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Regulation of physiological processes in winter wheat by growth regulators in conditions of powdery mildew infection

T. P. Mamenko*, R. A. Yakymchuk**

**Institute of Plant Physiology and Genetics, National Academy of Sciences of Ukraine, Kyiv, Ukraine*
***Pavel Tychyna Uman State Pedagogical University, Uman, Ukraine*

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Institute of Plant Physiology and Genetics, National Academy of Sciences of Ukraine, Vasylkivska st., 31/17, Kyiv, 03022, Ukraine. Tel.: +38-050-964-17-89. E-mail: t_mamenko@ukr.net

Pavel Tychyna Uman State Pedagogical University, Sadova st., 2, Uman, 20300, Ukraine. Tel. +38-097-341-89-12. E-mail: peoplenature16@gmail.com

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An important way of regulating the key units of metabolism in the plant organism under the action of stressors is the use of biologically active substances with regulating properties – plant growth regulators. They affect endogenous regulatory systems, altering key metabolic pathways and thus increasing the plant's sustainability and productive potential in adverse environmental conditions. The aim of the research was to establish the possibility of regulation of physiological processes in winter wheat varieties by exogenous treatment of plants with growth regulators of synthetic (salicylic acid) and natural (Emistim C and Biolan) origin to increase their resistance to the phytopathogen *Erysiphe graminis* DC f. sp. *tritici* Em. Marchal , the causative agent of powdery mildew. The intensity of the physiological processes in plants was evaluated by the dynamics of the activity of antioxidant enzymes – ascorbate peroxidase and catalase, the release of ethylene and the integrity of the cell membranes. The objects were selected varieties of soft winter wheat, which were grown in controlled growing conditions against the background of infection by powdery mildew. The treatment of plants was carried out using aqueous salicylic acid solutions in the concentration of 10^{-5} M (experimentally established by us), Emistim C and Biolan (manufacturer Agrobiotech) in the concentration specified by the manufacturer at the rate of 20 mL/ha, when the development of the disease reached 5% of the total natural background of the infection in the plants during the stages of heading-beginning of flowering. Infection of winter wheat with powdery mildew leads to disruption of cell membrane integrity, increased activity of catalase and ascorbate peroxidase in leaves of the susceptible winter wheat variety. The intensification of ethylene release by leaves of the resistant variety was observed, which was caused by the hypersensitive reaction of the hormone to the effect of stress. The use of plant treatment by growth regulators contributes to maintaining the integrity of membrane structures, adaptive changes in the activity of antioxidant enzymes and regulation of the synthesis of the stress hormone ethylene in both winter wheat varieties under stress. Such changes in the physiological processes induced by plant growth regulators are accompanied by the preservation of the grain productivity of winter wheat and the increase of their resistance to the development of the disease.

Keywords: ascorbate peroxidase; catalase; ethylene; electrolyte exosmosis; salicylic acid; Emistim C; Biolan.

Introduction

The realization of the potential productivity of winter wheat is often limited by the development of phytodiseases, among which the most harmful are septoria leaf spot, powdery mildew, brown leaf rust and soot diseases (Zozulya & Omelyanenko, 2010). Powdery mildew of wheat is one of the most harmful diseases of this crop, which can lead to a significant reduction of yield and quality in different regions of the country. Depending on the cultivars and climatic conditions of the year, the degree of damage can range 14–40%, which in turn leads to a loss of yield from 10% to 55% (Chernyayeva et al., 2012).

The causative agent of powdery mildew is the sac fungus (ascomycete) *Erusiphe graminis* DC f. sp. *tritici*, which is an obligate, highly specialized parasite. It manifests in the form of a bloom, affects crops during the entire growing season, beginning in the fall, and reaches maximum development in the flowering phase of cereals. Under the influence of the disease, the plants are depleted, the intensity of photosynthetic processes is disturbed, resulting in a decrease in their productivity (Zozulya & Omelyanenko, 2010; Nazarenko et al., 2018). There are two ways to solve the problem of powdery mildew – growing resistant varieties and protecting crops by spraying fungicides.

Fungicidal protection of cereals is becoming more and more urgent, as every year fungus diseases among cereals spread and increase. But it also has a disadvantage – the emergence of races of the pathogen resistant to the action of fungicides. Already resistance of powdery mildew to fungicides from the classes of triazoles, imidazoles, morpholines, spiroketalamines, strobilurins and quinolines has been determined. Therefore, it is important to search for new effective sources of disease resistance. There is a strong belief that the creation of sustainable varieties, recognized worldwide, is the most effective, economically sound and perfect method of plant protection from the point of view of environmental protection (Chernyayeva et al., 2012). However, research has shown that the resistance to powdery mildew of wheat varieties created by hybridization is effective for 7–10 years.

