Ukrainian Journal of Ecology

Ukrainian Journal of Ecology, 2018, 8(1), 148–152 doi: 10.15421/2018_199

ORIGINAL ARTICLE *UDC 581.131*

Activation of antioxidant enzymes and content of hydrogen peroxide in winter wheat leaves by deficit of soil nitrogen

T.P. Mamenko

Institute of Plant Physiology and Genetics,
National Academy of Science of Ukraine, Kyiv, Ukraine
31/17 Vasylkivska Str., Kyiv, 03022, Ukraine, (050)964-17-89. E-mail: t_mamenko@ukr.net
Submitted: 13.12.2017. Accepted: 18.01.2018

We proved that the insufficient supply of soil nitrogen, induces defense reactions in winter wheat, as evidenced by the increase in hydrogen peroxide and the activity of antioxidant enzymes in leaves. Treatment of plants with carbamide on a low nitrogen background, induced a decrease in the content of hydrogen peroxide in leaves of varieties of winter wheat, which may indicate a low level of oxidative processes in plants due to the inclusion of antioxidant enzymes in stress conditions. At the same time, the intensification of the activity of antioxidant enzymes in leaves of varieties of winter wheat for the low nitrogen nutrition of plants was recorded. Foliar dressing of winter wheat urea is regarded as a kind of stress on the plant, on the one hand, and on the other, as a factor that stimulates of inclusion the protective mechanisms, in the above privacy and activation of work in enzymes of ascorbate-glutathione cycle and superoxide dismutase. This contributes to a better realization of the genetic potential of winter wheat varieties.
Key words: *Triticum aestivum* L.; superoxide dismutase; glutathione reductase; ascorbate-peroxidase; hydrogen peroxide; nitrogen

Introduction

Support for high assimilation of nutrients is very important for increasing of yields, along with the creation of new varieties with high genetic productivity (Morgun et al., 2010). When cultivating wheat, it is necessary to consider great number of factors that affect the productivity and quality of the grain, which to a large extent depend on the genetic potential of the variety, soil and climatic conditions of cultivation, fertilizer systems, etc. (German, 2012; Hruskov, Svec, 2009; Li et al., 2014; Sedlar et al., 2015).
Applying mineral fertilizers, special attention should be paid to the provision of wheat with nitrogen fertilizers, since the protein is formed due to the mobilization of nitrogen stems and leaves. They need to be introduced so that the plants are supplied with nitrogen constantly and sufficiently during the vegetation (German, 2012; Li et al., 2014; Sedlar et al., 2015). Reducing the level of mineral nutrition, including nitrogen, leads to inhibition of assimilation activity of the plant and, ultimately, to reduce its biological and economic productivity (Morgun et al., 2010; Sokolovska-Serhiyenko, Kiriziy, 2013). Lack of power supplies also limits the ability of plant protective systems to respond to the action of stressors and mitigate their negative effects, that is is a kind of stressful factor of productivity (Sokolovska-Serhiyenko, Kiriziy, 2013).
Plants grow in constantly changing environmental conditions and undergo various stressors, causing a shift in the balance of oxidants-antioxidants in the direction of oxidants, which is the cause of internal oxidative stress (Kreslavskyy et al., 2012). The decisive role in the adaptation of plants to the effects of adverse environmental factors belongs to biochemical protection systems. Among them, considerable attention is paid to the clarification of the role of antioxidant enzymes in metabolism and the formation of plant resistance through stressors. Antioxidant enzymes take part in the neutralization of active forms of oxygen (AFO), the accumulation of which in the plant cell under stress causes initiation of the processes of oxidative destruction of membrane structures. Under optimal conditions, AFO is produced at a low level, mainly in chloroplasts, mitochondria and peroxisomes. Under conditions of stress, their formation can increase sharply and inhibit the body's protective systems (Desikan et al., 2001). At the same time, AFO can act as signaling molecules that are involved in the activation of protective systems under stress to induce the synthesis of enzymes of antioxidants (Mynybaeva, Gordon, 2003).
Superoxide dismutase is the primary link in protecting cells and tissues from oxidative degradation, which transforms the superoxide anion radical into a less potent hydrogen peroxide product (Baranenko, 2006), which is very harmful to cells. Destruction of hydrogen peroxide is carried out by a system of antioxidant protection, which involves many enzymes and substrates. In vegetative cells, an important substrate for the reduction of hydrogen peroxide is ascorbate, therefore, the functioning of the ascorbate-glutathione cycle plays an important role in the system of antioxidant protection (Konturska, Palladina, 2012).

