Content of malondialdehyde and activity of enzyme glutathione-S-transferase in the leaves of emmer wheat under the action of herbicide and plant growth regulator

V. Karpenko^{1,*}, S. Pavlyshyn¹, R. Prytuliak¹ and D. Naherniuk²

¹Uman National University of Horticulture, Faculty of Horticulture, Ecology and Plants Protection, Department of Biology, Instytutska street, 1, UA20300 Uman, Ukraine ²Uman National University of Horticulture, Faculty of Management, Department of Management, Instytutska street, 1, UA20300 Uman, Ukraine *Correspondence: unuh1844@gmail.com

Abstract. The article presents the results of vegetation experiment on the influence of different rates of herbicide Prima Forte 195 - 2-ethylhexyl ether 2.4-D + aminopyralid + florasulam (0.5; 0.6 and 0.7 L ha⁻¹) under different application methods of plant growth regulator of a natural origin Wuxal BIO Vita (*Ascophyllum nodosum* extract + microelements) on the content of malondialdehyde (MDA) as an important indicator of the intensity of peroxide oxidation of lipids in plants and on the activity of enzyme glutathione-S-transferase (GST) in the leaves of emmer wheat.

Experimental scheme included 16 experimental variants: 1 – without application of preparations and pre-sowing treatment of seeds (control); 2, 3, 4 – Prima Forte 195, applied to vegetative plants at the rates 0.5, 0.6 and 0.7 L ha⁻¹; 5 – Wuxal BIO Vita at the rate of 1.0 L ha⁻¹, applied to vegetative plants; 6, 7, 8 – Prima Forte 195 at the rates of 0.5, 0.6 and 0.7 L ha⁻¹ in tank mixtures with Wuxal BIO Vita at the rate of 1.0 L ha⁻¹, sprayed on vegetative plants; 9 – pre-sowing treatment of seeds with Wuxal BIO Vita at the rate of 1.0 L t⁻¹ (background); 10, 11, 12 – Prima forte 195, applied to vegetative plants at the rates of 0.5, 0.6 and 0.7 L ha⁻¹ at the background of pre-sowing treatment of seeds with Wuxal BIO Vita at the rate of 1.0 L t⁻¹; 13 – Wuxal BIO Vita at the rate of 1.0 L ha⁻¹ (applied to vegetative plants, treated before sowing with Wuxal BIO Vita at the rate of 1.0 L t⁻¹); 14, 15, 16 – Prima Forte 195 at the rates of 0.5, 0.6 and 0.7 L ha⁻¹ – spraying of plants and pre-sowing treatment of seeds with Wuxal BIO Vita at the rate of 1.0 L t⁻¹.

It has been found that under the application of Prima Forte 195 the redox state in the leaves of emmer wheat increased considerably in the direction of increasing the content of MDA. It has also been proved that herbicide Prima Forte 195 and its mixtures with plant growth regulator (PGR) Wuxal BIO Vita caused the changes in the activity of enzyme glutathione-S-transferase, which slightly increased in the initial period in the variants of integrated application of herbicide and plant growth regulator. However, later the GST activity in the variants of integrated application of herbicide and PGR decreased, which indicates stabilization and a positive influence of integrated application of preparations on the detoxification processes in the crops of emmer wheat.

Key words: MDA content, glutathione-S-transferase, oxidative stress, herbicide, plant growth regulator, emmer wheat (*Triticum dicoccum* (Schrank) Schuebl.)

INTRODUCTION

Weeds remain the main restricting factor of growing such an important crop as wheat. Therefore, over the last years in the technologies of growing wheat the volumes of using chemical plant protection products have increased, including herbicides, which has aggravated the problem of accumulating toxins in food chains (Hesammi, 2011). In this respect, the technological developments that provide transition from chemicalsdependent to biologically oriented arable farming are becoming more urgent. However, a drastic transition to biological arable farming leads to the increase in weed infestation of crops and a decrease of wheat yielding capacity. Therefore, nowadays when there is a shortage of foodstuffs worldwide, it is impossible to reject the application of herbicides completely. At the same time, it is necessary to look for the ways of decreasing their negative effect on farm ecosystems. Definitely, these ways should include the elements of biologization, which in the case of herbicides can be reached due to their integrated application with the preparations of natural origin, for example, with plant growth regulators (PGR), that are characterized by anti-stress and immune-stimulating properties. For the first time this possibility was proved on the example of spring barley (Karpenko et al., 2012). Though, a number of issues dealing with a comprehensive action of herbicides and PGR remains insufficiently studied. The choice and evaluation of the optimal combination of preparations in the mixtures, especially multi-component ones, are carried out without considering the mechanisms of their action on the key physiological reactions in a plant body, because herbicides, being highly-effective compounds, are able to penetrate in plants quickly, where they are subject to metabolic transformation on the part of fermentative systems.