In recent years, scientists have been focusing on molecular genetic studies of the natural resistance of plants to stress. Research is being conducted intensively to identify groups of genes responsible for resistance to biotic factors (Meyer et al., 2011). It is believed that resistant genotypes can be identified in the early stages of breeding using molecular biology methods (Meyer et al., 2011; Tripathi et al., 2013). Expanding and deepening research in this area involves the integrated use of physiological and genetic approaches for a more thorough study of the condition of the plant organism, revealing the essence of integral processes that determine the level and orientation of key units of metabolism and the functioning of regulatory systems of plants at different levels of their organization.

Biochemical protection systems play a decisive role in the adaptation of plants to the effects of adverse environmental factors. Among them, great attention is paid to the role of antioxidant enzymes in metabolism and the formation of plant resistance to stress factors (Zhang et al., 2004; Caverzan et al., 2016; Laxa et al., 2019). Antioxidant enzymes are involved in the neutralization of reactive oxygen species (ROS), the accumulation of which in the plant cell under the action of stress initiates the processes of oxidative destruction of membrane structures. The key enzymes to protect living organisms from oxidative degradation are superoxide dismutase, which catalyzes the formation of superoxide of hydrogen and oxygen from anion radicals (Alscher et al., 2002; Leonowicz et al., 2018). For the utilization of hydrogen peroxide, a complex of enzymes functions is involved: catalase, the peroxidase family, as well as ascorbate-glutathione cycle enzymes – ascorbate peroxidase and glutathione reductase (Willekens et al., 1997; Luna et al., 2004; De Gara et al., 2006; Khanra-Chopra & Semwal, 2011). It is known that reactive oxygen species function as peculiar signaling molecules in triggering a cascade of protective reactions (Mittler et al., 2011). In particular, it has been shown that stimulation of ethylene biosynthesis by the stress factors of a abiotic and biotic nature is involved in the generation of reactive oxygen species (Wang et al., 2002). The formation of "stress" ethylene is believed to be one of the fastest responses to external influences (Liu & Zhang, 2004). Its release is realized only in the presence of oxygen and indicates the transition of cellular metabolism to a stress state and the formation of stress-protective mechanisms (Wang et al., 2002; Ecker, 2004).

The leading role in the adaptation processes of plants belongs to the natural and synthetic analogues of phytohormones. Among them, salicylic acid plays an important role in regulating the development of protective reactions of the plant organism. Salicylic acid is one of the key regulators of protective reactions of plants under the action of stress factors of biotic and abiotic nature (Khan & Khan, 2013; Khan et al., 2015). Salicylic acid is now regarded as a compound that combines the properties of the signaling molecule and the phytohormone (Wang, & Li, 2006; Martynov et al., 2019). It can function as a primary signal and, in conjunction with plasma protein receptors, incorporate appropriate signaling systems, leading to the synthesis of protective compounds and the formation of plant resistance to adverse environmental conditions (Seyfferth & Tsuda, 2004). Scattered studies indicate that salicylic acid affects the generation of ROS, as well as the activity of antioxidant enzymes, causing intracellular changes to the antioxidant system in the plant body (Kawano et al., 2004; Wang & Li, 2006). Such changes are important for the pre-adaptation of plants to further stress. However, to date, there is insufficient experimental evidence regarding the effect of salicylic acid on changes in the activity of antioxidant processes in winter wheat plants due to the stress factors of a biotic and abiotic nature.

Previously, we have shown the possibility of regulation of water balance and prooxidant-antioxidant processes in winter wheat plants by exogenous treatment with salicylic acid in drought conditions. In particular, it was found that the treatment of winter wheat with salicylic acid during heading-flowering induced the preservation of watery tissues in the leaves, reducing the processes of lipoperoxidation and adaptive changes of antioxidant enzymes, which contributed to the increase in drought resistance and plant productivity (Mamenko, 2017).

One of the main tasks of modern researchers is to find new ways and means to improve the adaptive properties of plants and their stress tolerance under the effects of adverse environmental factors. Today, there is a large number of both synthetic and natural phytohormone analogues used to accomplish this task. Among modern preparationss, new growth regulators play an important role. In particular, the plant growth stimulator Emistim C is a highly effective growth regulator of natural origin with a broad spectrum of action. It is a product of biotechnological cultivation of fungi – endophytes isolated from the root system of sea buckthorn and ginseng, obtained on the basis of metabolites of endomycorrhizal fungi. The broad range of action of the preparation is due to its containing a balanced composition of physiologically active substances, including the phytohormones of an auxin, gibberellin, and cytokinin nature. Emistim effectively stimulates the growth and development of many cultivated plants (https://pikprom.com).

