

## GAMMA-AMINOBTYRIC ACID MODULATES ANTIOXIDANT AND OSMOPROTECTIVE SYSTEMS IN SEEDLINGS OF *TRITICUM AESTIVUM* CULTIVARS DIFFERING IN DROUGHT TOLERANCE

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*The stress-protective effects of plant neurotransmitters, including gamma-aminobutyric acid (GABA) have been intensively examined in recent years. However, studies on the GABA influence on stress protective systems in bread wheat cultivars with different drought adaptation strategies are still lacking. The aim of this work was to estimate the GABA effect on the state of antioxidant and osmoprotective systems in etiolated seedlings of two wheat cultivars differing significantly in drought tolerance, namely Doskonala (non-drought-resistant) and Tobak (drought-resistant) under model drought induced by PEG 6000. Two-day-old seedlings were transferred to 15% PEG 6000 and incubated for two days in the absence or presence of GABA. Treatment with 0.1 and 0.5 mM GABA significantly reduced the growth-inhibitory effect of PEG 6000 on the roots and shoots of both cultivars, but to a greater extent on those of non-resistant Doskonala. It was shown that GABA treatment reduced drought-induced accumulation of H<sub>2</sub>O<sub>2</sub> and MDA, stabilized SOD and GPX activity, the level of sugars, anthocyanins and flavonoids in seedlings of both cultivars. Meanwhile GABA treatment enhanced the stress-induced increase in proline content in the Doskonala cultivar, but decreased it in the Tobak, completely prevented stress induced decrease in anthocyanins and flavonoids level in the Tobak and only partially in Doskonala cultivar. Thus, the stabilization of the stress-protective systems functioning in the wheat cultivars and variety-dependent differences in response to GABA were revealed.*

**Key words:** *Triticum aestivum*, gamma-aminobutyric acid, drought resistance, oxidative stress, antioxidant system, proline, anthocyanins.

**D**rought is a major factor limiting the yield of one of the major food crops, wheat [1]. Increasing drought tolerance in wheat can be achieved through genetic improvement based on the search for quantitative trait loci (QTLs) closely associated with this trait [2], genetic manipulation (particularly by transforming genes for enzymes and transcription factors that provide important defense responses [3, 4], and exogenous influences that activate adaptive responses. The spectrum of physiologically active substances involved in the regulation of plant adaptive responses is constantly expanding. At present, the possibilities of using so-called “stress” plant hormones that activate adaptive and repara-

tive processes, such as salicylic and jasmonic acids, brassinosteroids, cytokinins, to increase the resistance of cultivated plants, including cereal grains, are being actively studied [5-7]. In addition, there is a growing body of knowledge on the regulation of adaptive responses by signaling mediators and stress metabolites [8-10]. One compound that combines stress metabolite and bioregulatory properties is  $\gamma$ -aminobutyric acid (GABA), referred to as the “plant neurotransmitters” [8, 11].

The position of GABA in the highly complex network of signal transduction and regulation of plant adaptive responses has not yet been determined. In recent decades, however, experimen-

tal evidence has emerged suggesting a role for the GABA shunt in maintaining respiration and reducing agent balance under stress conditions [12, 13]. GABA signaling effects may be related to its ability to interact specifically with membrane proteins (hypothetical receptors) and thereby influence ion homeostasis, including promoting calcium entry into the cytosol [14, 15]. There is also evidence that GABA enhances the expression of genes encoding the catalytic subunit of NADPH oxidase (RBOH) [16].

Protective effects of GABA on plants of different taxonomic groups have been found under the action of stressors of different nature: hypoxia, drought, salinity, low and high temperatures, heavy metals, and a number of biotic stresses [17, 18]. Application of exogenous GABA usually increases its endogenous content and induces a variety of changes at the molecular, biochemical, and organismal levels. A review by Shelp et al. [18] provides several examples suggesting that elevated endogenous GABA may enhance stressor-induced increases in antioxidant enzyme activity, low-molecular-weight antioxidants, and accumulation of osmolytes, including proline. All these effects are important for the development of drought tolerance in plants [19]. Another pathway for the GABA effect on drought resistance may be due to its involvement in the regulation of stomata [20].

The protective effects of exogenous GABA have been shown in white clover (*Trifolium repens*) [21], creeping bentgrass *Agrostis stolonifera* [22], and bean (*Phaseolus vulgaris*) [23]. Despite the importance and widespread use of bread wheat (*Triticum aestivum*), the effect of GABA on its drought tolerance is poorly understood. Farooq et al. [24] study conducted on wheat plants grown in soil culture in greenhouse showed that GABA treatment promoted membrane stability, increased their productivity index, chlorophyll content, and the amount of osmolytes proline and glycine betaine. Zhao et al. [25] showed that GABA treatment of 2-6-day-old wheat seedlings under conditions of PEG-induced osmotic stress reduced growth inhibition, decreased the content of lipid peroxidation (LPO) products, increased peroxidase activity, and promoted an increase in phenolic antioxidants.

At the same time, there are no works that have comprehensively studied the effect of GABA on the functioning of antioxidant and osmoprotective systems in bread wheat under drought. Meanwhile,

varietal specificities of GABA action have been investigated in single studies and are only concerned with integral physiological parameters such as membrane stability, water use efficiency, and productivity [24]. Related to the above, our work aimed to study the effect of GABA on the state of antioxidant and osmoprotective systems under model drought conditions in two wheat cultivars differing significantly in drought tolerance.

## Material and Methods

**Plant materials and treatments.** For the research we used etiolated seedlings of two cultivars of *Triticum aestivum* (L.): Doskonala (originator: Yuriyev Plant Production Institute of NAAS of Ukraine) and Tobak (originator: Saaten-Union GmbH, Isernhagen HB, Germany) of 2022 year reproduction. The Doskonala cultivar is mainly intended for cultivation in the Forest-steppe and has a low level of tolerance to drought [26]. The cultivar Tobak is intended for cultivation in Central Europe, but is capable of maintaining productivity under drought conditions [27]. A special study on the resistance of 4-day-old etiolated seedlings to the effects of model drought created by PEG 6000 confirmed a significantly higher resistance of the Tobak cultivar compared to Doskonala [26].

Seeds were disinfected in 70% ethanol for 2 min, then transferred to 2% sodium hypochlorite solution for 15 min, followed by rinsing 10 times with sterile distilled water and germination on water in Petri dishes in a thermostat at 24°C without light for 2 days. The seedlings of approximately equal length were then transferred to Petri dishes with two layers of filter paper moistened with 15% PEG 6000 solution. The concentration of PEG that caused 30-50% growth inhibition was chosen based on preliminary experiments (results not shown). The seedlings of control variants were transferred to Petri dishes with filter paper moistened with distilled water. GABA was dissolved in a small volume of ethanol; the solution was diluted with distilled water and added to Petri dishes to obtain working solutions with concentrations of 0.025-2.5 mM. In preliminary experiments, it was found that ethanol concentrations in GABA solutions did not significantly affect the growth and drought tolerance of seedlings and the biochemical parameters studied.

After two-day exposure of seedlings to PEG 6000 and/or GABA solutions, biochemical analyses were performed and shoot and root biomasses

of seedlings were estimated. Inhibition of seedling growth under model drought conditions was calculated using the formula [26]:

$$I = 100 - (E/C \times 100\%),$$

where  $I$  is growth inhibition (%);  $C$  and  $E$  are seedling organ fresh weight values in the control and experimental (drought) variants, respectively.