Great number of research has been carried out on the study of the functioning of the prooxidant-antioxidant system of plant protection by the actions of stress factors of different nature. However, the study of the influence of different levels of nitrogen feed on the functioning of enzymes antioxidant protection, considering the genotype features of the plant is devoted to not a significant number of works. It is believed that studies of the effects of variations in the level of mineral nutrition on the components of the wheat production process are relevant, especially relating to the problem of further improving the useful physiological features of this culture (Sokolovska-Serhiyenko, Kiriziy, 2013; Morgun, Kiriziy, 2012).
The purpose of our work was to investigate the changes in the content of hydrogen peroxide and the activity of the ascorbate-glutathione cycle enzymes (ascorbate peroxidase and glutathione reductase), as well as superoxide dismutase in the conditions of different levels of mineral nitrogen in the soil and carbamide treatment.

Materials and methods

The study subjects selected varieties of winter wheat (*Triticum aestivum L.*) that differed in their genetic potential for grain yield. Astarta - high-intensity variety, high-yielding direction, Kievskaya opastya and Malinovka - high-protein varieties of intensive type of universal use. The plants of winter wheat were grown in vegetable Wagner vessels with a capacity of 10 kg on a dark gray, podzolized soil, using a high background of nitrogen (N) nutrition at the rate of NPK of 160 mg of active substance per 1 kg of soil and a low background N of nutrition from NPK by 32 mg per 1 kg of soil. The plants were grown for optimal water supply and natural light. At the end of the flowering phase - the beginning of the milk-wax ripeness of winter wheat was sprayed with a solution of carbamide at a concentration of 1% at a rate of 7 kg ai/ha. To carry out research, the leaves of winter wheat were checked on the 7th day after the endocrine treatment of plants.
The content of hydrogen peroxide (H_2O_2) was determined by color reaction with rhodanium potassium spectrophotometrically at a wavelength of 480 nm (Sagisaka, 1976). The concentration of H_2O_2 was determined using a calibration curve constructed with known concentrations of H_2O_2. For extraction of enzymes, the leaves, pre-frozen in liquid nitrogen, were homogenized in 50 mM phosphate buffer (pH 7.2) containing 2 mM EDTA, 1 mM phenylmethylsulfonyl fluoride, 5 mM β-mercaptoethanol and 1% (w/o) polyvinylpyrrolidone. The homogenate was centrifuged at 10,000 rpm for 20 min at 4°C. The supernatant was used to determine the activity of enzymes spectrophotometrically. The activity of ascorbate peroxidase (APO) (1.11.1.11) was determined by Nakano and Asada (1981). The reaction mixture contained 50 mM potassium phosphate buffer (pH 7.2), 0.1 mM EDTA, 0.2 mM ascorbate, 0.1 mM H_2O_2. The reaction was initiated by the addition of a supernatant. The decrease in ascorbate content was recorded spectrophotometrically at 290 nm нм (ε = 2,8 мМ$^{-1}$см$^{-1}$). The activity of glutathione reductase (GR) (1.6.4.2) was determined as described in Halliwell and Foyer (1978). The reaction mixture contained 50 mM potassium phosphate buffer (pH 7.8), 0.12 mM NADF · H, 0.5 mM oxidized glutathione. The reaction was initiated by the addition of a supernatant. Reduction of NADP · H content in the incubation medium was recorded spectrophotometrically at 340 nm (ε = 6,2 мМ$^{-1}$см$^{-1}$). The activity of superoxide dismutase (SOD) (1.15.1.1) was determined by the ability of the enzyme to inhibit photochemical reduction of nitrosine blue tetrazolium. The optical density was measured at 560 nm. The unit of activity of SOD was taken by the amount of enzyme required to inhibit photodetection of nitrosine tetrazolium by 50% (Giannopolitis, Ries, 1977). The protein content was determined using the Bradford method (Bradford, 1976). Experiments were carried out in five biological and analytical repeats, the results were considered statistically significant at $P \leq 0.05$, according to Student's criterion.