In the literature, it is reported that Biolan (www.agrobiotech.com), a biostimulant of growth of natural origin, reliably protects plants against insect phytophages and phytonematodes, diseases associated with fungi-phytopathogens. This preparation is a product of biotechnological cultivation of micromycete fungi on the root system of ginseng. It contains a balanced mixture of free fatty acids, chitosan, oligosaccharides, phytohormones, amino acids, biogenic trace elements (Na, Mg, Ca, K, Cu, Fe) and vitamins.

Disclosure of physiological and biochemical features of the functioning of cultivated plants under the action of stress factors of a biotic and abiotic nature is important for understanding the peculiarities of formation of adaptive reactions in different plant genotypes, and the search for effective exogenous means of enhancing the adaptation potential of plants and improving the existing technologies of cultivating agricultural crops and developing new ones.

Therefore, the objective of the study was to assess the effect of exogenous treatment of plants with salicylic acid, Emistim C and Biolan on the dynamics of antioxidant enzymes (ascorbate peroxidase and catalase), the intensity of ethylene excretion and the electrolyte exosmosis in winter wheat varieties against the background of infection by *E. graminis*.

Materials and methods

The objects of the study were the selected varieties of soft winter wheat (*Triticum aestivum* L.) Favoritka and Poliska 90, which differ in their resistance to powdery mildew. Favoritka is resistant to powdery mildew, a mid season ripening variety, highly productive of highly intensive type, originated in the Institute of Plant Physiology and Genetics of the NAS of Ukraine and The V. M. Remeslo Myronivka Institute of Wheat of the NAAS of Ukraine. Poliska 90 is susceptible to powdery mildew, and originated in the Institute of Agriculture the UAAS.

The plants were grown in vegetation pots in dark grey podzolized soil with optimum water supply and natural light. Winter wheat infection by the phytopathogen *Erysiphe graminis* DC f. sp. *tritici* Em. Marchal was natural in the ear – flowering stages.

The treatment of plants was carried out with salicylic acid solutions in the concentration of 10^{-5} M (experimentally established by us), Emistim C and Biolan at the rate of 20 mL/ha specified by the manufacturer Agrobiotech (www.agrobiotech.com.ua) at the temperature of 27–29 ºC and relative humidity of 56–60%, when the development of the causative agent of powdery mildew reached 5% of the total natural background of the infection of plants in the heading-beginning of flowering stages. For the study, we selected flag leaves of winter wheat on the 14th day after the development of the disease. Controls were plants not treated with growth regulators, but which were infected by phytopathogen *E. graminis*.

Samples of plant material were placed in sealed glass vials with a capacity of 15 cm^3 and left in the dark for 24 hours. After incubation, the gas mixture containing ethylene was analyzed on a gas chromatograph Chromatograf-504 (Poland) with a flame ionization detector. The separation of the gases was carried out in a column 3 m long and 3 mm in diameter filled with Porapak Q at the temperature of 30 ºC. The carrier gas was helium (25 mL in 1 minute). The volume of the analyzed sample of gas mixture was 1 cm^3. The amount of ethylene formed in the sample was compared with a certified ethylene standard (Fluca, Germany), concentration of which was 10 μL/L (Guzman & Ecker, 1990). The results are presented in nmoles of isolated ethylene (C_2H_4) per hour per g of wet weight.

Permeability of cell membranes was determined by electrolytic method, by measuring the resistance of solutions of electrolytes which were washed from the tissue (exoosmosis). To do this, a portion of the leaves (1 g) was immersed for 4 h into 100 mL of distilled water. In the resulting solution, the resistance of the exosmosed electrolytes was measured using a slide-wire bridge and an X-38 electrolytic cell. The relative number of the exosmosed electrolytes was determined by the electrical conductivity of the same extract after the destruction of the membranes by boiling. The results of the electrolyte exoosmosis were calculated as a percentage of their total exoosmosis (Rossard et al., 2006).

A portion of the plant material was homogenized with cooled 60 mM phosphate buffer (pH 7.5) containing 2 mM ethylenediaminetetraacetic acid, 1 mM phenylmethylsulfonyl fluoride, 5 mM β-mercaptoethanol and 1% polyvinylpyrrolidone. The homogenate was centrifuged at 10,000 rpm for 20 minutes at 4 °C. The supernatant was used to determine enzyme activity using a Smart Spec Plus spectrophotometer (USA).

Ascorbate peroxidase (EC 1.11.1.11) – by reducing the optical density at the wavelength of 290 nm for a minute as a result of oxidation of ascorbate (Nakano & Asada, 1981). The reaction mixture contained 60 mM potassium phosphate buffer (pH 7.0), 0.1 mM ethylenediaminetetraacetic acid, 0.2 mM ascorbate, 0.1 mM hydrogen peroxide. The reaction was initiated by the addition of 150 μL of supernatant. The results are presented in μmol of ascorbate for protein concentration (mg) in the supernatant.

