scholar

Li, M., & Kim, C. (2022). Chloroplast ROS and stress signaling. *Plant Communications*, 3(1), 100264. doi:10.1016/j.xplc.2021.100264
Crossref ● PubMed ● PMC ● Google Scholar

Magwanga, R. O., Lu, P., Kirungu, J. N., Lu, H., Wang, X., Cai, X., Zhou, Z., Zhang, Z., Salih,ìH., Wang, K., & Liu, F. (2018). Characterization of the late embryogenesis abundant (LEA) proteins family and their role in drought stress tolerance in upland cotton. *BMC Genetics*, 19(1), 6. doi:10.1186/s12863-017-0596-1
Crossref ● PubMed ● PMC ● Google Scholar

Moustakas, M., Sperdouli, I., & Moustaka, J. (2022). Early drought stress warning in plants: color pictures of photosystem II photochemistry. *Climate*, 10(11), 179. doi:10.3390/cli10110179
Crossref ● Google Scholar

Nahar, K., Rhaman, M. S., Parvin, K., Bardhan, K., Marques, D. N., García-Caparrós, P., & Hasanuzzaman, M. (2022). Arsenic-induced oxidative stress and antioxidant defense in plants. *Stresses*, 2(2), 179–209. doi:10.3390/stresses2020013
Crossref ● Google Scholar

Nisar, N., Li, L., Lu, S., Khin, N. C., & Pogson, B. J. (2015). Carotenoid metabolism in plants. *Molecular Plant*, 8(1), 68–82. doi:10.1016/j.molp.2014.12.007
Crossref ● PubMed ● Google Scholar

Nxele, X., Klein, A., & Ndimba, B. K. (2017). Drought and salinity stress alter ROS accumulation, water retention, and osmolyte content in sorghum plants. *South African Journal of Botany*, 108, 261–266. doi:10.1016/j.sajb.2016.11.003
Crossref ● Google Scholar

Sadasivam, S., & Manickam, A. (2007). *Biochemical methods* (pp. 42–43). New Delhi: New Age International (P) Ltd Publishers.
Google Scholar

Skubacz, A., Daszkowska-Golec, A., & Szarejko, I. (2016). The role and regulation of ABI5 (ABA-Insensitive 5) in plant development, abiotic stress responses and phytohormone crosstalk. *Frontiers in Plant Science*, 7, 1884. doi:10.3389/fpls.2016.01884
Crossref ● PubMed ● PMC ● Google Scholar

Uarrota, V. G., Stefen, D. L. V., Leolato, L. S., Gindri, D. M., & Nerling, D. (2018). Revisiting carotenoids and their role in plant stress responses: from biosynthesis to plant signaling mechanisms during stress. In D. Gupta, J. Palma & F. Corpas, (Eds.), *Antioxidants and antioxidant enzymes in higher plants* (pp. 207–232). Springer, Cham. doi:10.1007/978-3-319-75088-0_10
Crossref ● Google Scholar

Ullah, A., Manghwar, H., Shaban, M., Khan, A. H., Akbar, A., Ali, U., Ali, E., & Fahad, S. (2018). Phytohormones enhanced drought tolerance in plants: a coping strategy. *Environmental Science and Pollution Research*, 25(33), 33103–33118. doi:10.1007/s11356-018-3364-5
Crossref ● PubMed ● Google Scholar

Voitsekhivska, O. V., Kapustian, A. V., Kosyk, O. I., Musiienko, M. M., Olkhovych, O. P., Parshykova, T. V. (Ed.). (2010). *Fiziolohiya roslyn. Praktychnyy kurs* [*Plant physiology. Practical course*]. Lutsk: Teren.