Estimation of total and relative water content of seedlings. The total water content of the tissues was determined by the gravimetric method with drying at 103°C to constant weight. The relative water content (RWC) was estimated after water saturation of shoots separated from seedlings for 12 h and calculated according to the formula [28]:

$$C = 100\% \times (MF - MD)/(MT - MD),$$

where  $MF$  is the mass prior to saturation,  $MT$  is the mass after saturation, and  $MD$  is the dry mass.

*Evaluation of hydrogen peroxide content.* For determination of  $H_2O_2$  content, seedling shoots were homogenized in cold with 5% trichloroacetic acid (TCA). Samples were centrifuged at 8000 g for 10 min at 2–4°C on an MPW 350R centrifuge (MPW MedInstruments, Poland). The concentration of  $H_2O_2$  in the supernatant was measured by the ferrothiocyanate method [29] with slight modifications. For this, 0.5 ml 2.5 M ammonium thiocyanate, 0.5 ml 50% TCA, 1.5 ml supernatant, and 0.5 ml 10 mM iron(II) ammonium sulfate were added to tubes. After mixing, the samples were transferred to cuvettes and the absorbance at 480 nm was determined.

*LPO products content evaluation.* The level of products reacting with 2-thiobarbituric acid (mainly malondialdehyde – MDA) was used to assess the rate of lipid peroxidation (LPO) in seedling shoots [30].

*Measurement of antioxidant enzymes' activity.* The activity of antioxidant enzymes – superoxide dismutase (SOD) (EC 1.15.1.1), catalase (CAT) (EC 1.11.1.6), and guaiacol peroxidase (GPX) (EC 1.11.1.7) was measured according to previously described protocols [30]. After homogenization of seedling samples in cold 0.15 M K, Na-phosphate buffer (pH 7.6) with the addition of EDTA (0.1 mM) and dithiothreitol (1 mM), the homogenate was centrifuged for 15 min in a centrifuge MPW 350R (MedInstruments, Poland) at 8000 g at 2–4°C to prepare the supernatant, which was then assayed. The activity of SOD was determined at pH 7.6 by a method based on the ability of the enzyme to compete with nitroblue tetrazolium for superoxide anions produced by the aerobic interaction between NADH (reduced

nicotinamide adenine dinucleotide) and phenazine methosulfate. CAT was evaluated by the amount of  $H_2O_2$  decomposed per unit time. GPX activity was estimated using guaiacol as the hydrogen donor and  $H_2O_2$  as the substrate.

*Analysis of osmolytes content.* Proline content of seedlings was determined using ninhydrin reagent according to the protocol described by Bates et al. (1970) with slight modifications [31]. The total sugar content of the plant material was determined by the Morris-Roe method based on the anthrone reagent [32] in our modification described previously [31].

*Determination of phenols and flavonoids content.* To determine total phenolics and flavonoids, seedlings were homogenized in 80% ethanol, extracted for 20 min at room temperature, and centrifuged at 8000 g for 15 min. For the evaluation of phenolic compounds, 0.5 ml of the supernatant, 8 ml of distilled water and 0.5 ml of Folin reagent were added to the reaction tubes, stirred and, after 3 min, 1 ml of 10% sodium carbonate was added. After 1 h, the reaction mixture absorbance was measured at 725 nm [33]. The content of phenolic compounds was expressed as gallic acid mg-equiv/g dry weight.

Prior to the determination of the content of anthocyanins and flavonoids that absorb in UV-B, the supernatant was acidified with HCl to a final concentration of 1%. The absorbance was determined at 530 and 300 nm [34]. Results are expressed as absorbance per dry weight of plant material.

*Replication of experiments and statistical processing.* Statistical processing of results was performed using analysis of variance (ANOVA) and Fisher's least significant difference (LSD) criterion. Figures show mean values from 3–4 biological replicates and their standard errors. Different letters indicate values with differences significant at  $P \leq 0.05$ .

## Results

*Effect of model drought and GABA treatment on seedling biomass accumulation.* Two-day exposure of seedlings to 15% PEG 6000 solution resulted in root and shoot growth inhibition of more than 50% in the non-tolerant cultivar Doskonala (Fig. 1). In the more tolerant cultivar, Tobak, root growth inhibition was 33% and shoot growth inhibition was approximately 43%.

GABA treatment attenuated the growth inhibitory effect of model drought. The stress-protective effect on root growth was significant at  $P \leq 0.05$  at concentrations of 0.1 and 0.5 mM, the range of con-

centrations that enhanced shoot growth was wider, from 0.1 to 2.5 mM (Fig. 1). In general, the protective effect of GABA was more prominent on shoot growth. Varietal differences were also observed: GABA had a more pronounced positive effect on seedling growth of the less resistant Doskonala cultivar.

It should be noted that in the absence of drought, GABA at concentrations of 0.1 and 0.5 mM had no significant effect on root and shoot growth of seedlings of both cultivars (results not shown). In this context, the effect of GABA on the water regime, the state of antioxidant and osmoprotective systems of wheat seedlings was further investigated against the background of drought. GABA was used at a concentration of 0.5 mM, which best alleviated the growth inhibiting effects of drought.

**Relative water content in shoots of wheat seedlings.** Model drought caused a decrease in the relative water content in the shoots of wheat seedlings of both cultivars, but this effect was more pronounced in the non-resistant Doskonala (Fig. 2). GABA treatment restored the relative water content of seedlings incubated on PEG 6000 solution to near control levels in both cultivars.

**Hydrogen peroxide content in wheat seedlings.** Exposure to model drought caused  $H_2O_2$  levels to increase 1.5-fold in shoots of the non-tolerant cultivar Doskonala (Fig. 3, A). At the same time, the amount of hydrogen peroxide increased by one third in the more resistant cultivar Tobak. GABA treatment almost completely eliminated the increase in  $H_2O_2$  content caused by drought.

**Content of TBA-active LPO products in wheat seedlings.** Seedling growth of the non-tolerant culti-

var Doskonala in the presence of the osmotic stress agent PEG 6000 was accompanied by a 1.7-fold increase in the content of TBA-active products (MDA is considered to be the main one) (Fig. 3, B). In contrast, the resistant cultivar Tobak showed only a 30% increase in this index of oxidative stress. GABA treatment reduced the drought-induced accumulation of LPO products in the Doskonala and almost completely eliminated this effect in the Tobak.

**Activity of antioxidant enzymes in shoots of wheat seedlings.** The baseline values of SOD activity in the two studied cultivars differed significantly: Tobak had a significantly higher enzyme activity than Doskonala (Fig. 4, A). Under the influence of osmotic stress, SOD activity decreased in both cultivars. GABA treatment prevented the stress-induced decrease in this enzyme activity.

The baseline catalase activity was higher in the Doskonala cultivar (Fig. 4, B). However, under osmotic stress conditions, it decreased significantly in this cultivar. In the drought-tolerant Tobak cultivar, the enzyme activity under stress was maintained at a level close to the control variant. GABA treatment had no significant effect on catalase activity under drought stress in either cultivar.

GPX activity in response to model drought stress was markedly decreased in the Doskonala cultivar, while this effect was less significant in the Tobak (Fig. 4, C). GABA treatment of seedlings of the Doskonala promoted a significant increase in the enzyme activity under drought conditions, while its effect on this indicator in the Tobak was at the level of tendency.

