UDC 581.143.01/07:577.125

Formation of Nonspecific Resistance in Winter Wheat Plants by the Hydrogen Peroxide Treatment

L. M. Batsmanova, M. M. Musienko

Kyiv Taras Shevchenko National University
64, Volodymyrska Str., Kyiv, Ukraine, 01033

e-mail: l.batsmanova@gmail.com

Received on April 16, 2014

Aim. The aim of the investigation was to study the effect of hydrogen peroxide on the activity of antioxidant enzymes, pigments' content and productivity of winter wheat in order to determine the biochemical mechanisms of induced winter wheat plant resistance during ontogenesis in the field. **Methods.** The study of physiological and biochemical parameters were carried out on winter wheat varieties of the wooded steppe (Poliska 90) and steppe (Skala) ecotypes at the tillering and flowering phases. The experimental plants were foliar treated with hydrogen peroxide (H_2O_2) in concentration of $1 \cdot 10^{-1}$ M twice, with a 3-day-interval, while the control ones – with distilled water. Spraying of plants was performed at spring (tillering phase). Lipid peroxidation (LPO) was determined as the formation of a peroxidation end product — malondialdehyde, which content was determined as the extinction of its condensation product with thiobarbituric acid. The activity of superoxide dismutase (SOD) was determined by a method based on the ability of SOD to inhibit nitroblue tetrazolium recovery by superoxide radicals in the light in the presence of riboflavin and methionine. The activity of catalase was calculated using the extinction coefficient of − 39.4 mM^{-1} cm^{-1}. **Results.** It was established that after 24 h of hydrogen peroxide treatment the processes of lipid peroxidation were intensified and antioxidant enzymes' activity was decreased in two varieties. In the next phase of ontogenesis (flowering) antioxidant enzymes' activity increased in both varieties: Poliska 90 and Skala, which suppressed the LPO growth. **Conclusion.** Treatment of plants with hydrogen peroxide promoted the formation of non-specific plant resistance and increased grain productivity of winter wheat varieties investigated.

Keywords: winter wheat, hydrogen peroxide, nonspecific resistance, superoxide dismutase, catalase, malonic dialdehyde.

INTRODUCTION

In Ukraine, just as in other regions of intensive agricultural production, the cultivation of high-quality agricultural products depends on the sharp fluctuations of weather conditions in the period of the plant vegetation. Nowadays, in terms of the progressive global climate changes on the planet the effect of the unfavorable factors of environment becomes more tangible and determines the position of the country on the world agrarian market.

In many countries of the world the limit of the ecologically permissible anthropogenic load on agricultural lands has been already reached. The application of technogeneous means (fertilizer, ameliorants, pesticides, herbicides, biologically active substances) in the agricultural technologies frequently leads to ecological imbalance and forming of the transformed agroecosystem and agro-landscapes. The intensification of plant growing and development of agrarian sector become the leading factors of negative influence on the biosphere (in particular, chemical and biogenic ones), including the genetic environmental pollution. Therefore, ecologically safe systems and technologies should be created on the basis of the study of pharmacology and biochemistry of plants. This will allow to create the diverse regulators of plant growing and fertilizer, antistress substances of natural origin, also to govern the endogenous regulator systems strictly of plants, avoiding the application of external chemical regulators.

An increase in the stability of the programmed harvests with the high quality of production in terms of deviation from the optimum meteorological factors (low and high temperatures, frost, ground and atmospheric droughts, hypoxia, anoxia, etc.) is one of the main trends in further development of agro-technologies because of the expansion of range of the standard for the physiological response of the plants through the disclosure of the endogenous mechanisms of the regulator systems in a plant organism, and also as a result of the use for this of exogenous, ecologically safe, natural physiologically active materials. It is possible to diminish the dependence of agricultural production on the unfavorable action of external environmental factors to a considerable extent due to the regulation of the plant adaptive capacities. The adaptive reconstructions of the protective compensatory responses of a plant organism to the stress factors play an important role in this process.

