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Salicylate-Induced Changes in Superoxide Dismutase and Catalase Activity in Cereals Seedlings in *Fusarium graminearum* Infection

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Abstract: Fusarium fungi are widespread in nature and cause various plant diseases. The phytohormone salicylic acid can increase plants' resistance to stress factors of various natures. Despite the large number of scientific publications devoted to the study of the functions of salicylic acid in plant organisms, data on the effect of salicylate on plants under conditions of biotic stress are few and contradictory. The aim of the work was to study the dynamics of the activity of individual enzymes of the antioxidant system in the roots and shoots of *Zea mays* L. and *Triticum aestivum* L. under the influence of salicylic acid during infection with the phytopathogen *Fusarium graminearum*. Physiological-biochemical, statistical research methods are applied. The effect of salicylic acid (SA) at a concentration of 0,5 mM on the dynamics of the activity of catalase and superoxide dismutase (SOD) in the roots and shoots of maize and wheat with lesions Fusarium graminearum in the early stages ontogenesis. It was found that under the action of SA, the defeat of phytopathogen increases the activity of SOD in the tissues of seedlings of corn and wheat and the activity of catalase in seedlings of corn.

Keywords: *Zea mays* L.; *Triticum aestivum* L.; salicylic acid; catalase; superoxide dismutase; *Fusarium graminearumi.*

1. Introduction

The problem of plant resistance to stresses of different natures occupies one of the central places in modern phytophysiology [1, 2]. Infection of plants with pathogenic organisms is common biotic stress. The first place in the number of diseases is occupied by phytopathogenic fungi, which cause numerous plant diseases. Fungi of the genus *Fusarium* are widespread and are the causative agents of various plant diseases [3-5].

The use of chemical plant protection products, despite their effectiveness, leads to environmental pollution, accumulation of dangerous doses of chemicals in human and animal health, and disturbance of the ecological balance in the biosphere. Therefore, the search for alternative, environmentally friendly means of protection is based on increasing the natural resistance of plants to disease [6, 7].

Such compounds include phytohormone-salicylic acid (SA), which can increase the resistance of plants to stressors of various natures [8, 9]. This action of growth regulator is associated primarily with its ability to induce the accumulation of reactive oxygen species,

which are involved in plant protection reactions against pathogens [10], cell wall lignification processes, and mediate the regulation of expression of certain genes of the antioxidant system [11, 12].

The great variety of functions of salicylic acid in plants makes it impossible to replace this compound in the plant protection system, which combines many different mechanisms, including the antioxidant system [6, 8, 13].

Despite the large number of scientific publications devoted to studying the functions of salicylic acid in the plant body, data on the effects of salicylate on plants under stress are contradictory [3, 14]. The effect of growth regulators on plant organisms can have a positive or negative effect, depending on the type of plant and the concentration of phytohormone [1, 15]. That is why the effect of salicylic acid on the antioxidant system of plants under the action of stressors of abiotic nature requires thorough study.

The key enzymes involved in the protection of the plant body from oxidative degradation are particularly superoxide dismutase (SOD) and catalase. Some studies suggest that salicylic acid affects both the generation of reactive oxygen species and the activity of the above antioxidant enzymes. This, in turn, causes changes in the state of the antioxidant system of plants [16 - 19].

The aim was to explore the dynamics of the activity of certain enzymes antioxidant but her system in the roots and shoots of *Zea mays* L. and *Triticum aestivum* L. under the action of salicylic acid when infected with the phytopathogen *Fusarium graminearum*.

2. Materials and Methods

2.1. Experimental design and treatments.

Corn plants *Zea mays* L varieties Zakarpattia yellow-toothed and wheat Triticum aestivum L. varieties Kryzhynka (https://minagro.gov.ua/file-storage/reyestr-sortiv-roslin), which are unstable to phytopathogens, were used for experiments.