**Osmolyte content in shoots of wheat seedlings.** The initial proline content was higher in the non-

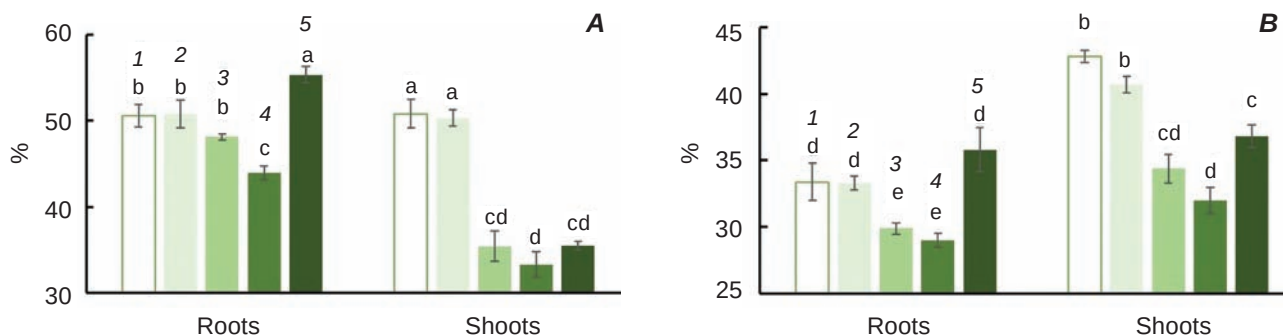


Fig. 1. Modulation of the growth-inhibitory effect (%) of 15% PEG 6000 on wheat seedlings of Doskonala (A) and Tobak (B) cultivars by GABA treatment. 1 – PEG; 2 – PEG + 0.025 mM GABA; 3 – PEG + 0.1 mM GABA; 4 – PEG + 0.5 mM GABA; 5 – PEG + 2.5 mM GABA. Different letters indicate values with significant differences ( $P \leq 0.05$ )



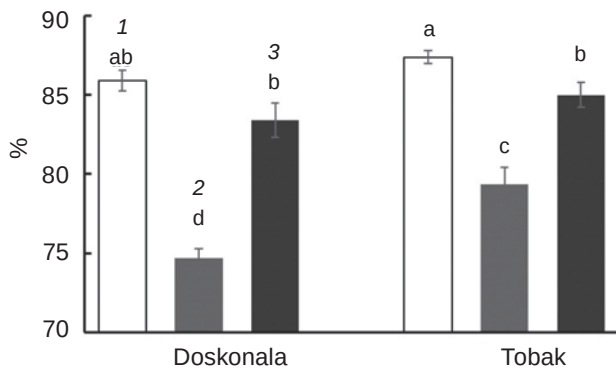


Fig. 2. Relative water content (%) in shoots of wheat seedlings. 1 – Control; 2 – PEG 6000 (15%); 3 – PEG 6000 (15%) + GABA (0.5 mM). Different letters indicate values with significant differences ( $P \leq 0.05$ )

tolerant cultivar Doskonala (Fig. 5, A). Exposure to model drought caused an increase in this index by 57% in the Doskonala and 47% in the Tobak. At the same time, the nature of the effect of GABA on this indicator differed significantly in the two varieties. While in the non-tolerant Doskonala the proline content increased under the influence of GABA, it decreased in the resistant cultivar Tobak.

The baseline content of soluble carbohydrates of the Tobak cultivar was higher than that of the Doskonala (Fig. 5, B). It was slightly decreased in both cultivars under the influence of drought. GABA treatment promoted the preservation of the sugar pool in seedlings of both cultivars under drought conditions.

*Content of phenolics and flavonoid compounds in wheat seedlings.* There was no significant difference between the two cultivars in the total content of phenolic compounds. It was almost unchanged in the

Doskonala and increased in the Tobak upon exposure to osmotic stress (Fig. 6, A). GABA treatment caused an increase in phenolic content in the Doskonala, but had no effect on this index in the Tobak.

The basic content of anthocyanins was almost the same in both cultivars (Fig. 6, B). Under the influence of drought, the non-tolerant cultivar Doskonala showed an almost 2-fold decrease in its content, while the resistant Tobak showed a less significant decrease in anthocyanin content. GABA treatment promoted the preservation of the anthocyanin pool in both varieties. At the same time, in the Tobak, their content under stress in the presence of GABA did not differ from the control values.

The content of colorless flavonoids absorbing in UV-B decreased in both cultivars under stress conditions (Fig. 6, C). GABA treatment had no effect on their amount in the Doskonala, but slightly ameliorated the effect of stress in the Tobak.

## Discussion

Treatment of etiolated wheat seedlings with GABA resulted in an increase in their tolerance to model drought, expressed as a reduction in its growth-inhibitory effect (Fig. 1), as well as a reduction in tissue water deficit (Fig. 2). In the non-tolerant cultivar Doskonala, the effects of GABA were more pronounced.

One reason for the stress-protective effect of GABA may be the inhibition of the development of dehydration-induced oxidative stress. Thus, GABA treatment completely eliminated the drought-induced increase in hydrogen peroxide levels in shoot tissues and reduced the accumulation of LPO products (Fig. 3). It should be noted that the effects of reducing the oxidative stress manifestation under

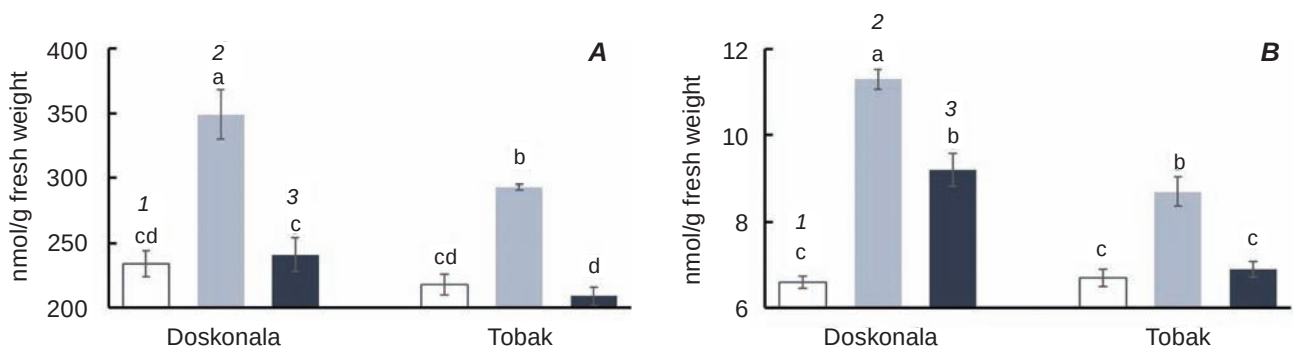


Fig. 3. Content of hydrogen peroxide (A) and TBA-active products (calculated by MDA – B) in shoots of wheat seedlings. 1 – Control; 2 – PEG 6000 (15%); 3 – PEG 6000 (15%) + GABA (0.5 mM). Different letters indicate values with significant differences ( $P \leq 0.05$ )