The study of the issues of physiological stability and adaptation of plants to oxidizing damages is an integral part of the basic researches devoted to the general adaptive response of an organism to the unfavorable environmental factors. The explanation of the mechanisms of the various extremely important for predicting the effect on the plants abiotic and biotic stress factors' influence is the basis of the search for the methods of decreasing their negative effect on agricultural crops. The physiological and biochemical changes, which define the plant immunity to the effect of oxide stress, is an objective test both for early diagnostics of stability level of the created forms and types, their selection under laboratory conditions and for the biochemical monitoring in transformed agrocoenosis.

There have been recorded instances for the data relative to the positive influence of exogenous hydrogen peroxide on the stability of plants to the effect of low [1] and high [2] temperatures, osmotic [3] and salt [4] stresses. We have already proven that the hydrogen peroxide treatment of winter wheat in the tillering phase increases the grain productivity of plants [5]. Since the majority of such studies are carried out in the simulation conditions, the special features of the unspecific stability forming in the winter wheat plants in ontogenesis in field conditions as a result of the natural shielding mechanisms, in particular, antioxidant systems, activation by hydrogen peroxide, has been insufficiently studied till yet. Therefore, the analysis of the biochemical mechanisms of the plant induced stability is extremely urgent. Due to it, the purpose of the current research has been the study of the hydrogen peroxide effect on the antioxidant ferments activity, pigments content and productivity of the winter wheat.

MATERIALS AND METHODS

Physiological biochemical indices were explored for the winter wheat plants of the wooded steppe (Poliska 90) and steppe (Skala) ecotypes in the tillering and flowering phases. Plants were cultivated in course of small-plot (10 m^2) experiments in the fields of the Institute of Agriculture NAAS of Ukraine in conditions of the Ukrainian Polissia on the gray forest light-clay soils. Agricultural methods of cultivation were conventional for the zone.

For studying the nonspecific resistance forming processes the experimental plants were foliar treated with H_2O_2 in concentration of $1 \cdot 10^{-4}$ M twice with the 3-day-interval, while the control ones – with distilled water. Plants were sprayed in spring (in the tillering phase), when the thickness of the cuticular layer of leaves and stems was insignificant, as that is the prerequisite of the acting substance penetration. The H_2O_2 solution expenditure for 1 m^2 was 1 l. The plants response to the stress factor was analyzed both after 24 h, and later (during the next ontogenesis phase – flowering) the plants adaptation degree.

The peroxide oxidation of endogenous lipids (LPO) was determined in the supernatant liquid of plant tissues' homogenate judging from the formation of one of the end products of peroxidation – the malonic dialdehyde (MDA), which content was determined due to the value of its condensation product extinction of with thiobarbituric acid [6]. The content of pigments was estimated by the spectrophotometer method [7].

The activity of superoxide dismutase (SOD) (KF 1.15.1.1) was estimated using to the Chevari *et al*. technique. [8]. The hinge of leaves with the mass of 300 mg was homogenized in 20 ml 50 mM of potassium phosphate buffer, pH 7.8. Furthermore, the homogenate was centrifuged during 15 min at 7000 g (with cooling); supernatant was used as the unpurified extract of SOD cytosol fraction. Incubation medium contained 1 ml of 1.3 mM riboflavin, 1 ml of 13 mM methionine, 1 ml of 63 M nitroblue tetrazolium (NBT), 1 ml of 0.1 mM edetic acid and 0.5 ml of fermentation extract. The reaction was initiated with the addition of riboflavin with the subsequent incubation during 20 min in white light (illumination of 4000 lx at the level of test tubes). A maximum amount of formazan was created in the variant without the fermentation extract (50 mM potassium

phosphate buffer, pH 7.8). The optical density at the wavelength of l = of 560 nm was measured against the control version, which was kept in darkness. The method is based on the SOD ability to inhibit the restoration of nitroblue tetrazolium with the radicals of superoxide in light in presence of riboflavin and methionine. The 50 per cent suppression of the formazan formation was taken as the unit of the ferment activity.