The seeds were germinated in a thermostat at a temperature of 24°C for three days. Before germination, part of the seeds was soaked in a salicylic acid solution at a concentration of 0,5 mM (Sphera Sim, Lviv, Ukraine) and the other – in distilled water (control) for three hours. The concentration of SA has a positive effect on seed germination and plant growth, which was determined in previous studies and based on the literature reports [8, 10]. After that, the seedlings were transferred to distilled water. On the fourth day, some of the seedlings were infected with the pathogen Fusarium graminearum. The control served seedlings whose seeds were soaked in distilled water and not infected. Catalase, peroxidase, and SOD activity were determined in the roots and shoots of seedlings at 3, 6, and 9 hours after infection.

2.2. Determination of catalase and superoxide dismutase activity.

Catalase activity (CF 1.11.1.6) was determined spectrophotometrically by decreasing the color intensity of the complex of hydrogen peroxide with ammonium molybdate [20]. To prepare the extract, the plant material was homogenized and extracted with 0.05 M Tris-HCl buffer (pH 7.8). The catalase reaction was started by adding 0.1 ml of homogenate to 2 ml of 0.03% H_2O_2 solution. The reaction was stopped after 10 min by adding 1 ml of ammonium molybdate. A solution of 0.1 ml of 4% ammonium molybdate was added to the blank sample instead of the homogenate. The color intensity of the blank and experimental samples was determined spectrophotometrically at a wavelength of 410 nm against a control sample in

which 2 ml of water was added instead of hydrogen peroxide. Enzyme activity was expressed in μmol/min·mg of protein. The activity of SOD (CF 1.15.1.1) was determined by the degree of its inhibition of autooxidation of quercetin [9]. Plant material was homogenized and extracted with 0.1 M potassium phosphate buffer (pH=7.8). The homogenate was centrifuged, and the supernatant was used to determine the enzyme activity. 1.0 ml of reagent C, 2.3 ml of distilled water, and 0.1 ml of homogenate were added to the test tube. 1.0 ml of reagent C and 2.4 ml of distilled water were added to the control tube. Immediately before the measurement, 0.1 ml of quercetin solution was added to both test tubes. Reagent C contained 0.1 M potassium phosphate buffer (pH 7.8), 0.08 M EDTA solution, and tetramethylethylenediamine (TEMED) solution, which is a superoxide radical donor. The TEMED solution was used to adjust the pH of reagent C to 10. When quercetin interacts with reagent C, the complete oxidation reaction occurs within 20 minutes. The quercetin oxidation reaction was recorded spectrophotometrically at a wavelength of 406 nm. The activity was expressed in μmol/min·mg protein. Protein concentration was determined by the Lowry method [21].

2.3. Statistical analyses.

The results were statistically processed using the Microsoft Excel software package; the arithmetic mean standard error was determined, and correlation analysis was performed. The statistical significance of the difference was evaluated with Student's t-test ($P<0,05$). Each experiment was performed in five replications.

3. Results and Discussion

The state of the antioxidant system largely determines the resistance of plants to adverse effects. It is proved that resistant species and varieties of plants have increased activity of SOD, catalase, glutathione reductase, and other enzymes involved in the elimination of oxygen radicals [3, 17, 22].

3.1. Catalase activity in corn and wheat seedlings.

The need to study the effect of salicylic acid on catalase activity is because the change in this indicator is directly related to changes in metabolic reactions in plant cells. Along with the decomposition of hydrogen peroxide, which protects the cell from its toxic effects, catalase catalyzes several metabolically important reactions. As a rule, the content and activity of catalase in the cell are sufficient to prevent a small amount of hydrogen peroxide, which is generated endogenously, from revealing potential toxicity [4, 23].

Analyzing the activity of catalase in the tissues of maize roots every three hours after infection with phytopathogen 4-day-old seedlings whose seeds were soaked in SA, it should be noted that the enzyme activity increases 4 times after three hours of infection, 15 times after 6 hours, and in 12 times after 9 s hours of infection. Thus, the highest catalase activity in the roots of corn seedlings, the seeds of which were treated with a growth regulator, is observed at 6 hours after infection (Figure 1a). Infection with a phytopathogen without the influence of SA also caused an increase in the activity of the enzyme.