It was proved that herbicides could cause oxidative stress (Van Camp et al.; 1994; Mitieva et al., 2010), which is accompanied by an intensive generation of active oxygen species (AOS). The latter, in their turn, lead to the structural damage of membranes, proteins, lipids, carbohydrates, and DNA, increase the level of peroxide oxidation of lipids (POL) (Hassan & Nemat Alla, 2005; Gill & Tuteja, 2010). Hence, the rate of detoxification of herbicides in cultivated plants and weeds determines their selectivity (Owen, 2000). It is in the process of destroying a toxic agent in cells when the activity of enzymes increases, as well as glutathione-S-transferase (GST) (EC 2.5.1.18) - the enzyme that metabolizes toxic products of peroxide oxidation of lipids, that damage DNA and other cell components (Liu et al., 2013). GST also catalyzes the creation of glutathione conjugates, that way decreasing the toxic level of alien compounds that are taken away from cells with the help of special ATF-dependent transport systems (Lamoureux & Rusness, 1989). Activation of glutathione-S-transferase enhances the ability of cells to detoxify toxic agents, which is explained by the participation of enzyme in the neutralization of AOS (Zama & Hatzios, 1986). In particular, GST catalyzes the conjugation of electrophilic and often hydrophobic toxic compounds with glutathione with the formation of non-toxic peptide compounds (Neuefeind et al., 1997; Dixon et al., 1998; Edwards & Dixon, 2000; DeRidder & Goldsbrough, 2006; Rochalska & Grabowska, 2007; Baimuhametova et al., 2016).

To eliminate AOS in plants there are other specific enzymes: peroxidase, catalase, and superoxide dismutase. According to our previous research (Karpenko & Pavlyshyn, 2018), under the application of herbicide Prima Forte 195 at the rates of 0.5–0.7 L ha⁻¹ the activity of peroxidase and catalase in the plants of emmer wheat can increase by

18–33 and 19–32% respectively, compared to the variant without the application of herbicide, which is the result of a considerable increase of H_2O_2 in the leaves of a plant as one of the forms of AOS. It is obvious, that GST also reacts in a proper way to the increased concentration of H_2O_2 in cells. However, there is no information available in scientific literature as to the influence of herbicide Prima Forte 195, that has been used in Ukraine only for the recent two years, on the activity of GST and development of oxidative stress in the plants of emmer wheat, which makes our experiment relevant.

The aim of our experiment was to study the influence of herbicide Prima Forte 195 $(0.5; 0.6; 0.7 \text{ L} \text{ ha}^{-1})$ in vegetative plants separately and in the mixtures with PGR Wuxal BIO Vita at the rate of 1.0 L ha⁻¹, and also application of the same compositions of herbicide and PGR at the background of pre-sowing treatment of seeds with PGR Wuxal BIO Vita at the rate of 1.0 L t⁻¹ on the accumulation of malondialdehyde (MDA), the product of peroxide oxidation of lipids and activity of glutathione-S-transferase. Taking into account oxidative and enzyme changes in emmer wheat we tried to determine the optimal combination of preparations, under which application the plants are subjected to the minimal stressful influence from the part of herbicide agent.

MATERIALS AND METHODS

The objects of the research were emmer wheat plants (*Triticum dicoccum* (Schrank) Schuebl.) of the cultivar Holikovska (originator – the Plant Production Institute named after V.Ya. Yuryev, Ukraine), herbicide Prima Forte 195, c.e. (Syngenta) (active substances – florasulam 5 g L⁻¹, aminopyralid 10 g L⁻¹, 2-ethylhexyl alcohol 2.4-D 180 g L⁻¹), plant growth regulator Wuxal BIO Vita (Unifer) (active substance – extract from seaweed *Ascophyllum nodosum*, nitrogen (N) – 52 g L⁻¹, manganese (Mn) – 38 g L⁻¹, sulphur (S) – 29 g L⁻¹, iron (Fe) – 6.4 g L⁻¹, zinc (Zn) – 6.4 g L⁻¹).