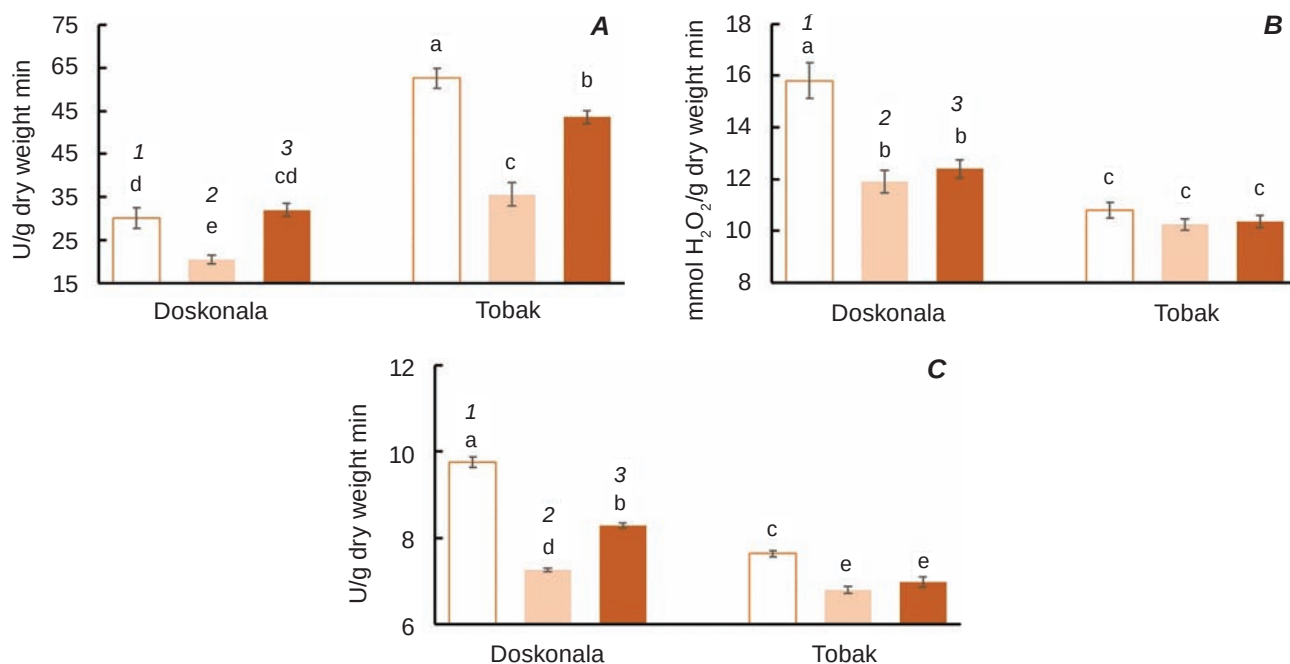


Fig. 4. Activity of SOD (A), catalase (B), and guaiacol peroxidase (C) in shoots of wheat seedlings. 1 – Control; 2 – PEG 6000 (15%); 3 – PEG 6000 (15%) + GABA (0.5 mM). Different letters indicate values with significant differences ( $P \leq 0.05$ )

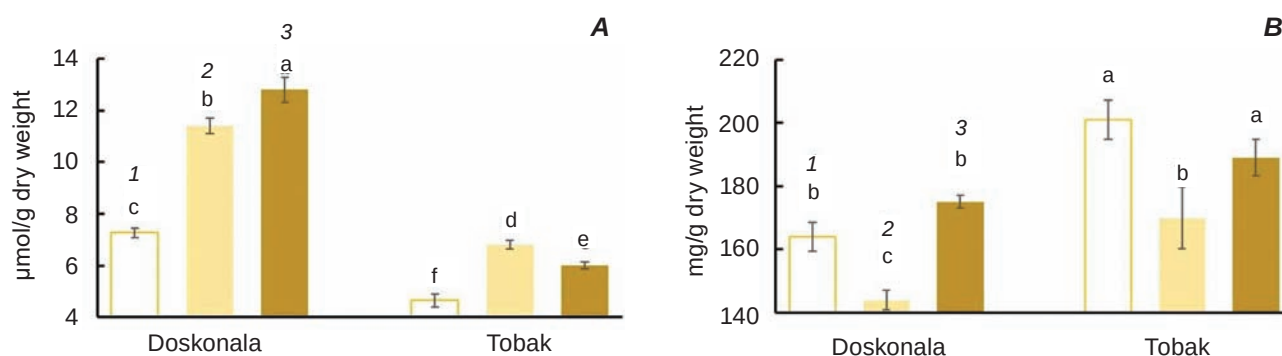


Fig. 5. Proline (A) and sugars (B) content in shoots of wheat seedlings. 1 – Control; 2 – PEG 6000 (15%); 3 – PEG 6000 (15%) + GABA (0.5 mM). Different letters indicate values with significant differences ( $P \leq 0.05$ )

the influence of GABA were registered in plants of different species under adverse effects of different nature. For example, reduction of hydrogen peroxide and MDA by GABA treatment has been shown in mung bean (*Vigna radiata*) plants under salt stress [35], and in maize (*Zea mays*) under flooding stress [36]. In rice, such an effect has been found under high temperature stress [37]. The results obtained by us agree well with the data of Zhao et al. [25], which showed a partial elimination of the effect of increasing MDA content in tissues of wheat seedlings under model drought conditions (PEG 6000).

The mechanisms of oxidative stress mitigation by GABA action are not completely clear, but the evidence in the literature suggests at least several reasons for this effect. The GABA shunt is now being considered as a possible regulator of ROS generation by mitochondria, which may be essential for normal redox signaling [13]. GABA is thought to prevent oxidative stress as a supplier of NADH in mitochondria under conditions of tricarboxylic acid cycle inhibition, respiration impairment, and ROS accumulation [12]. However, on the other hand, GABA treatment has been shown to transiently increase the expression of *CaGR60* gene, which en-