The catalase activity (KF 1.11.1.6) was determined according to Kumar [9]. 1 g of leaf tissue was grinded with 10 ml of 50 mM phosphate buffer, pH 7.0. The homogenate was filtered and centrifuged during 10 min at 8000 g (with cooling). 25 µl of fermentation extract and 90 µl of 3 per cent H_2O_2 solution were added to 2.9 ml of phosphate buffer. The optical density was measured at l = of 240 nm. The catalase activity was calculated with the use of an extinction coefficient – 39.4 mM^{-1} cm^{-1}.

The H_2O_2 influence on the production process of wheat was evaluated according to the elements of the harvest structure. A total number of plants and quantity of productive stems per unit of area (units/m^2), quantity of grains in the ear (pcs), height of plant (cm) and mass of 1000 grains (g) were determined [10]. The obtained results were processed by the variation statistics method [11].

RESULTS AND DISCUSSION

As the obtained results stated, the H_2O_2 action in concentration of $1 \cdot 10^{-4}$ M leads to the development of stress in the plants of both varieties: Poliska 90 and Skala, which is characterized with equilibrium shift between prooxidants and antioxidants due to the accumulation of products LPO, in particular MDA (Fig. 1). The LPO processes are more intensively developed in the photosynthetic tissues of the Skala variety, where the MDA content increases by 28 per cent, which suppresses the activity of the antioxidant ferments: the SOD activity decreases by 76 per cent, catalase − by 26 per cent. In the Poliska 90 experimental plants the MDA content raised by 17 per cent; however, this was not reflected in the SOD activity. Moreover, the plant responded to the stress by an increase in this ferment activity by 43 per cent. The catalase activity falls substantially down (by 26 per cent). Perhaps, that is connected to the hydrogen peroxide accumulation in the plant tissues, which is created as a result of the oxidizing explosion and SOD dismutation reaction. Nowadays there is no categorical data on the antioxidant system response to the hydrogen peroxide action in

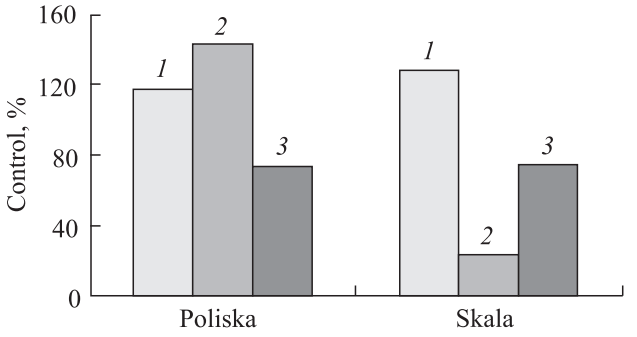

Fig. 1. Correlation between pro- and antioxidants in wheat laves at the H_2O_2effect ($1 \cdot 10^{-4}$ M, 24 h exposition): *1* – malonic dialdehyde; *2* – superoxide dismutase; *3* – catalase1

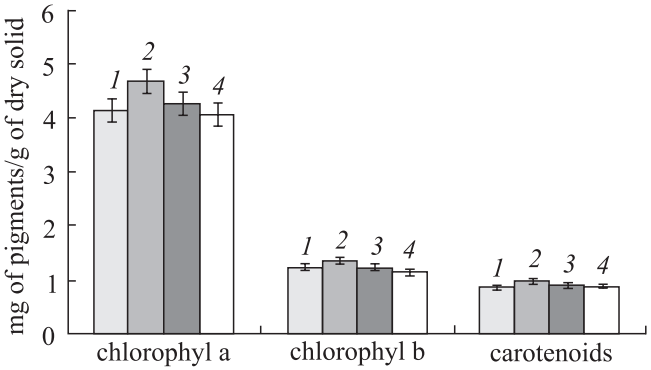

Fig. 2. Pigments content in wheat laves at the H_2O_2 effect (stem extension phase, 24 h exposition): *1* – Poliska 90, control; *2* – Poliska 90, experimental; *3* – Skala, control; *4* – Skala, experimental

the scientific literature. However, it is shown that the reaction depends on the species and variety of plant, their physiological state, and also duration of the stress factor impact. Thus, in leaves of the adult wheat plants treated with H_2O_2 an increase in the catalase activity was observed [12]. Simultaneously, in a number of works either decrease in the activity of antioxidant ferments or absence of changes in their activity in terms of H_2O_2 influence is shown [13].