Plants were grown in the laboratory in plastic containers with the capacity of 12 kg filled with absolutely dry soil, typical for a field experiment. The soil was a podzolized, heavy loamy black soil. The humus content in plowing horizon made up 3.4%; nitrogen content of alkaline hydrolysis compounds was low (103 mg kg⁻¹); the content of phosphorus movable compounds (96 mg kg⁻¹) was medium; the content of potassium movable compounds was higher; reaction of soil solution was weak acid (pH of salt solution is 5.9). (above mentioned indicators were determined according to the methods, described in (Hrycajenko et al., 2003). Soil moisture was maintained by the gravimetric method at the level of 60% soil moisture. Additional illumination with fluorescent lamps 800 lux (14-16 hours) was used under controlled conditions of plants growth and development. The temperature was maintained at the level of 25 °C. Relative humidity was 60%. Treatment of seeds with PGR was carried out according to the rates, calculated per seed weight, and spraying of vegetative plants was completed per area according to concentration in relation to the rates of application under field conditions. Treatment of seeds with PGR was carried out on the day of sowing (BBCH 00). Vegetative plants were treated with herbicide and PGR at the phase BBCH 29 with hand-operated sprayer. In order to provide an even illumination and temperature regime, the location of containers with plants was changed every two days. Vegetation experiment was carried out in a 3-time repetition, observing the requirements of vegetative method (Zhurbickij, 1968). The scheme of the experiment included 16 experimental variants: 1. C - without application of preparations and pre-sowing treatment of seeds (control); 2, 3, 4. H0.5, H0.6, H0.7 – herbicide Prima Forte 195 at the rates of 0.5, 0.6 and 0.7 L ha⁻¹; 5. PV – PGR Wuxal BIO Vita at the rate of 1.0 L ha⁻¹, treatment of vegetative plants; 6, 7, 8. H0.5, H0.6, H0.7 + PV – tank mixtures of Prima Forte 195 0.5, 0.6 and 0.7 L ha⁻¹ + Wuxal BIO Vita 1.0 L ha⁻¹, treatment of vegetative plants; 9. PS – PGR Wuxal BIO Vita 1.0 L t⁻¹ (pre-sowing treatment of seeds, background); 10, 11, 12. H0.5, H0.6, H0.7 + PS – Prima Forte 195 0.5, 0.6, 0.7 L ha⁻¹, treatment of vegetative plants at the background of pre-sowing seed treatment with PGR; 13. PS + PV –Wuxal BIO Vita 1.0 L ha⁻¹, treatment of vegetative plants with PGR at the background of pre-sowing seeds treatment with PGR, 14, 15, 16. H0.5, H0.6, H0.7 + PS + PV – Prima Forte 195 0.5, 0.6, 0.7 L ha⁻¹, treatment of vegetative plants with tank mixture of herbicide and PGR at the background of pre-sowing seed treatment with PGR.

The leaves of emmer wheat were selected for the analyses on the 3rd and on the 10th day after a post-germination application of preparations. Intensity of oxidative stress was evaluated by the reaction of POL – by the accumulation of a final product of peroxide oxidation of lipids - malondialdehyde (MDA), by the reaction with thiobarbituric acid (TBA) at 532 nm on spectrophotometer LEKI SS1104 according to the technique (Rogozhin, 2006). The method is based on determining the amount of a coloured product at the wave length 532 nm, obtained as the result of interaction of 2 molecules of TBA with one molecule of MDA as one of the by-products of POL. For this purpose, 1 g of leaves tissue was homogenized with 3 mL of 50% ethanol and centrifuged 10 min at 7,000 rpm. 0.5 mL of 1% triton X-100 solution, 0.2 mL of 0.6 M HCl and 0.8 mL 0.06 M of TBA were added to the obtained 0.5 mL of supernatant and heated in the boiling water bath (100 °C) for 10 min and then cooled to 15 °C for 30 min and added 0.2 mL 5 mM solution of Trilon B and 5-10 mL of 96% ethanol. As a control served test-tube in which all chemical reagents except TBA were added. MDA content was calculated, taking into account optical density of the sample and its corresponding dilutions under the coefficient of micro molar absorption TBA $\mathcal{E} = 155 \ \mu M^{-1} \ cm^{-1}$ at the wave length 532 nm and was expressed in μ mol g⁻¹ of raw substance.

The activity of GST was determined by the method of Habig et al. (1974) in the modification of Grishko & Syschikov (1999): for that purpose 1 g of raw tissue of leaves was crashed in mortar in 3 mL water cooled to 0 °C, homogenate was centrifuged 10 min at 7,000 rpm. 0.2 mL 0.015 M solution of reduced glutathione and 0.1 mL of supernatant were added in the cuvette (optical path length l = 1 cm) that contained 2.5 mL 0.1 M potassium phosphate buffer (pH = 6.5). Reaction was initiated by adding 0.2 mL 0.015 M 1-chloro-2.4-dinitrobenzene (DNCB) in the cuvette. Simultaneously, blank sample was prepared, in which distilled water was added instead of supernatant. The change in optical density was recorded in the third minute at wave length 340 nm on spectrophotometer LEKI SS1104.

Calculations of enzyme activity were carried out considering optical density of the sample, corresponding dilutions, the time of the reaction under the coefficient of micro molar absorbtion of DNCB at wave length of 340 nm $\mathcal{E} = 9.6 \,\mu M^{-1} \,\mathrm{cm^{-1}}$. Catalytic activity of GST was expressed in $\mu \mathrm{mol} \, \mathrm{g}^{-1}$ of raw substance per 1 minute.