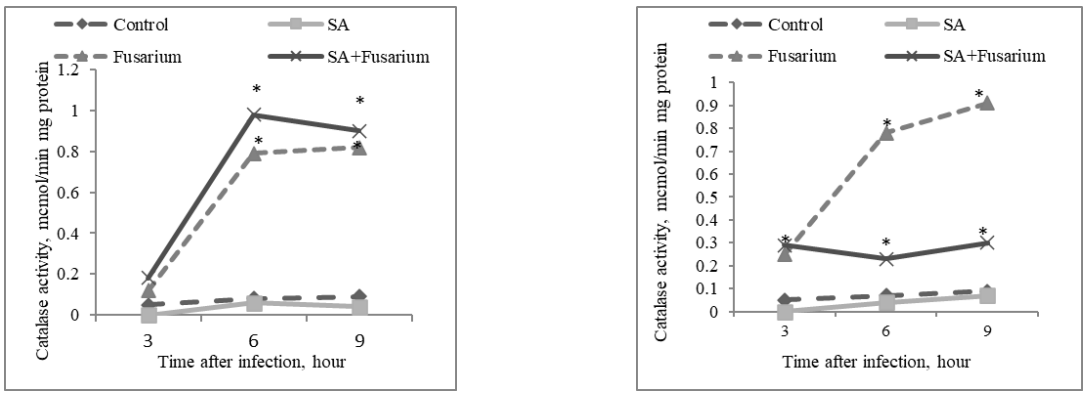

Figure 1. Effect of SA and *Fusarium graminearum* on catalase activity in 4-day-old seedlings of *Zea mays L.*: (**a**) roots; (**b**) shoots; laboratory experiment. * – P<0.05.

A slightly different tendency to change the catalase activity is observed in the organs of wheat seedlings under the action of salicylic acid, phytopathogen, and their combined effects. Under conditions of infection of wheat seedlings with phytopathogen, the enzyme activity in the roots of seedlings decreases every three hours after inoculation for control (Figure 2a). The highest catalase activity was observed in the third hour after inoculation. At the same time, pre-treatment of SA seeds caused a significant stable increase in the activity of the studied enzyme in the roots of seedlings.

When soaking wheat seeds in SA and infecting seedlings, the highest catalase activity in the shoots was observed after 3 hours of infection, and every three hours, this figure decreased significantly (Figure 2b).

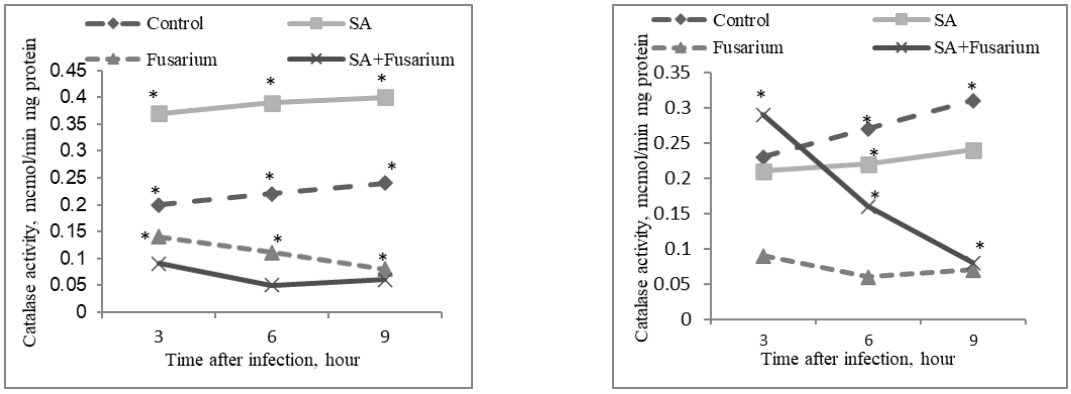

Figure 2 Effect of SA and *Fusarium graminearum* on catalase activity of 4-day-old seedlings of *Triticum aestivum* L.: (**a**) roots; (**b**) shoots; laboratory experiment. * – P<0.05.