Results and discussion

It was established that low nitrogen level in winter wheat varieties increased the level of H_2O_2 in the leaves, indicating the development of oxidative processes and the transition of the cellular metabolism of plants to the stress state (Fig. 1).

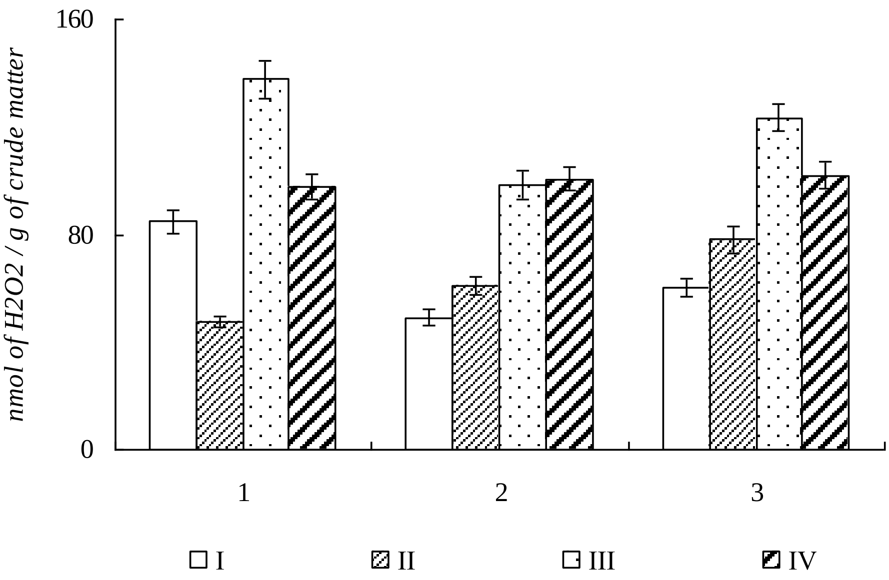

Fig. 1. Content of hydrogen peroxide in leaves of winter wheat at different levels of soil nitrogen. Here and further the varieties of winter wheat are presented: 1 - Astarta, 2 - Kievskaya opasty, 3 - Malinovka (I - high nitrogen background, II - high nitrogen background + carbamide treatment, III - low nitrogen background, IV - low nitrogen background + treatment carbamide), (M ± m, n = 5-7).

At the same time, the highest level of peroxide was recorded in plants of winter wheat Astarta, in comparison with other varieties, both for lack of nitrogen in the soil, and for optimal nutrition with nitrogen. This indicates the first-ever varietal differences in the metabolism of plants of this variety from two other varieties due to their genetic features.

Root treatment of plants with carbamide induced a decrease in the content of H_2O_2 in leaves of the Astarat variety, both in short supply and in the optimum level of nitrogen in the soil. In the varieties of Kyiv spruce and Malinovka, there was an increase in the content of peroxide in the leaves for sufficient nutrition with nitrogen and a slight increase and decrease in the lack of nitrogen in the soil (Fig. 1).