Catalase (EC 1.11.1.6) – by decrease in optical density at 240 nm for a minute due to the decomposition of hydrogen peroxide (Masuoka et al., 1996). The reaction mixture contained 60 mM potassium phosphate buffer (pH 7.0) and 10 mM hydrogen peroxide. The reaction was initiated by the addition of 100 μL of supernatant. The results are presented in μmol of hydrogen peroxide per protein concentration (mg) in the supernatant.

The content of total soluble protein in the enzyme extract was determined by the Bradford method (Bradford, 1976).

Analysis of the data was made using Statistica 6.0 (StatSoft Inc., USA) program. The data are presented in tables as x ± SD (x ± standard deviation). Differences between the values in the control and experimental groups were determined using the Tukey test, where the differences were considered reliable at $P < 0.05$ (taking into account the Bonferroni correction).

Results

It was found that during the stages of heading-flowering the average degree of infection by the phytopathogen *E. graminis* in the susceptible Poliska 90 variety exceeded twofold the development of the disease in the resistant Favoritka variety (Table 1). Treatment of plants with growth biostimulants did not cause significant changes in the disease development in the resistant variety Favoritka on the 3rd day and reduced the development of the pathogen on the 14th day. In particular, during the treatment with salicylic acid, the development of the infection by powdery mildew decreased by 15.8%, and by 9.7% during the treatment with Emistim C. No significant inhibition of the disease development during vegetation in winter wheat plants of the Favoritka cultivar by Biolan treatment was found.

Table 1
Development of powdery mildew infection on winter wheat on the 14th day after treatment with plant growth regulators (%, x ± SD, n = 16)

Variants	Favoritka	Poliska 90
E. graminis (control)	39.61 ± 3.14[a]	61.31 ± 4.21[a]
Salicylic acid	33.33 ± 2.31[b]	55.75 ± 4.50[b]
Emistim C	35.77 ± 2.51[ab]	53.81 ± 3.76[b]
Biolan	39.17 ± 2.74[a]	57.33 ± 4.12[ab]

Note: different letters indicate values that differ significantly from each other in the same columns of the table by comparison with the Tukey test, $P < 0.05$ (taking into account the Bonferroni correction).

In the winter wheat variety Poliska 90, susceptible to powdery mildew, the treatment of plants with growth regulators induced a slowdown in the disease on the 3rd day. In particular, in variants with salicylic acid the rate of development of the disease decreased by 14.5%, Emistim C reduced the infection by 7.8%, and Biolan by 12.3%. On the 14th day after the treatment of plants with growth regulators, the degree of the infection had decreased by 9.1%, 12.6% and 6.5%, respectively.

It was shown that the integrity of cell membranes was more significantly impaired in winter wheat of the susceptible variety, as evidenced by the increased exoosmosis of the electrolytes from the leaves in Poliska 90 by 31.2%, as compared with the resistant variety Favoritka (Fig. 1). Treatment of plants with growth regulators led to a decrease in the exoosmosis of leaf electrolytes in the resistent winter wheat variety equaling 9.6% for salicylic acid and 13.1% for Biolan. During the use of Emistim C, no significant difference between this indicator and the control was observed. The treatment of the susceptible variety of winter wheat with growth regulators caused decrease in the electrolyte exosmosis from the leaves, equaling 30.8% for salicylic acid, and 24.7% and 33.3% for Emistim C and Biolan. The data obtained indicate that the treatment of winter wheat by growth regulators in the stage of heading-flowering led to changes in plant metabolism, which positively affected the structure of cell membranes during the development of the disease.

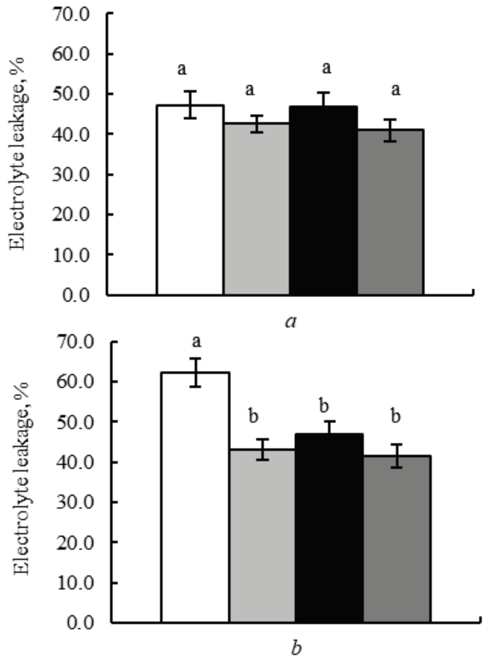

Fig. 1. Influence of growth regulators on the electrolyte exosmosis from leaves of winter wheat varieties Favoritka (*a*) and Poliska 90 (*b*) with *E. graminis* infection; columns are coloured according to variants: white – *E. graminis* (control), dark – salicylic acid, black – Emistim C, grey – Biolan; x ± SD, n = 8; different letters indicate values that differed significantly from each other in the same columns according to the results of the comparison using the Tukey test, $P < 0.05$ (taking into account the Bonferroni correction)