Reliability of the experiment and significance of the difference between indexes (LSD) in the experimental research were assessed according to the results of the analysis of variance (Ehrmantraut et al., 2000) with the application of Microsoft Excel. The graphs show arithmetic means of the analyses, that were carried out three times. Sampling error doesn't exceed 5% from mean values.

RESULTS AND DISCUSSION

Herbicides, violating the key physiological reactions in plants, including enzymological, cause the activation of free-radical oxidizing processes (Enan, 2009). The most representative among them is peroxide oxidation of lipids (POL), key index that shows the intensity of stressful influence of the factors of different nature, including herbicides. Therefore the accumulation of POL products in plants, in particular MDA, can serve as an indicator of the signal system of the adaptive protection of a plant against toxic action of xenobiotic (Agostinetto et al., 2016).

According to the results of the research into the MDA content in emmer wheat leaves it has been established, that herbicide treatment of plants led to the metabolic disturbance in plants, the level of which depended on a separate or integrated application of herbicide with PGR and correspondingly affected the level of MDA accumulation (Fig. 1).

In our experiment the highest MDA content in the leaves of emmer wheat was recorded in the variants with the application of herbicide without PGR, when under the rates of Prima Forte 195 0.5; 0.6 and 0.7 L ha⁻¹ its content exceeded control by 162, 190 and 226% on the 3rd day after application and by 111, 124 and 143% on the 10th day respectively.

It has been noted that along with the increase of the application rate of herbicide, there was the increase in the accumulation of MDA in the leaves of emmer wheat, which could serve as an indicator of the development of oxidative stress in the plants. Obtained results conform with the results of the experiments performed by other scientists (Rossikhina & Vinnichenko, 2004; Agostinetto et al., 2016), who established that the action of herbicides based on 2.4-Dichlorophenoxyacetic acid (which is one of the components of herbicide Prima Forte 195) in the crops of corn and wheat cause the activation of POL, which is manifested in the increased MDA accumulation.

Under the integrated application of Prima Forte 195 at the rates 0.5; 0.6 and 0.7 L ha⁻¹ with Wuxal BIO Vita 1.0 L ha⁻¹ the indexes of MDA content in the leaves of emmer wheat exceeded control by 78, 95 and 111% on the 3rd and by 79, 94 and 118% on the 10th day respectively.

When emmer wheat was sprayed with Prima Forte 195 at the rates of 0.5; 0.6 and 0.7 L ha⁻¹ at the background of pre-sowing treatment of seeds with Wuxal BIO Vita PGR 1.0 L t⁻¹, the indexes of MDA content compared to control increased by 141,161 and 185% on the 3^{rd} day and by 91, 107 and 135% on the10th day respectively.

Integrated application of herbicide Prima Forte 195 (0.5–0.7 L ha⁻¹) and PGR at the background of pre-sowing treatment of seeds with PGR increased MDA content compared to control in the plants of emmer wheat by 67, 91 and 103% on the 3rd and by 63, 73 and 89% on the 10th day respectively. It should be noted that MDA content in these variants was lower compared to the variants with the application on herbicide alone, which means the initial increased level of detoxification processes in plants, aimed at destroying the toxicant. It is obvious that complex application of PGR (treatment of seeds and plants) in this case was the factor of decreasing or stabilizing POL processes in the plants (Karpenko et al., 2012), which can be the result of the changes in the enzymatic system of plants in general.

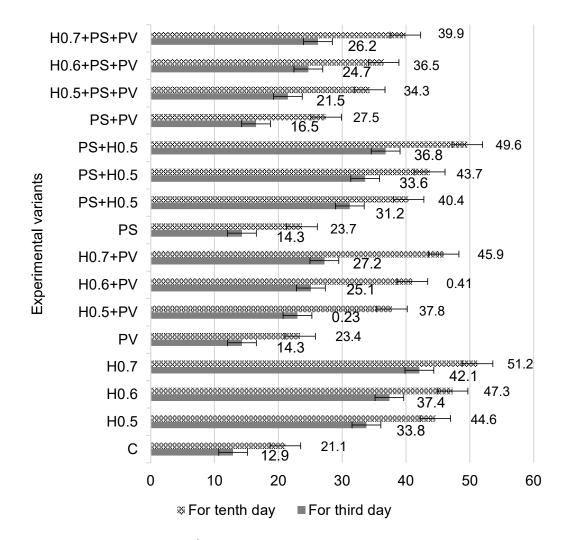


Figure 1. MDA content (μ mol g⁻¹ of raw substance) in the leaves of emmer wheat under the application of herbicide Prima Forte 195 and Wuxal BIO Vita PGR (LSD₀₅: for third day – 1.42; for tenth day – 2.08).