Reducing the content of hydrogen peroxide in the leaves for processing carbamide in winter wheat varieties may indicate a decrease in the level of oxidative processes in plants due to the inclusion of protective antioxidant systems, including antioxidant enzymes, under stress conditions - low nitrogen supply. Increasing the level of peroxide in the leaves treated with carbamide plants of wheat for optimal nitrogen supply, in our opinion, can be considered a protective reaction of plant metabolism to treatment. It has been proved that H_2O_2 plays the role of a secondary messenger in signaling schemes including plant protection systems against stress induction of synthesis of enzymes-antioxidants (Kreslavskyy et al., 2012; Kolupaev et al., 2011). AFO exhibit high activity and easily damage membranes and various cellular components, resulting in the mobilization of various protective systems that reduce the formation of AFK and enhance their neutralization. To do this, the cell induces the synthesis of de novo antioxidant enzymes (SOD, catalase, APO, GR) and / or the activation of their pre-existing forms, as well as the accumulation of low molecular weight antioxidants (ascorbate, glutathione, tocopherols, flavonoid (Kreslavskyy et al., 2012; Mynybaeva, Gordon, 2003; Kolupaev et al., 2011).

One of the main sources of AFO generation is the process of photosynthesis. Unfavorable environmental conditions inhibit the functioning of the Calvin cycle, which leads to a decrease in the speed of linear photosynthetic electron transport (ETL) and to the reversibility of components of the electron transport chain of photosynthesis and stromal acceptors of electrons (Kreslavskyy et al., 2012). The consequence is the development of oxidative stress in plants, which is accompanied by the formation of such AFO as superoxide radical ($O^{2-\cdot}$) and hydroxyl radical (OH·), as well as singlet oxygen ($^1O^2$) and H_2O_2 (Desikan et al., 2001; Baranenko, 2006; Kolupaev et al., 2011). Excessive AFC formation leads to a decrease in the electron transport speed in ETL in photosynthesis, which results in the activation of an alternative pseudocyclic transport of electrons and photo-breathing. Under these conditions, $O^{2-\cdot}$ - and $^1O^2$ are initially formed, after which the formation of H_2O_2 occurs or by the reaction of disproportionation of superoxide by means of SOD, or not enzymically in the process of diffusion of superoxide with a small reaction output. H2O2 is reduced to water with the participation of ascorbate peroxidase and ascorbate. The latter is oxidized and then regenerated by reduced glutathione at the expense of NADF · H (Kreslavskyy et al., 2012).

We found that insufficient nitrogen in the soil leads to an increase in the activity of antioxidant enzymes in leaves of winter wheat. At the same time, the activity of SOD in the leaves of Astarta and APO and GR in the varieties of Kiev spruce and Malinovka is particularly intensive (Figs 2-4).