On the 14th day of the infection with the phytopathogen, the ethylene release rate of resistant varieties was 66.1% higher, compared with the susceptible variety, indicating the hormone's sufficiently sensitive response to the phytopathogen's infection (Fig. 2). Treatment of plants with natural growth regulators induced a decrease in ethylene synthesis by leaves in the resistant winter wheat variety by 31.7% and 21.7%, respectively, under the action of Emistim C and Biolan, whereas ethylene synthesis increased by 38.1% under the action of salicylic acid. In plants of the susceptible variety Poliska 90, the intensity of ethylene release by leaves increased by 30.2% (Emistim C), 55.9% (Biolan), 50.5% (salicylic acid), regardless of the nature of the preparation. The response of the stress hormone ethylene to treatment with growth regulators differed in winter wheat varieties, especially in the resistant variety. This may be due to the formation of a specific adaptive response of plants to the development of the disease induced by the phytopathogen *E. graminis*.

Infection of winter wheat with powdery mildew led to an increase in catalase activity in the leaves of the resistant variety by 160.3% and a decrease in ascorbate peroxidase activity by 182.2%, compared with the susceptible variety (Fig. 3, 4). This indicates the inclusion of appropriate antioxidant systems in the formation of stress-protective responses of winter wheat of different varieties to the development of the disease.

Treatment of plants with growth regulators during the stage of heading-beginning of flowering led to increase in the activity of catalase in the leaves of winter wheat, regardless of the resistance of the variety to the disease. Therefore, on the 14th day of the development of powdery mildew in the leaves of the Favoritka variety, the activity of catalase increased by 75.3% during the treatment with Biolan and by 28.9% during the action of salicylic acid. Under these conditions, in the Poliska 90 variety, catalase activity increased by 380% under the action of Biolan and by

145.2% under the action of salicylic acid. We found no significant changes in enzyme activity in winter wheat leaves of either variety during the treatment with Emistim C.

Treatment by regulators of winter wheat growth induced intensification of ascorbate peroxidase activity in leaves of the resistant variety Favoritka by 255.2% (Emistim C), 73.0% (Biolan), 246.6% (salicylic acid) against the background of the disease development. The opposite reaction of the enzyme to the treatment with plant growth regulators was recorded in the leaves of the susceptible variety Polisska 90. In particular, the activity of ascorbate peroxidase decreased by 15.8 and 48.5% during the treatment with Emistim C and Biolan and by 36.9% during the treatment with salicylic acid.

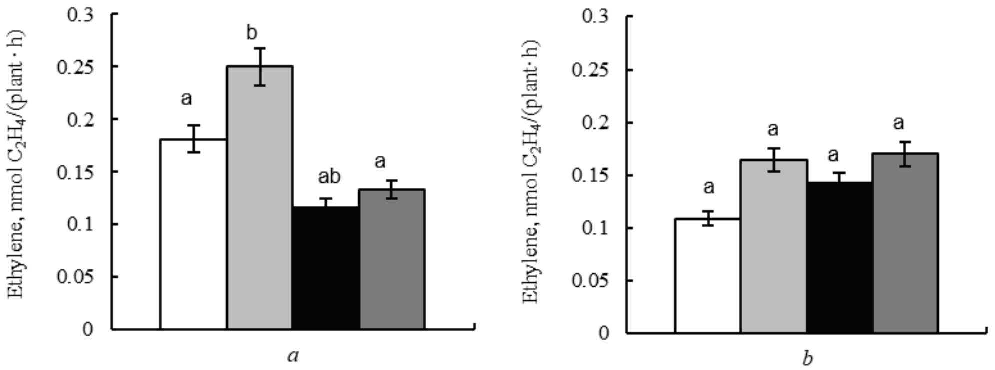

Fig. 2. Influence of growth regulators on the intensity of ethylene release by winter wheat leaves of variety Favoritka (*a*) and Poliska 90 (*b*) during *E. graminis* infection: columns are coloured according to variants: white – *E. graminis* (control), dark – salicylic acid, black – Emistim C, grey – Biolan; x ± SD, n = 8; different letters indicate values that differed significantly from each other in the same columns according to the results of the comparison using the Tukey test, $P < 0.05$ (taking into account the Bonferroni correction)