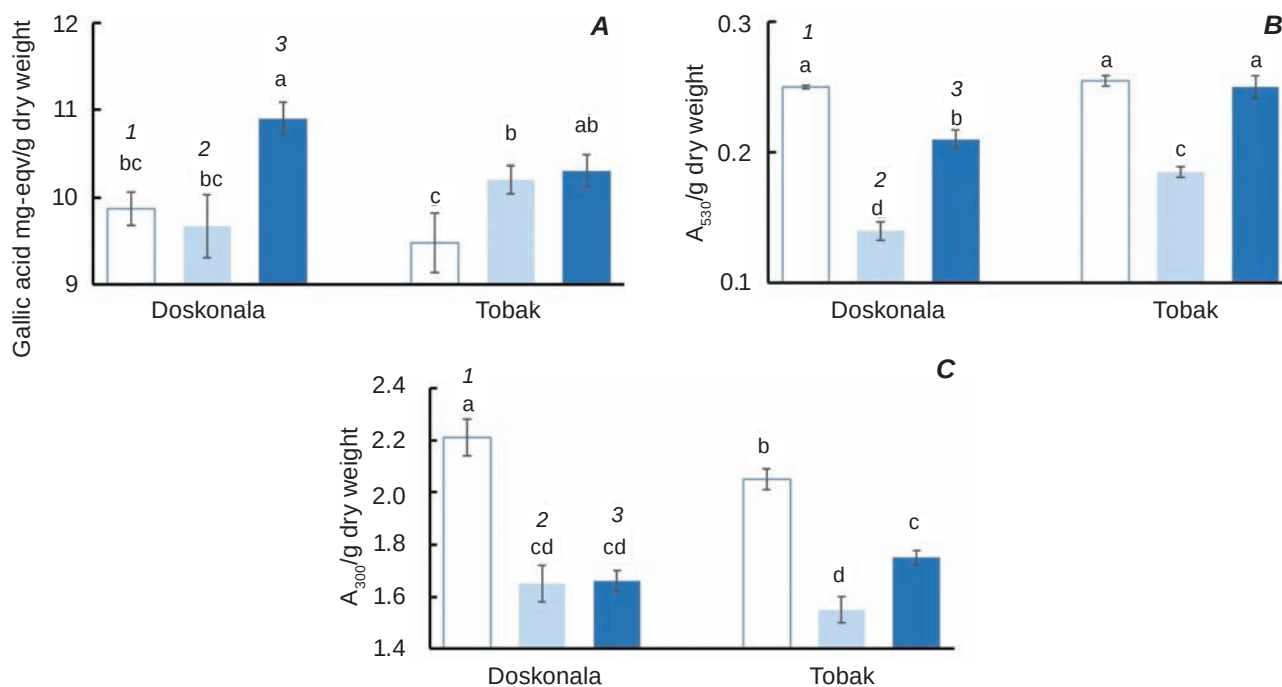


Fig. 6. Content of phenolic compounds (A), anthocyanins (B), and flavonoids absorbing in UV-B (C) in the shoots of wheat seedlings. 1 – Control; 2 – PEG 6000 (15%); 3 – PEG 6000 (15%) + GABA (0.5 mM). Different letters indicate values with significant differences ( $P \leq 0.05$ )

codes the catalytic subunit of NADPH oxidase, in roots of *Caragana intermedia* plants [38]. At the same time, the levels of hydrogen peroxide under salt stress in the leaves and roots of these GABA-treated plants were significantly lower than in untreated plants. The authors hypothesize that GABA, by transiently enhancing gene expression of the catalytic subunit of NADPH oxidase (RBOH), induces the generation of a signal that activates the antioxidant system and enhances resistance. Similar results were obtained on melon plants. Exogenous GABA-induced gene expression of a form of the catalytic subunit of NADPH oxidase (*RBOHD*) and  $H_2O_2$  accumulation under normal conditions, but also attenuated the development of oxidative stress under sodic salinity [16].

Under the conditions of our experiments, GABA treatment of wheat seedlings of both varieties stabilized the activity of the key antioxidant enzyme SOD, which was reduced by PEG 6000-induced tissue dehydration (Fig. 4). Also under the influence of GABA, GPX activity stabilized in the non-tolerant Doskonala cultivar. These effects may be due to the formation of signals inducing antioxidant system with GABA participation. Stabilization of antioxidant enzyme activities by GABA treatment has been shown in *Agrostis stolonifera* plants during tissue

dehydration with PEG 6000 [22]. In wheat under salt stress, an increase in SOD and catalase activity was observed with GABA treatment [39]. *Trifolium repens* under the influence of GABA during salt stress showed enhanced gene expression and increased activity of various forms of SOD, catalase, and ascorbate-glutathione cycle enzymes [40]. However, GABA application had no significant effect on SOD activity in perennial ryegrass grown under drought conditions [41], which may indicate that the effects of GABA depend on plant species characteristics. However, it is possible that the manifestation of GABA protective effects may also depend on the experimental conditions.

GABA also appears to be involved in regulating the accumulation of low-molecular-weight multifunctional compounds with stress-protective effects. Thus, under our experimental conditions, proline accumulation under osmotic stress increased in the non-resistant Doskonala cultivar under GABA influence (Fig. 5, A). At the same time, GABA treatment against drought stress slightly decreased proline content in the resistant cultivar Tobak. An increase in proline content is known to be a plant response to fairly severe stresses [42]. It is possible that under moderate stress, the increase in proline content is activated as an adaptive response in non-tolerant

rant cultivars, whereas under more severe stress, its accumulation becomes more pronounced in resistant plants. For example, in 20-day-old wheat plants, the leaf proline content of the most drought-tolerant cultivar exceeded that of other cultivars only under very severe drought conditions [42].

Apparently, against the background of 15% PEG action, the reaction of proline content increase is more pronounced in less resistant wheat variety and GABA contributes to its strengthening. The effect of GABA on proline content under stress has also been found in other cereal species. For example, treating rice plants with GABA increased proline accumulation under heat stress [37]. On the other hand, plants of the same species showed a decrease in proline content under osmotic stress when treated with GABA [17, 18]. It can be assumed that the effect of GABA on proline content involves various mechanisms that may be related to modulation of energy processes under its influence, formation of redox signals, and utilization of a part of the GABA pool for metabolic processes. Naturally, such an assumption requires special research.

The stabilization of the soluble carbohydrate content in wheat seedlings can be considered as one of the protective effects of GABA under the conditions of our experiments. In both cultivars, GABA treatment eliminated the stress-induced decrease in the pool of sugars (Fig. 5, B). The effects of increasing the sugar content or preventing the decrease of their pool under stress conditions under the action of GABA have been reported on various objects. Thus, the treatment of white clover seeds increased their sugar content during germination under water deficit conditions [43]. When rice seeds germinated under salt stress conditions, the content of sugars in seedlings decreased, and GABA treatment promoted the maintenance of their pool [17]. GABA treatment of corn (*Zea mays*) and mung bean (*Vigna radiata*) plants also increased or stabilized soluble carbohydrate content under saline conditions [35, 44]. Such effects may be due to the positive effects of GABA shunt activation on energy metabolism under stress conditions [13].

Stabilization of the pools of key osmolytes, proline and soluble carbohydrates may be one of the reasons for the increased relative water content in seedlings of both wheat cultivars under stress conditions under the action of GABA (Fig. 2).

GABA appears to be involved in regulating the levels of another important group of compounds with multifunctional stress-protective effects – phe-

nols and flavonoids. Thus, under the influence of GABA, an increase in total phenolic compounds was observed under stress conditions in the non-resistant Doskonala cultivar (Fig. 6, A). At the same time, GABA treatment prevented the reduction of the anthocyanin pool induced by drought stress in both cultivars (Fig. 6, B). Presumably, the depletion of the anthocyanin pool under stress is associated with their antioxidant functions [45], and maintaining a sufficient pool under stress conditions may be an important factor in providing antioxidant defense. Zhao et al. [25] showed a significant increase in the activities of the key enzymes of secondary metabolite synthesis, phenylalanine ammonia lyase, cinnamic acid 4-hydroxylase, and 4-coumarate-CoA ligase, in 4-day-old wheat seedlings when they were treated with GABA, indicating its important role in regulating the synthesis of a variety of phenolic antioxidants.

In our work, we investigated the effect of GABA on the enzymatic antioxidant system, as well as the content of multifunctional defense compounds (proline, sugars, and secondary metabolites) combining antioxidant and osmoprotective properties in wheat seedlings under model drought conditions. Quite naturally, this does not limit the effects of GABA on stress-protective systems in wheat. It is now known that an important component of the GABA protective effect may be modulation of the formation of heat shock proteins with a wide range of molecular masses [46], dehydrins [43], and effects on gene expression of a number of transcription factors that control adaptation to dehydration [43]. In addition, the involvement of GABA in the regulation of stomata has been demonstrated in several objects, which may be very important for the manifestation of drought tolerance [20]. The results obtained in our work indicate that the stress-protective effect of GABA can be manifested at the earliest developmental stages and in the absence of developed photosynthetic and stomatal apparatuses. An important part of the protective effects of GABA in young wheat seedlings may be its involvement in regulating ROS formation and neutralization, as well as maintaining cellular osmotic balance. The mechanisms of these effects of GABA require special studies; in particular, it is of interest to study the involvement of the main components of the signaling network in the realization of the effect of GABA on the state of antioxidant and osmoprotective systems of plants.