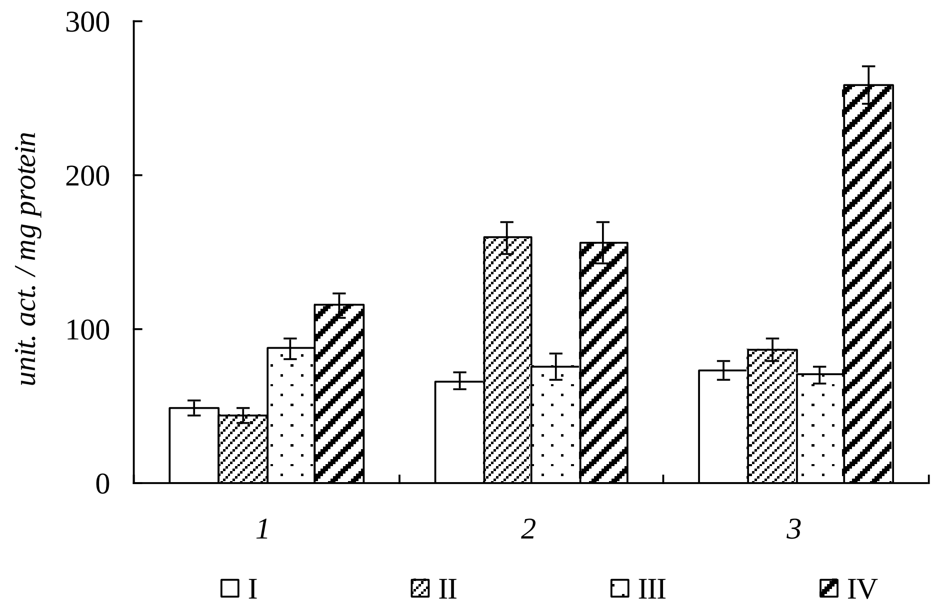

Fig. 2. Activity of superoxide dismutase in leaves of winter wheat at various levels of soil nitrogen.

It was noted that the increase in the activity of APO and GR in leaves of plants that suffered from lack of nitrogen in the soil was accompanied by a slight increase in the activity of SOD in the leaves, which is obviously due to the effective work of the ascorbate-glutathione cycle enzymes in the disinfection of hydrogen peroxide resulting from the reaction dismutations of superoxide radicals ($O_2^{\cdot-}$).

It is known that the formation of the photosynthesis and respiration superoxide radical $O_2^{\cdot-}$ formed during the work of the electron transport chain is neutralized with the participation of SOD with the formation of hydrogen peroxide (Baranenko, 2006). In the elimination of peroxide, a complex of enzymes is involved: catalase, the family of peroxidases, as well as ascorbate-glutathione cycle enzymes - APO and GR. The cycle of APO, which requires two ascorbate molecules to restore H_2O_2 to water, is required, which is followed by the formation of two molecules of monodehydroascorbate with the subsequent rapid formation of ascorbate and dehydroascorbate. Recovery of dehydroascorbate to ascorbate carries dehydroascorbate reductase (1.8.5.1), which uses glutathione as a reducing agent and forms glutathione disulfide. Completes the GR cycle, which restores glutathione disulfide to glutathione with NADPH (Konturska, Palladina, 2012). A large amount of data on the functioning of the ascorbate-glutathione cycle enzymes has been accumulated due to the actions of various stressors (Sokolovska-Serhiyenko, Kiriziy, 2013; Kreslavskyy et al., 2012; Mynybaeva, Gordon, 2003; Konturska, Palladina, 2012).

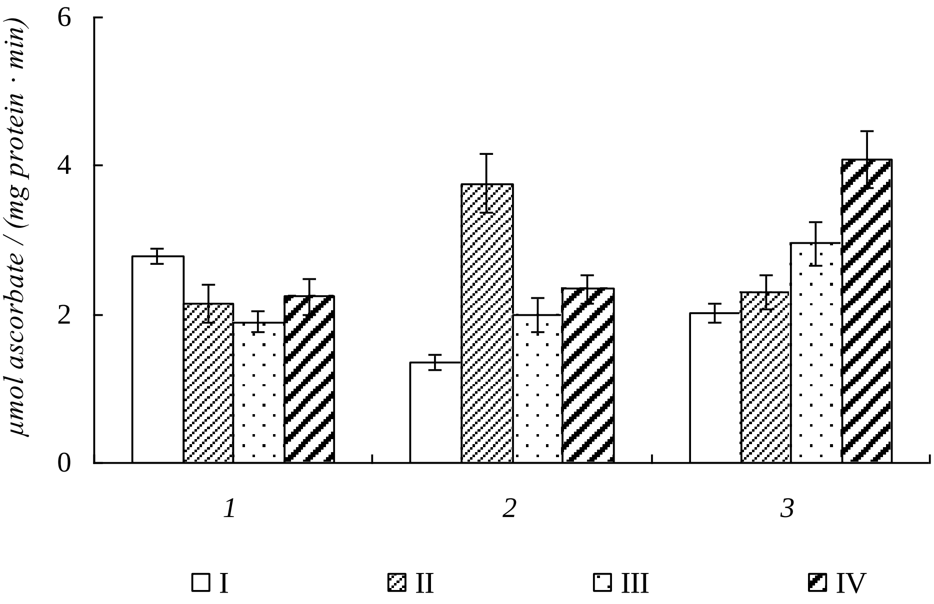

Fig. 3. Activity of ascorbate peroxidase in leaves of winter wheat at different levels of soil nitrogen.