C – without application of preparations (control); H0.5, H0.6, H0.7 – Prima Forte 195 0.5, 0.6, 0.7 L ha⁻¹; PV – Wuxal BIO Vita – 1.0 L ha⁻¹; H0.5, H0.6, H0.7 + PV – Prima Forte 195 0.5, 0.6, 0.7 L ha⁻¹ + Wuxal BIO Vita 1.0 L ha⁻¹; PS – Wuxal BIO Vita 1.0 L t⁻¹ (pre-sowing treatment of seeds, background); H0.5, H0.6, H0.7 + PS – Background + Prima Forte 195 0.5, 0.6, 0.7 L ha⁻¹; PS + PV – Background + Wuxal BIO Vita 1.0 L ha⁻¹; H0.5, H0.6, H0.7 + PS + PV – Background + Prima Forte 195 0.5, 0.6, 0.7 L ha⁻¹ + Wuxal BIO Vita 1.0 L ha⁻¹; H0.5, H0.6, H0.7 + PS + PV – Background + Prima Forte 195 0.5, 0.6, 0.7 L ha⁻¹ + Wuxal BIO Vita 1.0 L ha⁻¹.

Enzymatic reactions in the mechanism of protective processes are leading and the most powerful, because they prevent not only the development of free-radical reactions but also support high intensity of oxidant-renewing processes, provide elimination of final oxygen metabolites with their involvement into the energetic exchange and activation of synthesis processes (Kobylinska & Tymochko, 2000). Metabolic response of a cell to the action of the irritant depends on its oxidizing-renovating state, that is able to influence the formation of adaptive reactions through the variability of the level of renovation of low-molecular compounds and proteins and the efficiency of the system of antioxidant protection (Kulinskii & Kolesnichenko, 1993). According to the data, obtained by the scholars (Kurganova et al., 1997; Kalashnikov et al., 1999), POL

products – 'initial mediators' of stress as a special state of an organism, can trigger the corresponding mechanisms of protection in the plants, including enzymatic ones. For the reasons, that haven't been studied up to now, main agricultural crops such as wheat, sorghum, corn, soybean et al. manifest a considerably higher level of GST in the process of detoxification of herbicides than weeds, which provides the possibility to manage their selective action in relation to weeds (Dixon & Edwards, 2010).

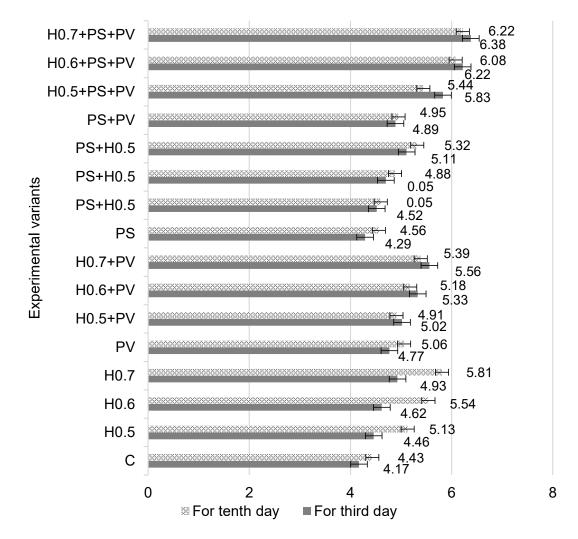


Figure 2. Activity of GST (μ mol g⁻¹ of raw substance per 1 min) in the leaves of emmer wheat under the action of the herbicide Prima Forte 195 and Wuxal BIO Vita PGR (LSD₀₅: for third day – 0.54; for tenth day – 0.48).

C – without application of preparations (control); H0.5, H0.6, H0.7 – Prima Forte 195 0.5, 0.6, 0.7 L ha⁻¹; PV – Wuxal BIO Vita 1.0 L ha⁻¹; H0.5, H0.6, H0.7 + PV – Prima Forte 195 0.5, 0.6, 0.7 L ha⁻¹ + Wuxal BIO Vita 1.0 L ha⁻¹; PS – Wuxal BIO Vita 1.0 L t⁻¹ (pre-sowing treatment of seeds, background); H0.5, H0.6, H0.7 + PS – Background + Prima Forte 195 0.5, 0.6, 0.7 L ha⁻¹; PS + PV – Background + Wuxal BIO Vita 1.0 L ha⁻¹; H0.5, H0.6, H0.7 + PS + PV – Background + Prima Forte 195 0.5, 0.6, 0.7 L ha⁻¹; PS + PV – Background + Wuxal BIO Vita 1.0 L ha⁻¹; H0.5, H0.6, H0.7 + PS + PV – Background + Prima Forte 195 0.5, 0.6, 0.7 L ha⁻¹; H0.5, H0.6, H0.7 + PS + PV – Background + Prima Forte 195 0.5, 0.6, 0.7 L ha⁻¹; H0.5, H0.6, H0.7 + PS + PV – Background + Prima Forte 195 0.5, 0.6, 0.7 L ha⁻¹ + Wuxal BIO Vita 1.0 L ha⁻¹.