It should be noted that the development of such an intensive stress does not make an impact on the content of basic photosynthetic pigments in the plants of the Skala variety, whereas the increasing tendency as for their content (11 per cent) has been noted in the Poliska 90 experimental plants (Fig. 2), which provides the nonspecific resistance of photosynthetic apparatus to the stress factor impact. The H_2O_2 role both as a signal molecule and expression regulator for some chloropast and nuclear genes began to be examined relatively recently [14−16]. The literature provides the data on the H_2O_2 regulatory impact both in the photosynthetic ap-

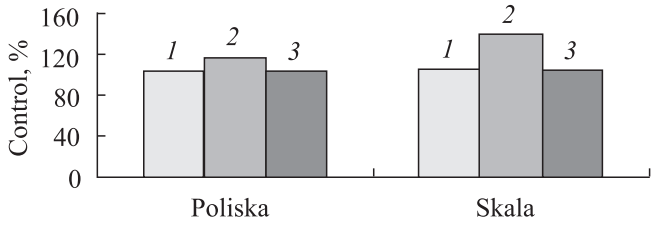

Fig. 3. Correlation between pro- and antioxidants in wheat laves after the H_2O_2 effect ($1 \cdot 10^{-4}$ M, flowering phase): *1* – malonic dialdehyde; *2* – superoxide dismutase; *3* – catalase

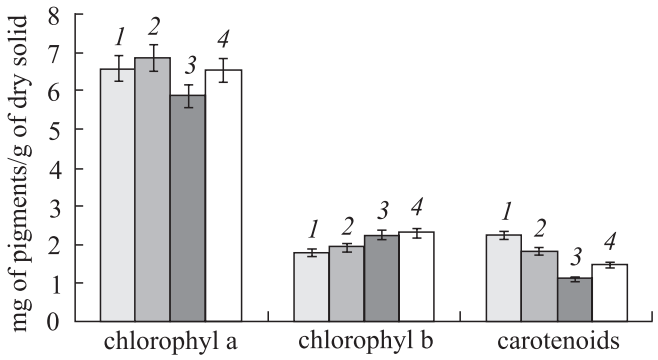

Fig. 4. Pigments content in wheat laves at the H_2O_2 effect (flowering phase): *1* – Poliska 90, control; *2* – Poliska 90, experimental; *3* – Skala, control; *4* – Skala, experimental

paratus functioning [17] and its stress-tolerance forming [18].

Thus, the Poliska 90 winter wheat plants proved to be more resistant in the early development stages to the oxidative stress induced by hydrogen peroxide than the Skala plants.

The issue on the duration period after the stress factor impact, during which the capability for the increased resistance remains, is also noteworthy. As proved by the current research, during the next phase (flowering) the processes of peroxidation decrease and their levels correspond to the control versions (Fig. 3). The increased level of the SOD activity and restoration of the catalase activity in both varieties give evidence on the fact that hydrogen peroxide in concentration of $1 \cdot 10^{-4}$ M serves as the inductor of nonspecific resistance forming in plants due to the activation of endogenous protection systems. No reliable difference between the control and experimental versions in the pigments content of in the Poliska 90 plants during this phase of ontogenesis has been observed (Fig. 4); however, an increase in the chlorophyll content 11 per cent was fixed in the Skala plants.

Since hydrogen peroxide caused an increase in the nonspecific resistance of plants of both types, its positive influence on their production process has been also assumed. As the analysis of the obtained results stated (Table), hydrogen peroxide does not influence on the morphometric indices of the plants of the varieties being investigated. However, 1000 grains' mass in the examined Poliska 90 plants increased by 18 per cent, and in the Skala steppe plants – by 8 per cent. An increase in 1000 grains mass can be caused by the fact that due to the hydrogen peroxide actions up to 20 forms of proteins are *de novo* synthesized [19].