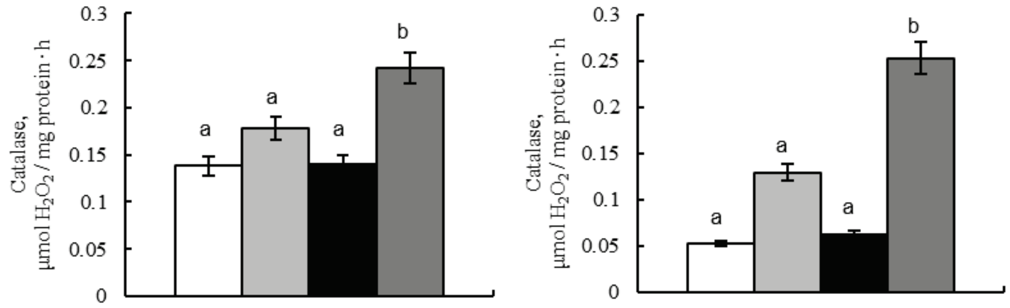

Fig. 3. Effect of plant growth regulators on the activity of catalase in leaves of winter wheat of the Favoritka (*a*) and Poliska 90 (*b*) varieties during *E. graminis* infection: columns are coloured according to variants: white – *E. graminis* (control), dark – salicylic acid, black – Emistim C, grey – Biolan; x ± SD, n = 8; different letters indicate values that differed significantly from each other in the same columns according to the results of the comparison using the Tukey test; $P < 0.05$ (taking into account the Bonferroni correction)

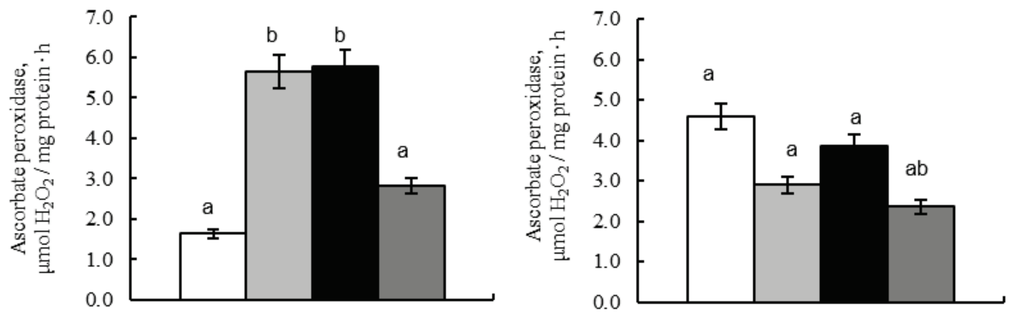

Fig. 4. Effect of plant growth regulators on the activity of ascorbate peroxidase in leaves of winter wheat of the Favoritka (*a*) and Poliska 90 (*b*) varieties during *E. graminis* infection: columns are coloured according to variants: white – *E. graminis* (control), dark – salicylic acid, black – Emistim C, grey – Biolan; x ± SD, n = 8; different letters indicate values that differed significantly from each other in the same columns according to the results of the comparison using the Tukey test; $P < 0.05$ (taking into account the Bonferroni correction)

The observed changes in the activity of antioxidant enzyme – catalase and ascorbate peroxidase in winter wheat leaves during the treatment with growth regulators are obviously orientated towards regulating antioxidant processes under stress and indicate the formation of the adaptive response of the plants.

The analysis of grain productivity of winter wheat infected by powdery mildew showed that in the susceptible variety Poliska 90 the grain weight of spikelets and the weight of 1,000 grains was lower by 29.1% and 14.4%, respectively, compared to the resistant variety Favoritka (Table 2, 3). Treatment of the plants by the growth regulators in the heading-flowering start stage helped to increase the grain yield of winter wheat in both varieties. Thus, in powdery mildew resistant variety Favoritka the treatment with growth regulators of natural origin contributed to the increase of grain weight of spikelets by 5.3% and 30.1% under the action of Emistim C and Biolan, while the mass of 1,000 grains increased by 7.1% and 13.3% respectively. The tendency of the action of natural origin preparations to increase the grain productivity of Poliska 90, susceptible to powdery mildew, was similar to that of the resistant Favoritka variety. In particular, in the Poliska 90 variety, the grain weight of the ear increased by 4.1% and 27.7%, respectively, due to the actions of Emistim C and Biolan, while the mass of 1,000 grains increased by 8.2% and 14.1%, respectively.