Determining the GST activity in the leaves of emmer wheat under the application of Prima Forte 195 at the rates of 0.5; 0.6 and 0.7 L ha⁻¹ showed that the index exceeded control by 7; 10 and 18 % on the 3rd day and by 16; 25 and 31% on the 10th day (Fig. 2).

Such increase in the GST activity and other enzymes of antioxidant protection of emmer wheat plants under the action of herbicides were recorded by other researchers (Yin et al., 2008; Jiang & Yang, 2009; Song et al., 2010).

When plants of emmer wheat were sprayed with Prima Forte 195 at the rates of 0.5; 0.6 and 0.7 L ha⁻¹ with Wuxal BIO Vita 1.0 L ha⁻¹, the indexes of GST activity exceeded control by 20; 28 and 33% on the 3^{rd} day and by 11, 17 and 22% compared to control on the 10^{th} day correspondingly.

According to the data of Shorning et al. (2000), the plants constantly need AOS for the regulation of growth and development, therefore the dynamics of GST activity in the control variant and some variants testifies this process on the tenth day. However, in the variants with combined application of herbicide and PGR, compared to the indexes on the 3^{rd} day, GST activity decreased on the 10^{th} day, which indicates the stabilization of detoxification processes in emmer wheat plants and conforms with the data of other scientists (Bilonozhko et al., 2012).

When the plants of emmer wheat were sprayed with Prima Forte 195 at the rates of 0.5; 0.6 and 0.7 L ha⁻¹ at the background of pre-sowing treatment of seeds with Wuxal BIO Vita at the rate of 1.0 L t⁻¹ the GST activity increased in relation to the control by 8; 13 and 23 % (3^{rd} day) and by 4, 10 and 20% (10^{th} day).

Under spraying the plants with a tank mixture of Prima Forte 195 at the rates of 0.5; 0.6 and 0.7 L ha⁻¹ with Wuxal BIO Vita 1.0 L ha⁻¹ at the background of pre-sowing treatment of seeds with Wuxal BIO Vita at the rate 1.0 L t⁻¹ the indexes of GST activity increased on the 3rd day compared to the control by 40; 49 and 53%, and on the 10th day by 23; 37 and 40% respectively. Obtained results of GST activity in the variants of a combined application of herbicide and PGR at the background of pre-sowing treatment of seeds with PGR show the balancing of pro-antioxidant status of the plants of emmer wheat plants.

The experimental data, presented in this article, give us grounds to state that under separate application of herbicide Prima Forte 195 (without PGR) MDA content in the leaves of emmer wheat is higher than in the case of a combined application of herbicide with PGR in the seeds both treated and untreated before sowing with the same PGR. The same factual findings were demonstrated on the example of pea with the application of 2.4-Dichlorophenoxyacetic acid (which is one of the components of herbicide Prima Forte 195), where MDA level increased considerably and which served as a destroying factor of haloid phenoxyacetic acids (Shevchenko et al., 1980). At the same time, combined application of herbicide and PGR at the background of pre-sowing treatment of seeds with PGR fostered the decrease of MDA content in plants. Thus, we can summarize that under complex application of herbicide and PGR the level of oxidative stress in plants slightly decreases. As to the GST activity in the leaves of emmer wheat the experiment showed its increase under lower MDA content, in particular, in the variant with the complex application of herbicide and PGR, especially at the background of pre-sowing treatment of seeds with PGR. We can, therefore, state that there is activation of metabolic processes in plants, including detoxification processes. As our previous research showed, herbicides and PGR, having different mechanisms and course of action, don't vie for the common sites (biological targets). At the same time, their complex action shows a specific form of interaction, that can be characterized as antidotal, which implements through the activation of detoxification systems of plants (Karpenko et al., 2016).

CONCLUSIONS

1. Herbicide action of Prima Forte 195, especially under the higher norms of its application, causes oxidative stress in the plants of emmer wheat that manifests in the accumulation of MDA, the product of peroxide oxidation of lipids. MDA content in the leaves of emmer wheat as a component of a signal system of the adaptive protection of a plant body considerably increases under separate action of herbicide, while it decreases under complex action of herbicide Prima Forte and PGR Wuxal BIO Vita.

2. GST activity in the leaves of emmer wheat is aimed at the decrease of oxidative stress and the formation of plant resistance to the action of herbicide agent, as evidenced by the decrease of MDA content in the corresponding variants of the experiment, where GST activity was the highest.

3. Complex application of herbicide Prima Forte 195 at the rates of 0.5; 0.6 and 0.7 L ha⁻¹ with the plant growth regulator Wuxal BIO Vita at the rate of 1.0 L ha⁻¹ at the background of pre-sowing treatment of seeds with the same PGR at the rate of 1.0 L t⁻¹, obviously causes faster pace of detoxification of xenobiotic in plants, because in the present variants of the experiment there is increased GST activity under decreased MDA content.