All mentioned above allows to make the conclusion that the plant adaptation strategy to the oxidative stress effect induced by hydrogen peroxide is aimed to the oxidation-reduction homeostasis support implemented in its turn with the aid of a whole series of physiological mechanisms. In particular, hydrogen peroxide causes the expression of many protection genes [20], what causes the forming of the induced stability temporary phenotypic stable, and therefore, unspecific. Thus, the exogenous application of H_2O_2 in low concentrations (~ 10 mM) induces the accumulation of osmoprotector – betaine glycine in barley leaves [21], increases salt resistance in rice sprouts as a result of the antioxidant ferments activation and expression of transcripts, so-called stress-genes, coding saccharine phosphate synthase, pirolin-5-carboxylate synthase and the thermal shock protein 26 [21].

Winter Wheat Varieties Harvest Structure At the Effect of Hydrogen Peroxide

Wheat Variety	Plants per/m^2	Yielding Plants, per/m^2	Plant Height, cm	Grain number per ear	1000 Grain Mass, g	Yield, t^{-1}/ha
Poliska 90,						
control	44 ± 0.6	73 ± 2.3	93.0 ± 1.9	41.1 ± 0,9	38.4 ± 0.9	48.0 ± 1.8
experimental	54 ± 0.9	76 ± 3.4	98.0 ± 1.6	42.2 ± 0,8	45.4 ± 0.7	56.0 ± 1.6
Skala,						
control	40 ± 0.9	85 ± 1.3	73.3 ± 0.3	31.3 ± 0,8	30.1 ± 1.1	43.2 ± 0.9
experimental	30 ± 0.6	71 ± 1.2	78.0 ± 0.3	34.0 ± 0,5	32.3 ± 1.2	46.7 ± 1.2

Thus, taking into account the obtained results and scientific data, it is worthwhile to note that hydrogen peroxide in low concentrations can perform a signal role in the plant organisms in stress conditions, influencing on the expression of transcripts, what induces the synthesis of the proteins, antioxidant ferments among them, and other stress proteins. Hydrogen peroxide can be considered as one of the chemical substances able to participate in the physiological biochemical processes in the plants increasing their adaptive potential. This is promising for its use in the agriculture, since molar and nano-concentrations of acting substances are considered in the contemporary technologies as the perspective means for directed control of ontogenesis.

CONCLUSIONS

The results of the performed explorations give evidences on the fact that the hydrogen peroxide action in concentration of $1 \cdot 10^{-4}$ M causes the increase in the winter wheat adaptive potential, both for the wooded steppe and steppe ecotypes, due to reduction in the intensity of LPO processes and the antioxidant ferments activation. The hydrogen peroxide treatment contributes to an increase of 1000 grains mass in the tested varieties.

Формування неспецифічної резистентності у рослин озимої пшениці за дії пероксиду водню

Л. М. Бацманова, М. М. Мусієнко

Київський національний університет
імені Тараса Шевченка
Вул. Володимирська, 64, Київ, Україна, 01033

e-mail: l.batsmanova@gmail.com

Мета. Дослідження впливу пероксиду водню на активність антиоксидантних ферментів, вміст пігментів і продуктивність озимої пшениці для з'ясування біохімічних механізмів індукованої стійкості рослин озимої пшениці в онтогенезі за польових умов. **Методи.** Фізіолого-біохімічні показники вивчали на рослинах озимої пшениці лісостепового (Поліська 90) і степового (Скала) екотипів у фазах кущіння та цвітіння. Дослідні рослини позакоренево обробляли пероксидом водню (H_2O_2) у концентрації $1 \cdot 10^{-4}$ М двічі з інтервалом у 3 доби, контрольні –дистильованою водою. Рослини обприскували навесні (фаза кущіння). Пероксидне окиснення ліпідів (ПОЛ) визначали за утворенням одного з кінцевих продуктів пероксидації – малонового діальдегіду, вміст якого встановлювали за величиною екстинкції продукту його конденсації з тіобарбітуровою кислотою. Активність супероксиддисмутази (СОД) виявляли методом, який базується на здатності СОД інгібувати відновлення нітросинього тетразолію радикалами супероксиду на світлі за присутності рибофлавіну і метіоніну. Активність каталази розраховували за коефіцієнтом екстинкції – 39,4 $мМ^{-1}см^{-1}$. **Результати.** Встановлено, що за дії пероксиду водню на 24-ту год після обробки інтенсифікуються процеси ПОЛ та знижується активність антиоксидантних ферментів в обох сортів. У наступну фазу онтогенезу (цвітіння) активність антиоксидантних ферментів зростає як у рослин сорту Поліська 90, так і Скала, що пригнічує розвиток ПОЛ. **Висновки.** Обробка рослин пероксидом водню сприяє формуванню неспецифічної стійкості рослин та підвищенню зернової продуктивності дослідних сортів озимої пшениці.