Analyzing the results of the study of catalase activity, we must first pay attention to the specific characteristics of the reaction of seedlings of corn and wheat to the effects of phytopathogen and SA. If, in the organs of *Zea mays* L., the infection caused a sharp increase in the enzyme activity in the tissues of *Triticum aestivum* L., the changes were opposite. Pre-treatment of SA seeds and the phytopathogen also caused opposite reactions in the two studied plant species - a decrease in catalase activity in wheat seedlings (especially in the last stages of research) and an increase in this indicator for control in maize tissues. In general, the effect of both SA and the combined effect of SA and Fusarium wilt on the enzyme activity had similar trends [24, 25].

Data from the literature on the effect of salicylic acid on catalase activity in plant organs are ambiguous. Some authors note that exogenous salicylic acid does not inhibit catalase, while

others claim a decrease in the activity of this enzyme in plant organs [2, 9, 10]. Such differences can probably be explained by various reasons: the type of plants, the duration of action of the phytohormone, its concentration, and the presence of stress factors of different natures.

The substrate is known to induce catalase synthesis, so the manifestation of catalytic function requires a large amount of hydrogen peroxide [15]. It can be assumed that the accumulation of hydrogen peroxide in the organs of wheat seedlings under the action of a stress factor - phytopathogen, does not exceed the physiological norm and, therefore, does not require significant activation of antioxidant enzymes, particularly catalase. At the same time, the increase in catalase activity in maize seedlings under the action of fusarium wilt and SA may manifest this plant species' resistance to stress.

3.2. SOD activity in corn and wheat seedlings.

Given the importance of the role of SOD in the mechanisms of plant adaptation to different types of stress [16, 26, 27], we studied the effect of salicylic acid on the activity of this enzyme in the organs of corn and wheat seedlings under infection of *Fusarium graminearum* seedlings.

Under *Fusarium graminearum* maize seedling infection, it was found that after three hours, the SOD activity in the roots was higher than in the control, decreased almost to the control level after 6 hours of infection, and increased again after 9 hours (Figure 3a). Regarding the combined effect of SA and phytopathogen, the combined action of these factors caused a significant increase in the activity of the enzyme in the roots and shoots of seedlings both in terms of control and in terms of exposure to phytopathogen alone (Figure 3b).

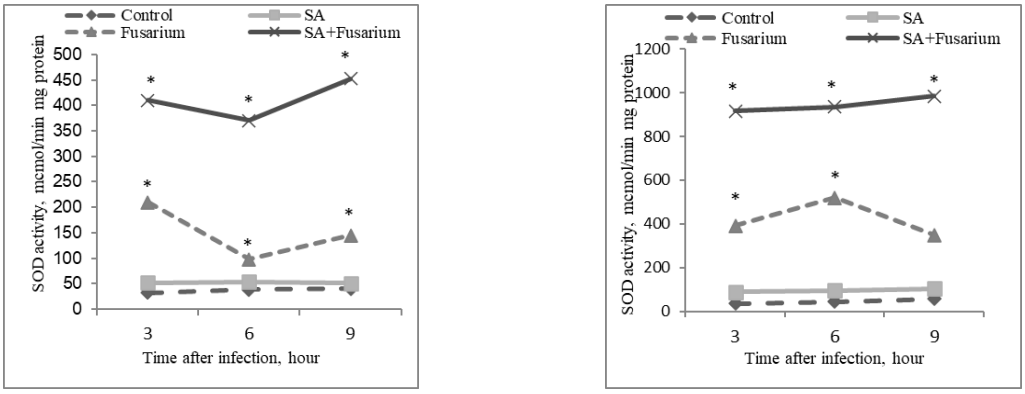

Figure 3. Effect of SA and *Fusarium graminearum* on SOD activity of 4-day-old seedlings of *Zea mays L.*: (**a**) roots; (**b**) shoots; laboratory experiment. * – P<0.05.