REFERENCES

- Agostinetto, D., Perboni, L.T., Langaro, A.C., Gomes, J., Fraga, D.S. & Franco, J.J. 2016. Changes in Photosynthesis and Oxidative Stress in Wheat Plants Submmited to Herbicides Application. *Planta Daninha*. **34**(1), 1–9.
- Baimuhametova, E.A., Taipova, R.M. & Kuluev, B.R. 2016. Glutathione and glutathione stransferases: key components of the antioxidant protection system of plants. *Biomics*. **8**(4), 311–322 (in Russian).
- Bilonozhko, V.Ya., Karpenko, V.P., Poltoretskyi, S.P. & Prytuliak, R.M. 2012. Physiological and biochemical processes in the plants of spring barley under separate and integrated application of herbicides and plant growth regulators. *Bulletin of Poltava state agrarian academy* **2**, 7–13 (in Ukrainian).
- DeRidder, B.P. & Goldsbrough, P.B. 2006. Organ-Specific Expression of Glutathione S-Transferases and the Efficacy of Herbicide Safeners. Arabidopsis. Plant Physiology 140(1), 167–175.
- Dixon, D.P. & Edwards, R. 2010. Glutathione transferases. *The arabidopsis book* 8:e0131. doi:10.1199/tab.0131.
- Dixon, D.P., Cummins, I., Cole, D.J. & Edwards, R. 1998. Plant glutathione S-transferases and herbicide detoxification. *Current Opinion in Plant Biology* 1(3), 258–266.
- Edwards, R. & Dixon, D.P. 2000. The role of glutathione transferases in herbicide metabolism. In Cobb, A.H. and Kirkwood, R.C. (eds.): *Herbicides and Their Mechanisms of Action*. Sheffield Academic Press, Sheffield, pp. 38–71.
- Ehrmantraut, E., Shevchenko, I. & Nenyn, P. 2000. Mathematical analysis and interpretation of research. *Coll. Science. works Institute for Sugar Beet UAAS.* **2**, 189–205 (in Ukraine).
- Enan, M.R. 2009. Genotoxicity of the herbicide 2,4-dichlorophenoxyacetic acid (2,4-D): Higher plants as monitoring systems. *Am.-Eurasian J. Sustain. Agric.* **3**(3), 452–459.
- Gill, S.S. & Tuteja, N. 2010. Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. *Plant. Physiol. Biochem.* **48**(12), 909–930.