Ключові слова: озима пшениця, пероксид водню, активність супероксиддисмутази, каталаза, малоновий діальдегід, неспецифічна резистентність.

Формирование неспецифической резистентности у растений озимой пшеницы при действии пероксида водорода

Л. М. Бацманова, М. М. Мусиенко

Киевский национальный университет
имени Тараса Шевченко
Ул. Владимирская, 64, Киев, Украина, 01033

e-mail: l.batsmanova@gmail.com

Цель. Изучение действия пероксида водорода на активность антиоксидантных ферментов, содержание пигментов и урожайность озимой пшеницы для определения биохимических механизмов индуцированной устойчивости растений озимой пшеницы в онтогенезе в полевых условиях. **Методы.** Физиолого-биохимические показатели изучали на растениях озимой пшеницы лесостепного (Полесская 90) и степного (Скала) экотипов в фазах кущения и цветения. Опытные растения внекорневым способом обрабатывали пероксидом водорода в концентрации $1 \cdot 10^{-4}$ М дважды с интервалом в 3 сут; контрольные − дистиллированной водой. Растения опрыскивали весной (фаза кущения). Пероксидное окисление липидов (ПОЛ) определяли по образованию одного из конечных продуктов пероксидации − малонового диальдегида, содержание которого определяли по величине экстинкции продукта его конденсации с тиобарбитуровой кислотой. Активность супероксиддисмутазы (СОД) выявляли методом, основанным на способности СОД ингибировать восстановление нитросинего тетразолия радикалами супероксида на свету в присутствии рибофлавина и метионина. Активность каталазы рассчитывали с использованием коэффициента экстинкции − 39,4 $мМ^{-1}см^{-1}$. **Результаты.** Установлено, что под действием пероксида водорода через 24 ч после обработки интенсифицировались процессы ПОЛ и снижалась активность антиоксидантных ферментов у обоих сортов, в следующую фазу онтогенеза (цветение)

активность антиоксидантных ферментов увеличивалась как у растений сорта Полесская 90, так и Скала, что подавляло развитие ПОЛ. **Выводы.** Обработка растений пероксидом водорода способствует формированию неспецифической устойчивости растений и повышению зерновой продуктивности исследованных сортов озимой пшеницы.

Ключевые слова: озимая пшеница, пероксид водорода, активность супероксиддисмутази, каталаза, малоновый диальдегид, неспецифическая резистентность.