Another issue that requires special study and may be of practical importance is the reasons for varietal differences in the response of wheat seedlings to the action of exogenous GABA. In addition to the peculiarities of the functioning of antioxidant and osmoprotective systems [19], varieties with different resistance may also have differences in the endogenous GABA content, dynamics of its change under stress conditions and, accordingly, in response to the action of exogenous GABA. Thus, a positive relationship between endogenous GABA levels and their heat tolerance has been shown in different cultivars of *Agrostis stolonifera* plants [47]. However, for other plant species, including wheat, the relationship between endogenous GABA content and their tolerance to certain abiotic stresses remains largely unexplored.

**Conclusions.** GABA treatment increased the resistance of etiolated wheat seedlings to osmotic stress induced by PEG 6000. The growth-inhibiting effect of drought was more markedly attenuated by GABA treatment in the non-drought-tolerant cultivar Doskonala.

The stress-induced accumulation of hydrogen peroxide and MDA was reduced under the influence of GABA in both the resistant cultivar Tobak and the non-resistant Doskonala (Fig. 7).

In both cultivars, GABA treatment prevented the stress-induced decrease in SOD activity as well as the decrease in sugars and anthocyanins. At the same time, GABA promoted GPX activity, proline, and total phenolic compounds in the non-drought-tolerant cultivar Doskonala and had little effect on these parameters in the resistant cultivar Tobak.

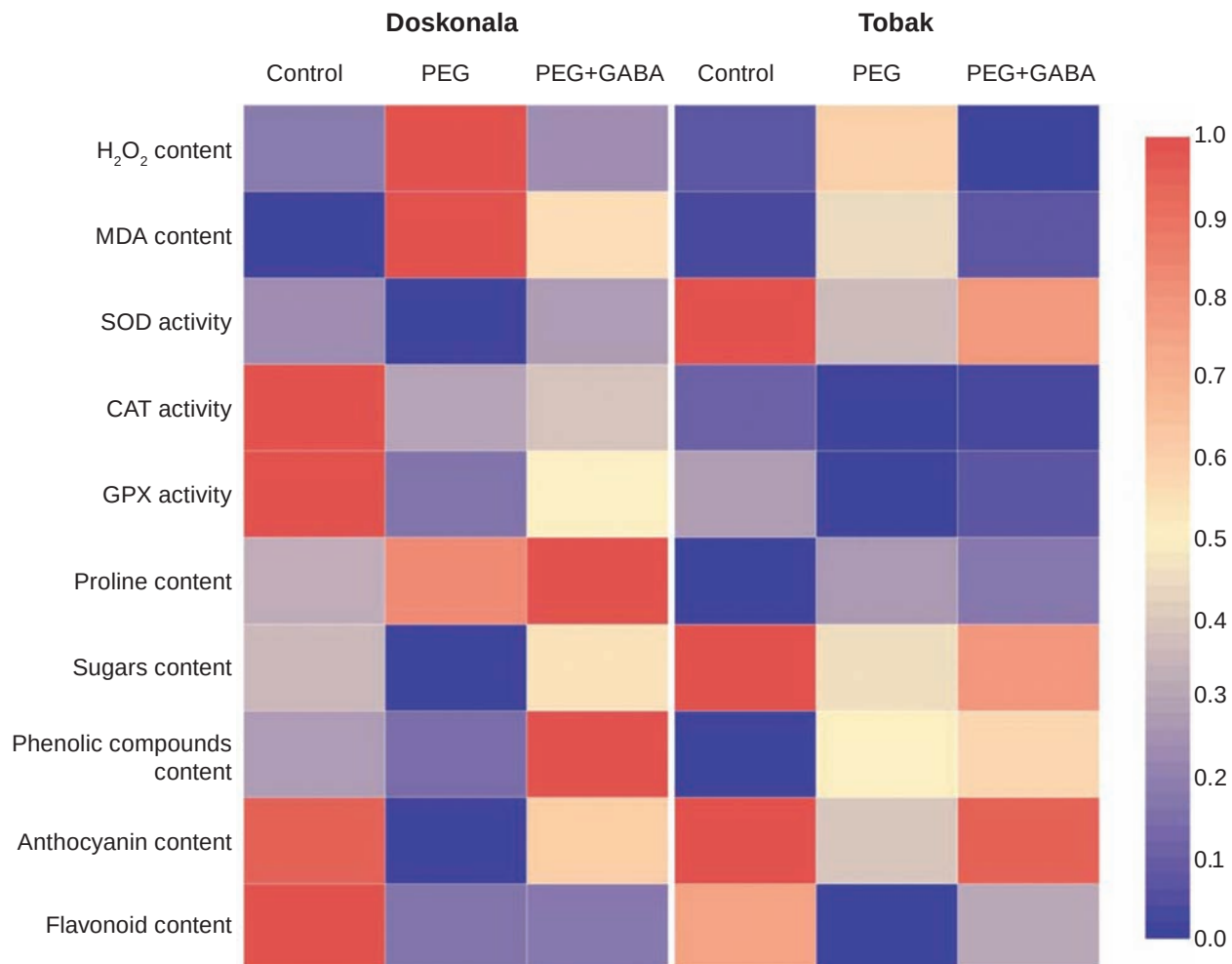


Fig. 7. Heat map of changes in the state parameters of antioxidant and osmoprotective systems of wheat seedlings under the action of model drought (15% PEG) and their modulation by GABA treatment. When constructing the map, each indicator was normalized from 0 to 1

Thus, a differentiated manifestation of the stress-protective effect of GABA depending on the adaptive strategies of specific cultivars was recorded.

**Conflict of interest.** The authors have completed the Unified Conflicts of Interest form at [http://ukrbiochemjournal.org/wp-content/uploads/2018/12/coi\\_disclosure.pdf](http://ukrbiochemjournal.org/wp-content/uploads/2018/12/coi_disclosure.pdf) and declare no conflict of interest.

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### ГАММА-АМІНОМАСЛЯНА КИСЛОТА МОДУЛЮЄ АНТИОКСИДАНТНУ ТА ОСМОПРОТЕКТОРНУ СИСТЕМИ У ПРОРОСТКІВ СОРТІВ *TRITICUM AESTIVUM* З РІЗНОЮ ПОСУХОСТІЙКІСТЮ

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Останніми роками у світі інтенсивно досліджують стрес-протекторну дію рослинних нейротрансмітерів, одним з яких є гамма-аміномасляна кислота (GABA). Проте ще недостатньо вивчений вплив GABA на стрес-протекторні системи у сортів м'якої пшениці з різними стратегіями адаптації до посухи. Метою роботи було оцінити вплив GABA на стан антиоксидантної та осмопротекторної систем етіологованих проростків двох сортів пшениці, які суттєво відрізняються за посухостійкістю, а саме Досконала (непосухостійкий) і Тобак (посухостійкий) за умов посухи. Індукованої PEG 6000. Дводобові проростки переносили в 15% розчин PEG 6000 та інкубували протягом двох днів за відсутності або присутності GABA. Обробка 0,1 і 0,5 мМ GABA суттєво зменшила інгібуючу дію PEG 6000 на ріст коренів і пагонів

обох сортів, але більшою мірою впливала на нестійкий сорт Досконала. Показано, що обробка GABA зменшувала спричинене посухою накопичення  $H_2O_2$  та MDA, стабілізувала активність SOD та GPX, рівень цукрів, антоціанів та флавоноїдів у проростках обох сортів. Водночас обробка GABA посилювала стрес-індуковане збільшення вмісту проліну у сорту Досконала, але зменшувала його у сорту Тобак, повністю запобігла стрес-індукованому зниженню рівня антоціанів і флавоноїдів у сорту Тобак і лише частково у сорту Досконала. Таким чином, виявлено стабілізацію функціонування стрес-протекторних систем у проростків пшениці та сортозалежні відмінності у відповіді на GABA.