We have investigated that treatment of plants with carbamide on a low nitrogen background induces an increase in the activity of SOD, APO and GR in leaves of varieties of winter wheat, as compared to untreated plants. Particularly noteworthy is the Malinovka variety, which has the highest activity of all these enzymes. For the treatment of plants with urea on the background of optimal plant maintenance with nitrogen in the soil, there was a slight intensification of the work of antioxidant enzymes, compared with untreated plants. Under such conditions, the cultivar was distinguished by the Kyiv spinach variety, which had the highest activity of SOD, APO and GR.
According to the results obtained for the treatment of plants with carbamide, there is an increase in the activity of antioxidant enzymes, regardless of the level of plant nutrition. In our opinion, the endocrine processing of winter wheat by carbamide in the concentrations used by us can be compared with the peculiar stress for plants, which stimulates the development of protective reactions and the restructuring of plant metabolism to appropriate conditions of cultivation.

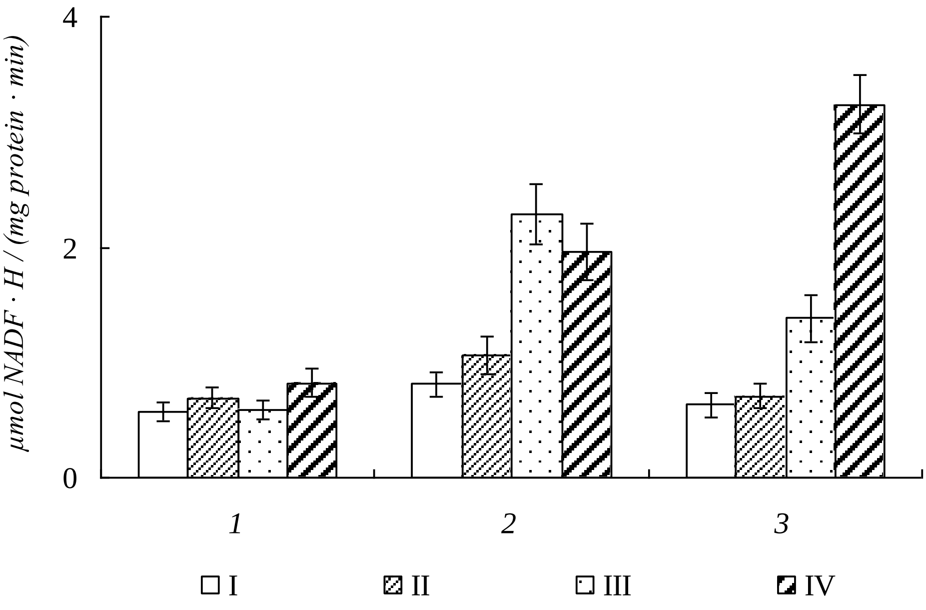

Fig. 4. Activity of glutathione reductase in leaves of winter wheat at various levels of soil nitrogen.

Thus, insufficient provision of winter wheat by nitrogen in the soil induces the development of stress-protective reactions in plants, as evidenced by the increase in the level of hydrogen peroxide and the activity of enzymes in antioxidant protection in the leaves. Extracurricular treatment of winter wheat carbamide is considered as a peculiar stress for plants, on the one hand, and on the other hand, as a factor stimulating the inclusion of protective mechanisms, the activation of the work of the ascorbate-glutathione cycle enzymes and superoxide dismutase.