Table 2
Effect of plant growth regulators on winter wheat grain yield of Favoritka during infection with powdery mildew (x ± SD, n = 4)

Variants	Weight of grain from the spike, g	% to control	Weight of 1000 grains, g	% to control
E. graminis (control)	0.932 ± 0.061[a]	100.0	42.8 ± 3.1[a]	100.0
Salicylic acid	1.151 ± 0.072[a]	123.5	47.5 ± 3.3[a]	110.9
Emistim C	0.981 ± 0.061[a]	105.2	45.8 ± 3.2[a]	107.0
Biolan	1.212 ± 0.082[a]	130.0	48.5 ± 3.4[a]	113.3

Note: see Table 1.

Table 3
Effect of plant growth regulators on winter wheat grain yield of Poliska 90 during infection with powdery mildew (x ± SD, n = 4)

Variants	Weight of grain from the spike, g	% to control	Weight of 1000 grains, g	% to control
E. graminis (control)	0.721 ± 0.051[a]	100.0	37.4 ± 2.6[a]	100.0
Salicylic acid	0.814 ± 0.064[a]	112.9	42.2 ± 3.1[a]	112.8
Emistim C	0.753 ± 0.053[a]	104.4	40.4 ± 2.8[a]	108.0
Biolan	0.921 ± 0.061[a]	127.7	42.7 ± 3.2[a]	114.1

Note: see Table 1.

Treatment of plants with the synthetic analogue of phytohormones – salicylic acid led to an increase in grain productivity in the resistant variety Favoritka: weights of grains from spikelets by 19.8% and masses of 1,000 grains by 11.1%, whereas in the susceptible variety Poliska 90: weight of grain from spikelets increased by 12.5% and the mass of 1,000 grains increased by 12.9%.

Discussion

The effect of stressors of abiotic and biotic nature on the intensity and orientation of physiological processes is accompanied by changes in the state of cytoplasmic structures, which is particularly clear when determining the permeability of the cytoplasm (Rossard et al., 2006; Hohenberger et al., 2011). Disruption of cytoplasmic structures, in particular cell membranes, is characterized by a sharp increase in the electrolyte exosmosis from the cell. It is believed that the value of the electrolyte exosmosis can be an indicator of the degree of ordering of intracellular structures (Rossard et al., 2006).

During the development of winter wheat powdery mildew, an increase in the electrolyte exosmosis was observed, especially in plants of the susceptible variety. This indicates a significant disruption of the integrity of the cell membranes. The use of plant treatment with growth regulators leads to the preservation of the integrity of membrane structures under stress and is accompanied by a slowing of the electrolyte exosmosis from leaves of winter wheat, which is more evident in the leaves of the susceptible variety. It is obvious that the metabolism of plants in the resistant winter wheat can adapt more quickly to unfavourable growing conditions by mobilizing its own protective mechanisms.

The existence of a sequence of changes in cell membranes under unfavourable conditions has been proved: phase transition of part of the lipids of membranes, disruption of the structure in the areas of interphase boundaries and increase of their permeability within defective areas (Veselov, 2002; Demidchik, 2015). The inhibition of membrane-bound enzymes, including transport ATPases, change in pH, activation of membrane phospholipases, and lipid peroxidase oxidation, have a direct effect on the increase in the exoosmosis of electrolytes (Hohenberger et al., 2011). It is known that the end product of lipid peroxidase oxidation – malonic dialdehyde interacts with free amino groups of proteins, components of phospholipids, causes the appearance of ethylene membranes, which leads to changes in the properties of both individual components and membranes on the whole.

An increase in the intensity of ethylene release under adverse environmental conditions was observed after the onset of the impact of the stressor, long before the appearance of external signs of damage. Ethylene release is necessary for the transduction of the stress signal that initiates the formation of general and specialized adaptation mechanisms (Wang et al., 2002; Ecker, 2004). The intensification of ethylene release by leaves of the resistant winter wheat cultivar during the development of damage by the phytopathogen was revealed. This is a consequence of the faster response of the plant metabolism to the effects of stress and its stress tolerance.