**Ключові слова:** *Triticum aestivum*, гамма-аміномасляна кислота, посухостійкість, окислювальний стрес, антиоксидантна система, пролін, антоціани.

### References

1. Adel S, Carels N. Plant Tolerance to Drought Stress with Emphasis on Wheat. *Plants (Basel)*. 2023; 12(11): 2170.
2. Andleeb T, Shah T, Nawaz R, Munir I, Munsif F, Jalal A. QTL mapping for drought stress tolerance in plants. In: Hasanuzzaman M, Tanveer M. (Eds.) Salt and Drought Stress Tolerance in Plants. Signaling and Communication in Plants. Springer: Cham, Switzerland; 2020. p. 383-403.
3. Khan S, Anwar S, Yu S, Sun M, Yang Z, Gao ZQ. Development of Drought-Tolerant Transgenic Wheat: Achievements and Limitations. *Int J Mol Sci*. 2019; 20(13): 3350.
4. Punchkhon C, Plaimas K, Buaboocha T, Siangliw JL, Toojinda T, Comai L, De Diego N, Spíchal L, Chadchawan S. Drought-Tolerance Gene Identification Using Genome Comparison and Co-Expression Network Analysis of Chromosome Substitution Lines in Rice. *Genes (Basel)*. 2020; 11(10): 1197.
5. Kosakivska IV, Vedenicheva NP, Babenko LM, Voytenko LV, Romanenko KO, Vasyuk VA. Exogenous phytohormones in the regulation of growth and development of cereals under abiotic stresses. *Mol Biol Rep*. 2022; 49(1): 617-628.
6. Kavulych Y, Kobyletska M, Romanyuk N, Terek O. Stress-protective and regulatory properties of salicylic acid and prospects of its use in plant production. *Studia Biologica*. 2023; 17(2): 173-200.

7. Kolupaev YE, Yastreb TO, Dmitriev AP. Signal Mediators in the Implementation of Jasmonic Acid's Protective Effect on Plants under Abiotic Stresses. *Plants (Basel)*. 2023; 12(14): 2631.
8. Akula R, Mukherjee S. New insights on neurotransmitters signaling mechanisms in plants. *Plant Signal Behav*. 2020; 15(6): 1737450.
9. Kolupaev YE, Karpets YV, Shkliarevskiy MA, Yastreb TO, Plohovska SH, Yemets AI, Blume YB. Gasotransmitters in plants: Mechanisms of participation in adaptive responses. *Open Agricult J*. 2022; 16(Suppl-1, M5): e187433152207050.
10. Jurkonienė S, Mockevičiūtė R, Gavelienė V, Šveikauskas V, Zareyan M, Jankovska-Bortkevič E, Jankauskienė J, Žalnierius T, Kozeko L. Proline Enhances Resistance and Recovery of Oilseed Rape after a Simulated Prolonged Drought. *Plants (Basel)*. 2023; 12(14): 2718.
11. Seifikalhor M, Aliniaiefard S, Hassani B, Niknam V, Lastochkina O. Diverse role of  $\gamma$ -aminobutyric acid in dynamic plant cell responses. *Plant Cell Rep*. 2019; 38(8): 847-867.
12. Bouché N, Fait A, Bouchez D, Møller SG, Fromm H. Mitochondrial succinic-semialdehyde dehydrogenase of the gamma-aminobutyrate shunt is required to restrict levels of reactive oxygen intermediates in plants. *Proc Natl Acad Sci USA*. 2003; 100(11): 6843-6848.
13. Bor M, Turkan I. Is there a room for GABA in ROS and RNS signalling? *Environ Exp Bot*. 2019; 161: 67-73.
14. Ramesh SA, Tyerman SD, Gilliam M, Xu B.  $\gamma$ -Aminobutyric acid (GABA) signalling in plants. *Cell Mol Life Sci*. 2017; 74(9): 1577-1603.
15. Suhel M, Husain T, Pandey A, Singh S, Dubey NK, Prasad SM, Singh VP. An appraisal of ancient molecule GABA in abiotic stress tolerance in plants, and its crosstalk with other signaling molecules. *J Plant Growth Regul*. 2023; 42(2): 614-629.
16. Jin X, Liu T, Xu J, Gao Z, Hu X. Exogenous GABA enhances muskmelon tolerance to salinity-alkalinity stress by regulating redox balance and chlorophyll biosynthesis. *BMC Plant Biol*. 2019; 19(1): 48.
17. Sheteiwy MS, Shao H, Qi W, Hamoud YA, Shaghaleh H, Khan NU, Yang R, Tang B. GABA-Alleviated Oxidative Injury Induced by Salinity, Osmotic Stress and their Combination by Regulating Cellular and Molecular Signals in Rice. *Int J Mol Sci*. 2019; 20(22): 5709.
18. Shelp BJ, Aghdam MS, Flaherty EJ.  $\gamma$ -Aminobutyrate (GABA) Regulated Plant Defense: Mechanisms and Opportunities. *Plants (Basel)*. 2021; 10(9): 1939.
19. Kolupaev YE, Yastreb TO, Ryabchun NI, Kokorev AI, Kolomatska VP, Dmitriev AP. Redox homeostasis of cereals during acclimation to drought. *Theor Exp Plant Physiol*. 2023; 35(2): 133-168.
20. Xu B, Long Y, Feng X, Zhu X, Sai N, Chirkova L, Betts A, Herrmann J, Edwards EJ, Okamoto M, Hedrich R, Gilliam M. GABA signalling modulates stomatal opening to enhance plant water use efficiency and drought resilience. *Nat Commun*. 2021; 12(1): 1952.
21. Yong B, Xie H, Li Z, Li YP, Zhang Y, Nie G, Zhang XQ, Ma X, Huang LK, Yan YH, Peng Y. Exogenous Application of GABA Improves PEG-Induced Drought Tolerance Positively Associated with GABA-Shunt, Polyamines, and Proline Metabolism in White Clover. *Front Physiol*. 2017; 8: 1107.
22. Tang M, Li Z, Luo L, Cheng B, Zhang Y, Zeng W, Peng Y. Nitric Oxide Signal, Nitrogen Metabolism, and Water Balance Affected by  $\gamma$ -Aminobutyric Acid (GABA) in Relation to Enhanced Tolerance to Water Stress in Creeping Bentgrass. *Int J Mol Sci*. 2020; 21(20): 7460.
23. Abd El-Gawad HG, Mukherjee S, Farag R, Abd Elbar OH, Hikal M, Abou El-Yazied A, Abd Elhady SA, Helal N, ElKelish A, El Nahhas N, Azab E, Ismail IA, Mbarki S, Ibrahim MFM. Exogenous  $\gamma$ -aminobutyric acid (GABA)-induced signaling events and field performance associated with mitigation of drought stress in *Phaseolus vulgaris* L. *Plant Signal Behav*. 2021; 16(2): 1853384.
24. Farooq M, Nawaz A, Chaudhry MAM, Indrasti R, Rehman A. Improving resistance against terminal drought in bread wheat by exogenous application of proline and gamma-aminobutyric acid. *J Agron Crop Sci*. 2017; 203(6): 464-472.
25. Zhao Q, Ma Y, Huang X, Song L, Li N, Qiao M, Li T, Hai D, Cheng Y. GABA Application Enhances Drought Stress Tolerance in Wheat Seedlings (*Triticum aestivum* L.). *Plants (Basel)*. 2023; 12(13): 2495.