Wang, Z., Li, G., Sun, H., Ma, L., Guo, Y., Zhao, Z., Gao, H., & Mei, L. (2018). Effects of drought stress on photosynthesis and photosynthetic electron transport chain in young apple tree leaves. *Biology Open*, 7(11), bio035279. doi:10.1242/bio.035279
Crossref ● PubMed ● PMC ● Google Scholar

Yang, X., Lu, M., Wang, Y., Wang, Y., Liu, Z., & Chen, S. (2021). Response mechanism of plants to drought stress. *Horticulturae*, 7(3), 50. doi:10.3390/horticulturae7030050
Crossref ● Google Scholar

Yilmaz, C., & Gökmen, V. (2016). Chlorophyll. In B. Caballero, P. M. Finglas, F. Toldrá (Eds.), *Encyclopedia of food and health* (pp. 37–41). Academic Press. doi.org/10.1016/B978-0-12-384947-2.00147-1
Crossref ● Google Scholar

АДАПТИВНІ РЕАКЦІЇ РОСЛИН ПШЕНИЦІ ДО УМОВ ПОСУХИ ЗА ВПЛИВУ САЛІЦИЛОВОЇ КИСЛОТИ

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Обґрунтування. Посуха є одним із найважливіших факторів впливу на ріст і продуктивність рослин. Досліджено перспективи використання стреспротекторної сполуки – саліцилової кислоти (СК) – з метою підвищення адаптивного потенціалу рослин *Triticum aestivum* L. за умов посухи. Встановлено вплив саліцилової кислоти на фотосинтетичний апарат рослин пшениці в умовах посухи з подальшим поступовим відновленням поливу.

Матеріали та методи. Об'єктами дослідження були рослини пшениці (*Triticum aestivum* L.) сорту Подолянка. Попередньо насіння замочували у розчині саліцилової кислоти (50 мМ) на 3 год. Насіння спочатку пророщували в інкубаторі, а на 3-й день пересаджували у пластикові горщики (d = 14 см). Рослини вирощували на ґрунтовому субстраті, вологість якого підтримували на рівні 60 % від повної вологоємності – оптимальне водопостачання. Модель посухи створювали одночасним припиненням зрошення (30 % вологоємності ґрунту) упродовж 12 днів. Опісля вологість ґрунту в горщиках доводили до 60 % від повної вологоємності ґрунту. Контрольні рослини вирощували з насіння, не обробленого саліциловою кислотою, за умов оптимального водопостачання (60 %). Для досліджень відбирали зразки із проростків пшениці на 7, 9 і 12-ту добу посухи та в першу добу після відновлення поливу (14-та доба). Визначали концентрацію ТБК-активних продуктів, хлорофілу, каротиноїдів, глюкози, сахарози й активність хлорофілази.

Результати. Встановлено вплив саліцилової кислоти на вміст хлорофілу, каротиноїдів і ТБК-активних продуктів, а також на активність хлорофілази у проростках пшениці за умов посухи на ранніх етапах онтогенезу. Виявлено, що посуха й саліцилова кислота змінюють вміст хлорофілу і каротиноїдів у пагонах *Triticum aestivum* L., очевидно, впливаючи на їхній метаболізм. Важливу роль в адаптації фотосинтетичного апарату до умов посухи відіграє хлорофілаза. Наші дослідження доводять, що саліцилова кислота зумовлює збільшення вмісту фотосинтетичних пігментів і зниження активності хлорофілази за умов посухи. Саліцилат також спричинював нагромадження глюкози та сахарози у тканинах пагонів рослин пшениці за стресових умов.

Висновки. Наші дослідження свідчать про участь саліцилової кислоти в адаптаційних процесах рослин за умов дефіциту вологи. Також встановлено, що під дією саліцилової кислоти в пагонах рослин за умов посухи відбувається зниження вмісту ТБК-активних продуктів. З огляду на те, що вміст ТБК-активних продуктів є важливим показником інтенсивності перекисного окиснення ліпідів, зниження цього показника свідчить про зниження активності вільнорадикальних процесів у рослинних тканинах під дією саліцилової кислоти. Можна припустити, що СК позитивно впливає на метаболізм рослин за умов посухи.

Ключові слова: адаптація, посуха, *Triticum aestivum* L., ТБК-активні продукти, фотосинтетичні пігменти, хлорофілаза, вуглеводи

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