Given infected seedlings, *Triticum aestivum* L. with pathogen observed decreased activity of SOD in roots and shoots of seedlings of control (Figure 4). However, under the combined influence of SA and Fusarium graminearum, the enzyme activity was significantly higher in the shoots of seedlings but lower in their roots, but increased sharply at 9 hours after infection.

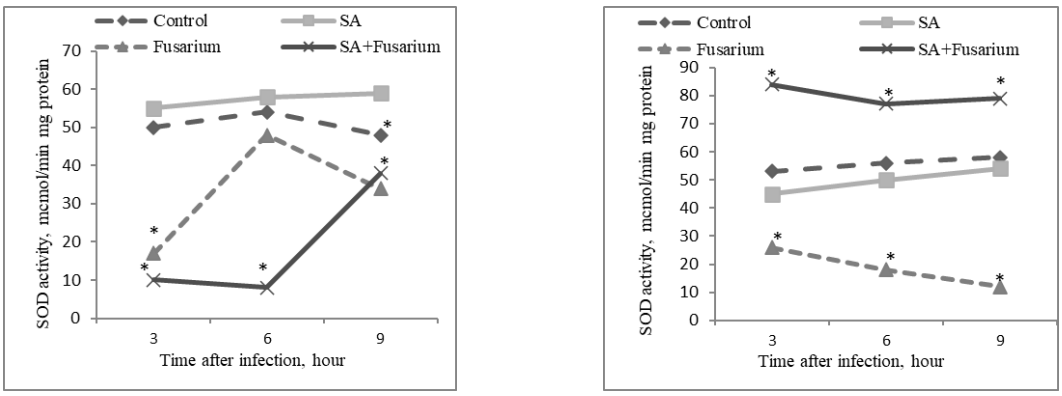

Figure 4. Effect of SA and *Fusarium graminearum* on SOD activity of 4-day-old seedlings of *Triticum aestivum* L.: (**a**) roots; (**b**) shoots; laboratory experiment. * – P<0.05.

It should be noted that the activity of SOD in plants that differ in resistance to stress is different. Resistant plants are characterized by higher activity of SOD and less pronounced oxygen damage compared to unstable ones [13, 17, 28]. The results determine SOD activity suggests that the chosen research chosen corn Transcarpathian yellow-toothed characterized by almost an order of magnitude higher activity of the enzyme when infected with pathogens compared to the wheat of the Kryzhynka variety, particularly in the early stages of ontogeny, which could be evidence of higher resistance of this variety to *Fusarium*.

And the activation of SOD under adverse environmental conditions is associated with a response to increased production of superoxide radicals under such conditions that protect cells and tissues of plant organisms from oxidative degradation [6, 9, 23, 29], so the growth of enzyme activity in seedlings by SA and Fusarium graminearum infection can be considered a positive process that underlies the formation of plant resistance to phytopathogens. This section may be divided by subheadings. It should provide a concise and precise description of the experimental results, their interpretation, and the conclusions that can be drawn.

4. Conclusions

Summarizing the obtained results, we note that SA under the conditions of infection of *Fusarium graminearum* seedlings causes an increase in the effect of "oxidative stress" due to an increase in the activity of SOD, which converts the superoxide radical into a more stable form. Against the background of the increase in SOD activity, it becomes clear that the increase in catalase activity in the tissues of corn seedlings, which at the early stages of ontogenesis, were characterized by higher resistance to Fusarium wilt compared to wheat seedlings. These processes play an important role in forming plant resistance to pathogens, particularly *Fusarium graminearum*. The results of this study will be useful for growing plants on fusarium-contaminated soils. The use of exogenous SA can be recommended for pre-treatment wheat and corn seeds to increase the resistance of plants to pathogens.

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Conflicts of Interest

The authors declare no conflict of interest.

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