The treated plants of the susceptible winter wheat variety were observed to have an increase in ethylene synthesis in the leaves. This indicates a clear positive effect of the use of the preparations to induce stress-protective reactions. At the same time, treatment of the resistant variety with natural growth promoters led to a decrease in ethylene excretion in the leaves during the development of powdery mildew. This specific response of the stress hormone to treatment with growth regulators is related to the specifics of metabolism of the variety itself, its ability to quickly mobilize and realize its adaptive potential during stress, which does not require additional growth-promoting factors. The action of the synthetic analogue of the phytohormones of salicylic acid in the resistant and susceptible varieties was similar and initiated an increase in the synthesis of the stress hormone ethylene. In the literature there is no data on the effect of salicylic acid on the intensity of ethylene release under the action of stress factors of abiotic and biotic nature. However, it has been found that under optimal plant growing conditions, salicylic acid inhibits ethylene synthesis, probably by inhibiting the enzyme amino-cyclopropanecarboxidase, which is involved in the transformation of 1-amino-cyclopropanoic acid into ethylene (Liu & Zhang, 2004). However, it is known that salicylic acid affects the generation of reactive oxygen species and also induces an increase in the activity of antioxidant enzymes (superoxide dismutase, catalase and peroxidase), which are involved in their metabolism (Khan et al., 2015). It is believed that changes in the antioxidant system caused by salicylic acid, accompanied by a slight increase in the level of reactive oxygen species, can be important for the adaptation of plants to stress (Kawano et al., 2004; Seyfferth & Tsuda, 2014). It has been found that excess in the tissues of hydrogen peroxide and other reactive oxygen species can stimulate amino-cyclopropanecarboxylase or induce the formation of its isoforms (Mittler et al., 2011). Therefore, it is believed that the increase in ethylene biosynthesis in stress conditions depends on the rapid transformation of 1-amino-cyclopropanoic acid into ethylene, the activity of amino-cyclopropanecarboxidase which catalyzes the last stage of ethylene biosynthesis (Liu & Zhang, 2004).

An important role in neutralizing the effects of oxidative stress is played by the antioxidant system. One of the key enzymes involved in protecting the plant body from oxidative degradation is catalase and the peroxidase family. Catalase in the cell is a synergist of superoxide dismutase and prevents the accumulation of the product of superoxide dismutase reaction – hydrogen peroxide, superoxide dismutase inhibitor. The catalase is compartmentalized in special micro-bodies of peroxisomes. They have an oxidative type of metabolism characterized by plasticity, whereby the enzyme composition of peroxisomes can vary depending on the organism, type of cells, tissues and external conditions (Mhamdi et al., 2012). As is known from the literature, the synthesis of catalase in peroxisomes is induced by its substrate and a sufficiently high amount of hydrogen peroxide is required to exhibit enzyme activity, and in the absence of the latter, the enzyme activity may be low (Mhamdi et al., 2010; Mhamdi et al., 2012).

Ascorbate peroxidase is a key enzyme of the ascorbate-glutathione cycle, which involves a complex of enzymes and substrates which take part in the elimination of hydrogen peroxide (Shigeoka et al., 2002; Caverzan et al., 2012). The ascorbate peroxidase cycle begins, which requires two ascorbate molecules for reduction of hydrogen peroxide to water, which is accompanied by the formation of two monodehydroascorbate molecules, followed by the rapid formation of ascorbate and dehydroascorbate. The reduction of dehydroascorbate to ascorbate is carried out by dehydroascorbate reductase which uses glutathione as a reducing agent and forms glutathione disulfide. The cycle is completed by glutathione reductase which reduces glutathione disulfide to glutathione with NADP·H (Sofo et al., 2015).

The sort specificity of the reaction of antioxidant enzymes – catalase and ascorbate peroxidase in winter wheat leaves under the action of the biotic stress factor, infection by powdery mildew, has been investigated. In particular, there was an increase in the activity of catalase in leaves of resistant winter wheat and an increase in ascorbate peroxidase activity in leaves of susceptible winter wheat against the background of the disease. Treatment of plants with growth regulators led to adaptive changes in the activity of antioxidant enzymes in leaves of both winter wheat varieties under stress. The activity of catalase increased more intensively in leaves of the susceptible variety of winter wheat, which induces increase in their protective properties during the development of the disease. Under such conditions, the intensification of the activity of ascorbate peroxidase in the leaves of the susceptible variety was leveled out due to the action of the treatment, while in the resistant variety its activity was significantly increased. The observed changes in the activity of catalase and ascorbate peroxidase in varieties of winter wheat induced adaptive rearrangements of plant metabolism, contributed to the formation of the plant's resistance during the development of the disease, and were accompanied by an increase in the grain productivity of winter wheat.

Conclusions

The resistance of winter wheat to the action of phytopathogenic pathogens – the causative agent of powdery mildew is manifested in the ability of the variety to quickly activate stress-protective reactions, the components of which are the intensification of the release of the stress hormone ethylene, the increase in the activity of antioxidant enzymes – catalase and ascorbate peroxidase, which is accompanied by the stabilization of electrolyte exosmosis from the plant's leaves.

Treatment of the plants with growth regulators of synthetic and natural origin against the background of infection of the plants by powdery mildew helps preserve the integrity of cell membranes, and also induces rapid ethylene synthesis in the leaves and adaptive changes in the activity of antioxidant enzymes, which leads to an increase in the grain productivity of winter wheat under biotic stress.

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