References

Baranenko, V.V. (2006). SOD in the cells of plants. Cytology, 48(6), 465–474. (In Ukrainian)
Bradford, M.A (1976). Rapid and sensitive method for the quantitation of the microgram quantities of protein utilising: the principle of protein–dye binding. Anal. Biochem, 72, 248–254.
Desikan, R., Mackerness, S.A.–H., Hancock, J.T., Neill, S.J. (2001). Regulation of the Arabidopsis Transcriptome by Oxidative Stress. Plant Physiol, 127, 159–72.
German, M. (2012). Effect of fertilizer and seed treatment preplant the formation of the physical properties of dough and baking quality of wheat soft winter. Bulletin of Poltava State Agrarian Academy, 1, 99–102. (In Ukrainian)
Giannopolitis, C.N., Ries, S.K. (1977). Superoxide Dismutase. 1. Occurrence in higher plants. Plant Physiol, 59(2), 309–314.
Halliwell, B., Foyer, C.H. (1978). Properties and physiological function of a glutathione reductase purified from Spinach leaves by affinity chromatography. Planta, 139, 388–396.
Hruskov, M., Svec, I. (2009). Wheat Hardness in Relation to Other Quality Factors. Czech J. Food Sci, 27(4), 240–248.
Kolupaev, J.E., Karpets, U.V., Oboznyi, A.I. (2011). Antioxidant system of plants, participate in cell signaling system and for adaptation to action stressors. Bulletin of Kharkiv National Agrarian University, 1(22), 6–34 (in Ukrainian).
Konturska, A.A., Palladina, T.A. (2012). Activity enzyme ascorbate–glutathione cycle in the leaves of maize seedlings under conditions of salinity and processing adaptogenic agents. Ukr. Biochem. J, 84(6), 139–144. (In Ukrainian)
Kreslavskyy, V.D., Los, D.A., Allakhverdiev, S.I., Kuznetsov, V.I. (2012). Syhnalnaya role of reactive oxygen at syresse in plants. Physiology of plants, 59(2), 163–178. (In Russian)
Li, M., Yang, X.W., Tian, X.H., Wang, S.X., Chen, Y.L. (2014). Effect of nitrogen fertilizer and foliar zinc application at different growth stages on zinc translocation and utilization efficiency in winter wheat. Cereal Res. Com, 42(1), 81–90.
Morgun, V.V., Kiriziy, D.A. (2012). Prospects and modern strategies of improving the physiological characteristics of wheat to improve performance. Physiology and biochemistry cult. Plants, 44(6), 463–483. (In Ukrainian)
Morgun, V.V., Schwartau, V.V., Kyryzyy, D.A. (2010). Physiological principles of formation peak productivity grain cereal. Physiology and biochemistry cultural plants, 42(5), 371–393. (In Ukrainian)
Mynybaeva, F.V., Gordon, L.K. (2003). Products extracellular superoxide and peroxidase activity in plants tissues under the stress. Physiology of plants, 50(3), 459– 464. (In Russian)
Nakano, Y., Asada, K. (1981). Hydrogen peroxidase is scavenged by ascorbate–specific peroxidase in spinach chloroplasts. Plant Cell Physiol, 22(5), 867–880.
Sagisaka, S. (1976). The occurrence of peroxide in a perennial plant, Populus gelrica. Plant Physiol, 57(2), 308–309.
Sedlar, O., Balik, J., Cerny, J., Peklova, L., Kulhanek, M. (2015). Nitrogen uptake by winter wheat (Triticum aestivum) depending on fertilizer application. Cereal Res. Com, 43(3), 515–524.
Sokolovska–Serhiyenko, A.G., Kiriziy, D.A. (2013). The intensity of photosynthesis and antioxidant enzymes leaves of winter wheat under different conditions of mineral nutrition physiology and biochemistry. Physiology and biochemistry cultural plants, 45(3), 206–214. (In Ukrainian)

Citation:
Mamenko, T.P. (2018). Activation of antioxidant enzymes and content of hydrogen peroxide in winter wheat leaves by deficit of soil nitrogen. *Ukrainian Journal of Ecology, 8*(1), 148–152.