REFERENCES

1. Zauralov O. A. The effect of hydrogen peroxide on the cold resistance of heat-loving plants // Agrokhimiya. – 2003. – N 12. – P. 42–45.
2. Loppez-Delgado H., Dat J. F., Foyer C. H., Scott I. M. Induction of thermotolerance in potato microplants by acetylsalicylic acid and H_2O_2 // J. Exp. Bot. – 1998. – 49, N 321. – P. 713–720.
3. Xu Y. C., Wang J., Shan L. Effect of H_2O_2 stress hardening on drought resistance of wheat seedlings // Acta Bot. Boreali-Occident. Sin. – 2000. – 20, N 3. – P. 382– 386.
4. Narimanov A. A. The effect of hydrogen peroxide on the salt tolerance of plants // Agrokhimiya. – 2001. – N 7. – P. 57–60.
5. Taran N. Ju., Batsmanova L. M., Grudyna N. S., Makarenko V. I., Musienko M. M., Kononjuk L. M. Hydrogen peroxide effect upon winter wheat grain productivity in the conditions of the Polissia // Visnyk Agrarnoyi Nauky. – 2008. – N 9. – P. 21–25.
6. Andreeva L. I., Kozhemiakin L. A., Kishkun A. A. Modification of the method of determining lipid peroxidation in a test using thiobarbituric acid // Lab. Delo. – 1988. – N 11. – P. 41– 43.
7. Porra R. J., Thompson W. A., Kriedemann P. E. Determination of accurate extinction coefficients and simultaneous equations for assaying chlorophylls a and extracted with four different solvents: verification of the concentration of chlorophyll standards by atomic absorption spectroscopy // Biochim. Biophys. Acta. – 1989. – 975. – P. 384−394.
8. Chevari S., Chaba I., Sekej I. Role of superoxide dismutase in cellular oxidative processes and method of its determination in biological materials // Lab. Delo. – 1985. – N 11. – P. 678−681.
9. Kumar C. N., Knowles N. R. Changes in lipid peroxidation and lipolytic and free-radical scavenging enzyme during ageing and sprouting of potato (Solanum tuberosum L.) Seed-Tubers // Plant Physiol. – 1993. – 102, N 1. − P. 115 –124.
10. Metodika gosudarstvennogo sortoispytaniya selskohoziaistvennykh kultur. – Moscow, 1971. – Vol. 1. – P. 45− 47.
11. Maslov Ju. I. Ustanovlenie stepeni dostovernosti (znachimosti) razlichiy mezhdu seriyami izmereniy // Metody biokhimicheskogo analiza rasteniy. – Leningrad : Leningr. Univ. Press, 1978. – P. 415–424.
12. Sairam R. K., Srivastava G. C. Induction of oxidative stress and antioxidant activity by hydrogen peroxide treatment in tolerant and susceptible wheat genotypes // Biol. Plant. – 2000. – **43**, N 3. – P. 381–386.
13. Kolupaev Ju. E., Karpec Ju. V. Plant adaptive reaction formation against abiotic stress action. – Kiev : Osnova, 2010. – 352 p.
14. Galvez-Valdivieso G., Mullineaux P.M The role of reactive oxygen species in signalling from chloroplasts to the nucleus // Physiol. Plant. – 2010. – **138**, N 4. – P. 430–439.
15. Jaspers P., Kangasjarvi J. Reactive oxygen species in abiotic stress signaling // Physiol. Plant. – 2010. –138, N 4. – P. 405–413.
16. Hung S.-H., Yu C.-W., Lin C.H. Hydrogen peroxide functions as a stress signal in plants // Bot. Bull. Acad. Sin. – 2005. – **46**. – P. 1–10.
17. Kaluev A. V. On the problem of a regulatory role of active oxygen species in cells // Biochemistry (Mosc). – 1998. – **63**, N 9. − P. 1114−1115; Discussion 1116−1117.
18. Kreslavski V. D., Carpentier R., Klimov V. V., Murata N., Allakhverdiev S. I. Molecular mechanisms of stress resistance of photosynthetic apparatus // Biol. Membrany. − 2007. − **24**, N 3. − P. 195−217.
19. Scandalios J. G. Oxidative stress: molecular perception and transduction of signals triggering antioxidant gene defenses// Braz. J. Med. Biol. Res. – 2005. – **38**, N 7. – P. 995−1014.
20. Foyer C. H., Noctor G. Redox homeostasis and antioxidant signaling: A metabolic interface between stress perception and physiological responses // Plant Cell. – 2005. – **17**, N 7. – P. 1866−1875.
21. Uchida A., Takabe T., Takabe T., Jagendorf T. Induction of biosynthesis of osmoprotectants in higher plants by hydrogen peroxide and its application to agriculture // Abiotic stress tolerance in plants: Toward the im-provement of global environment and food / Eds A. K. Rai, T. Takabe. – Dordrecht : Springer, 2006. − P. 153−159.