26. Kolupaev YE, Yastrebo TO, Ryabchun NI, Kuzmyshyna NV, Shkliarevskyi MA, Barabolia O, Pysarenko VM. Response of *Triticum aestivum* seedlings of different ecological and geographical origin to heat and drought: relationship with resistance to oxidative stress and osmolyte accumulation. *Agricult Forest*. 2023; 69(2): 83-99.
27. Urban O, Hlaváčová M, Klem K, Novotná K, Rapantová B, Smutná P, Horáková V, Hlavinka P, Škarpa P, Trnka M. Combined effects of drought and high temperature on photosynthetic characteristics in four winter wheat genotypes. *Field Crops Res*. 2018; 223: 137-149.
28. Tambussi EA, Nogués S, Araus JL. Ear of durum wheat under water stress: water relations and photosynthetic metabolism. *Planta*. 2005; 221(3): 446-458.
29. Sagisaka S. The Occurrence of Peroxide in a Perennial Plant, *Populus gelrica*. *Plant Physiol*. 1976; 57(2): 308-309.
30. Kolupaev YuE, Horielova EI, Yastrebo TO, Ryabchun NI. State of antioxidant system in triticale seedlings at cold hardening of varieties of different frost resistance. *Cereal Res Commun*. 2020; 48(2): 165-171.
31. Yastrebo TO, Kolupaev YuE, Kokorev AI, Makaova BE, Ryabchun NI, Zmiiivska OA, Pospelova GD. Indices of antioxidant and osmoprotective systems in seedlings of winter wheat cultivars with different frost resistance. *Ukr Biochem J*. 2023; 95(1): 73-84.
32. Zhao K, Fan H, Zhou S, Song J. Study on the salt and drought tolerance of *Suaeda salsa* and *Kalanchoe clavigremontiana* under iso-osmotic salt and water stress. *Plant Sci*. 2003; 165(4): 837-844.
33. Bobo-García G, Davidov-Pardo G, Arroqui C, Virseda P, Marín-Arroyo MR, Navarro M. Intra-laboratory validation of microplate methods for total phenolic content and antioxidant activity on polyphenolic extracts, and comparison with conventional spectrophotometric methods. *J Sci Food Agric*. 2015; 95(1): 204-209.
34. Nogués S, Baker NR. Effects of drought on photosynthesis in Mediterranean plants grown under enhanced UV-B radiation. *J Exp Bot*. 2000; 51(348): 1309-1317.
35. Ullah A, Ali I, Noor J, Zeng F, Bawazeer S, Eldin SM, Asghar MA, Javed HH, Saleem K, Ullah S, Ali H. Exogenous  $\gamma$ -aminobutyric acid (GABA) mitigated salinity-induced impairments in mungbean plants by regulating their nitrogen metabolism and antioxidant potential. *Front Plant Sci*. 2023; 13: 1081188.
36. Salah A, Zhan M, Cao C, Han Y, Ling L, Liu Z, Li P, Ye M, Jiang Y.  $\gamma$ -Aminobutyric Acid Promotes Chloroplast Ultrastructure, Antioxidant Capacity, and Growth of Waterlogged Maize Seedlings. *Sci Rep*. 2019; 9(1): 484.
37. Nayyar H, Kaur H, Kaur S, Singh R.  $\gamma$ -Aminobutyric acid (GABA) imparts partial protection from heat stress injury to rice seedlings by improving leaf turgor and upregulating osmoprotectants and antioxidants. *J Plant Growth Regul*. 2014; 33(2): 408-419.
38. Shi SQ, Shi Z, Jiang ZP, Qi LW, Sun XM, Li CX, Liu JF, Xiao WF, Zhang SG. Effects of exogenous GABA on gene expression of *Caragana intermedia* roots under NaCl stress: regulatory roles for  $H_2O_2$  and ethylene production. *Plant Cell Environ*. 2010; 33(2): 149-162.
39. Li MF, Guo SJ, Yang XH, Meng QW, Wei XJ. Exogenous gamma-aminobutyric acid increases salt tolerance of wheat by improving photosynthesis and enhancing activities of antioxidant enzymes. *Biol Plant*. 2016; 60(1): 123-131.
40. Cheng B, Li Z, Liang L, Cao Y, Zeng W, Zhang X, Ma X, Huang L, Nie G, Liu W, Peng Y. The  $\gamma$ -Aminobutyric Acid (GABA) Alleviates Salt Stress Damage during Seeds Germination of White Clover Associated with  $Na^+/K^+$  Transportation, Dehydrins Accumulation, and Stress-Related Genes Expression in White Clover. *Int J Mol Sci*. 2018; 19(9): 2520.
41. Krishnan S, Laskowski K, Shukla V, Merewitz EB. Mitigation of drought stress damage by exogenous application of a non-protein amino acid  $\gamma$ -aminobutyric acid on Perennial Ryegrass. *J Amer Soc Hort Sci*. 2013; 138(5): 358-366.
42. Nasirzadeh L, Sorkhilaleloo B, Hervan EM, Fatehi F. Changes in antioxidant enzyme activities and gene expression profiles under drought stress in tolerant, intermediate, and susceptible wheat genotypes. *Cereal Res Commun*. 2021; 49(1): 83-89.
43. Zhou M, Hassan MJ, Peng Y, Liu L, Liu W, Zhang Y, Li Z.  $\gamma$ -Aminobutyric Acid (GABA) Priming Improves Seed Germination and Seedling Stress Tolerance Associated With Enhanced Antioxidant Metabolism, DREB



- Expression, and Dehydrin Accumulation in White Clover Under Water Stress. *Front Plant Sci.* 2021; 12: 776939.
44. Jalil SU, Ansari MI. Physiological role of gamma-aminobutyric acid in salt stress tolerance. In: Hasanuzzaman M, Tanveer M, eds. Salt and Drought Stress Tolerance in Plants. Signaling and Communication in Plants. Springer, Cham., 2020. p. 337-350.
45. Kolupaev YuE, Karpets YuV, Yastrebo TO, Shemet SA, Bhardwaj R. Antioxidant system and plant cross-adaptation against metal excess and other environmental stressors. Eds. Landi M, Shemet SA, Fedenko VS. In: Metal toxicity in higher plants. New York: Nova Science Publishers, 2020. p. 21-66.
46. Zeng W, Hassan MJ, Kanga D, Peng Y, Li Z. Photosynthetic maintenance and heat shock protein accumulation relating to  $\gamma$ -aminobutyric acid (GABA)-regulated heat tolerance in creeping bentgrass (*Agrostis stolonifera*). *South Afr J Bot.* 2021; 141: 405-413.
47. Li Z, Tang M, Hassan MJ, Zhang Y, Han L, Peng Y. Adaptability to High Temperature and Stay-Green Genotypes Associated With Variations in Antioxidant, Chlorophyll Metabolism, and  $\gamma$ -Aminobutyric Acid Accumulation in Creeping Bentgrass Species. *Front Plant Sci.* 2021; 12: 750728.