- Grishko, V.N. & Syshchikov, D.V. 1999. Peroxide oxidation of lipids and functioning of some antioxidant enzyme systems in maize and oat under severe affection by hydrogen fluoride. *Ukrainian biochemical journal* **71**(3), 51–57 (in Russian).
- Habig, W.H., Pabst, M.J. & Jakoby, W.B. 1974. Glutathione S-transferase. The first step in mercapturic acid formation. *Journal Biol. Chem.* 249(22), 7130–7139.
- Hassan, N.M. & Nemat Alla, M.M. 2005. Oxidative stress in herbicide-treated broad bean and maize plants. *Acta Physiol. Plant.* **27**(4), 429–438.
- Hesammi, E. 2011. Evaluation of herbicides Nicosulphron and Glayphusit On some weeds. *Advances in Environmental Biology* **5**, 2680–2682.
- Hrycajenko, Z.M., Hrycajenko, A.O., Karpenko, V.P. 2003. Soil Nutrient Analysis. In Hrycajenko, Z.M (ed.): *Methods of biological and agrochemical investigations of plants and soil*. Nichlava, Kyiv, pp. 197–201 (in Ukrainian).
- Jiang, L. & Yang, H. 2009. Prometryne-induced oxidative stress and impact on antioxidant enzymes in wheat. *Ecotoxicol. Environ. Saf.* **72**(6), 1687–1693.
- Kalashnikov, Yu.Ye., Balakhnina, N.I. & Bennichelli, R.P. 1999. Activity of antioxidant system and intensity of peroxide oxidation of lipids in the plants of wheat in relation to resistance to water-logged soil. *Plant physiology* **46**(2), 268–275 (in Russian).
- Karpenko, V.P. & Pavlyshyn, S.V. 2018. Activity of antioxidant enzymes in plants of amelcorn under the influence of Prima Forte 195 herbicide and Wuxal BIO Vita plant growth regulator. *Bulletin of agrarian science of Black Sea region* 99 (3), 61–65 (in Ukrainian).
- Karpenko, V.P., Hrytsayenko, Z.M., Prytuliak, R.M., Poltoretskyi, S.P., Mostoviak, I.I. & Fomenko, O.O. 2012. *Biological principles of integrated action of herbicides and plant* growth regulators. Sochinskyi, Uman, 357 pp. (in Ukrainian).
- Karpenko, V.P., Prytuliak, R.M., Datsenko, A.A. & Ivasiuk, I.I. 2016. Physiological and biochemical mechanisms integrated herbicide and plant growth regulator. *Bulletin of Uman National University of Horticulture*. 1. 72–75 (in Ukrainian).
- Kobylinska, L.I. & Tymochko, M.F. 2000. The role of peroxide-antioxidant balance in the adaptational processes of an organism. *Experimental and clinical phisiol. and biochem.* 12(4), 52–57 (in Ukrainian).
- Kulinskii, V.I. & Kolesnichenko, L.S. 1993. Structure, properties, biological role and regulation of glutathione peroxidase. *Achievements of modern biology* **113**(1), 107–122 (in Russian).
- Kurganova, L.N., Veselov, A.P., Goncharova, T.A. & Sinitsyna, Yu.V. 1997. Peroxide oxidation of lipids and antioxidant protection system in chloroplast of peas under heat shock. *Plant physiology* **44**(5), 725–730 (in Russian).
- Lamoureux, G.L. & Rusness, D.G. 1989. The role of glutathione and glutathione S-transferases in pesticide metabolism, selectivity, and mode of action in plants and insects. In Dolphin, D., Poulson R., Avramovic, O. (eds): *Glutathione: chemical biochemical and medical aspects*. John Wiley & Sons, New York, pp. 153–196.
- Liu, L., Liu, Y., Rao, J., Wang, G., Li, H., Ge, F. & Chen, C. 2013 Overexpression of glutathione S-transferase gene from the fruits of *Pyrus pyrifolia* raises resistance of transgenic tobacco plants to abiotic stress. *Molecular biology*. 47(4), 591–601 (in Russian).
- Mitieva, L.P., Ivanov, S.V. & Aleksiyeva, V.S. 2010. Change of pool of glutathione and some enzymes of its metabolism in the leaves and roots of pea, treated with herbicide glyphosate. *Plant physiology* **57**(1), 139–145 (in Russian).
- Neuefeind, T., Reinemer, P. & Bieseler, B. 1997. Plant glutathione S-transferases and herbicide detoxification. *Biol. Chem.* 378(3–4), 199–205.
- Owen, W.J. 2000. Herbicide metabolism as a basis for selectivity. In Roberts, T. (ed.): *Metabolism of Agrochemicals in Plants*. John Wiley and sons Ltd., London, pp. 211–258
- Rochalska, M. & Grabowska, K. 2007. Influence of magnetic fields on the activity of enzymes: α - and β - amylase and glutathione S-transferase (GST) in wheat plants. *International Agrophysics.* **21**, 185–188.

- Rogozhin, V.V. 2006. *Practical guide on biological chemistry*. Lan. St. Petersburg, 256 pp. (in Russian).
- Rossikhina, A. & Vinnichenko, O. 2004. Herbicide treatment influence on lipoperoxidation and its regulation system in maize grain. *Visnyk of L'viv Univ. Biology series.* **37**, 227–231. (in Ukraine).
- Shevchenko, N.V., Pohosian, S.I. & Merzliak, M.N. 1980. Peroxide oxidation of membrane lipid under the action of haloid phenoxyacetic acids. *Plant physiology*. **27**(2), 363–369 (in Russian).
- Shorning, B.Yu., Smirnova, Ye.G., Yaguzhynskii, L.S. & Vaniushyn, B.F. 2000. Necessity of superoxide formation for the development of etiolated of wheat sprouts. *Biochemistry*. 65(12), 1612–1618 (in Russian).
- Song, N.H., Zhang, S., Hong, M. & Yang, H. 2010. Impact of dissolved organic matter on bioavailability of chlorotoluron to wheat. *Environ. Pollut.* **158**(3), 906–912
- Van Camp, W., Van Montagu, M. & Inze, D. 1994. Superoxide dismutase. In Foyer, C.H. and Mullineaux, P.M. (eds.): *Causes of Photooxidative Stress and Amelioration of Defense Systems in Plants.* Boca Raton, FL: CRC Press, Florida, pp. 317–341.
- Yin, X.L., Jiang, L., Song, N.H. & Yang, H. 2008. Toxic reactivity of wheat (*Triticum aestivum*) plants to herbicide isoproturon. J. Agric. Food. Chem. **56**(12), 4825–4831.
- Zama, P. & Hatzios, K.K. 1986. Effects of CGA-92194 on the chemical reactivity of metolachlor with glutathione and metabolism of metolachlor in grain sorghum (*Sorghum bicolor*). Weed Science. **34**(6), 834–841.
- Zhurbickij, Z.I. 1968. Theory and practice of vegetation method. Nauka, Moskva. 268 pp. (in Russian).