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INFLUENCE OF METHYL JASMONATE AND SALICYLIC ACID AS COMPONENTS OF THE CULTIVATION MEDIUM FOR RHIZOBIUM ON FORMATION OF DIFFERENT-EFFICIENCY SYMBIOTIC SYSTEMS *GLYCINE MAX — BRADYRHIZOBIUM JAPONICUM*

Phytohormones are important in the main pathways of transduction of symbiotic signals between macro- and microsymbionts, and understanding of their participation in integration with other metabolic pathways, including prooxidant-antioxidant systems, is crucial in the formation of different-efficiency symbiotic systems. ***Aim****. To investigate the effect of salicylic acid (SA, 50* μM*) and methyl jasmonate (MJ, 0.75* μM*) as components of the cultivation media of different in the activity and virulence rhizobia 604k and B1-20 on the peculiarities of the formation of symbiotic systems in terms of intensity and activity of catalase, as well as on the course of nodulation and nitrogen fixation.* ***Methods****. Microbiological (cultivation of nitrogen-fixing microorganisms, seed inoculation), physiological (vegetation experiment), biochemical (spectrophotometry, gas chromatography), and statistical.* ***Results****. It was found that the use of SA (50* μM*) as an additional component of the culture medium of active rhizobia Tn5-mutant B1-20 for seed inoculation induces increased levels of peroxide production and catalase activity in soybean roots in the early stages of symbiosis, which contributes to the effectiveness of its symbiotic apparatus. Addition of MJ (0.75* μM*) to the culture medium of rhizobia B1-20 does not affect changes in peroxide content and catalase activity in the roots, however, stimulates the processes of nodulation and reduces nitrogen fixation. It has been shown that modification of the cultivation medium of inactive rhizobia of the highly virulent strain 604k using SA (50* μM*) or MJ (0.75* μM*) does not change the peroxide content and leads to an increase in catalase activity in soybean roots during the formation of an ineffective symbiotic system with activation of nodulation processes.* ***Conclusions.*** *When using SA (50* μM*) or MJ (0.75* μM*) as components of the culture medium for rhizobia of different activity and virulence (604k and B1-20), differences were recorded in the levels of functioning of pro-antioxidant systems, in particular, in the production of peroxides and active catalase complexes, at the early stages of the formation of symbiotic systems of Glycine max — Bradyrhizobium japonicum, which affects the intensity of the processes of nodulation and nitrogen fixation.*

Keywords: *Glycine max (L.) Merr., Bradyrhizobium japonicum, symbiotic system, virulence, nitrogen-fixing activity, growth regulators.*

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During the formation of legume-rhizobial symbiosis, there is a clear regulation of the interaction between the partners of symbiosis: rhizobia with their associated molecular patterns (MAMPs) and a plant that forms two types of immune response to infection, namely MAMP-triggered immunity (MIT) and effector-triggered immunity (ETI), which leads to the activation of the main pathways for the transduction of symbiotic signals in the host plant and inhibition of the functioning of its defense systems [1].

The development of protective reactions of legume plants to the invasion of rhizobia is significantly similar to the processes of pathogenesis accompanied by the intensification of oxidative processes in plant cells and increased generation of reactive oxygen species (ROS) [2]. Despite their toxic potential, ROS are key components of signal transduction pathways that trigger protective responses and participate in plant development processes and plant-microbial interactions [3]. It has been shown that peroxides are produced in response to exogenous factors of various natures and can act as signaling molecules and induce (apparently through protein kinase activation and protein phosphorylation) the expression of «protective» genes, which is important for adaptive plant responses, including the establishment of mutualist interactions [4]. Antioxidant enzyme systems play a crucial role in maintaining homeostasis and utilization of excess ROS production by cells [5], a key role among which is played by the catalase enzyme complex, which prevents the accumulation of peroxides — products of the superoxide dismutase reaction [6].

Nodule bacteria are highly sensitive to ROS; however, they are symbiotic microorganisms and, during differentiation in bacteroids, use the host plant's defense reactions against toxic forms of oxygen [1]. Therefore, it is believed that this stage of legume-rhizobial symbiosis is one of the most important for the successful infection of bacteria in the root system and the formation of nodules, which depends primarily on regulation by macrosymbionts [7].

In establishing the immune response of plants, phytohormones occupy a special place — salicylic and jasmonic acids and ethylene, the transduction pathways of which are associated with cytokinin and auxin signals, as well as with DELLA proteins, as described in scientific studies [8, 9]. In this case, the cross-interaction between the transduction pathways of ethylene, jasmonic and salicylic acids modulates the response of plants to infection and leads to the formation of systemically acquired resistance induced by rhizobacteria [10].

To date, it has been proven that jasmonates and salicylates are primarily involved in protecting plants from pathogenic microorganisms, and there have been many studies on their high efficiency in the formation of increased plant resistance to extreme factors [11—13].

Jasmonic acid (JA) and its derivatives, in particular methyl ester MJ, can act as positive and negative regulators of the processes of nodulation and nitrogen fixation depending on the type of legumes, concentration of JA, and phytohormone application [14, 15]. However, it is not clear whether the reaction of plants, the reaction of rhizobia, or combination of both partners is responsible for certain effects of JA on the processes of nodulation [16, 17]. Therefore, additional research is needed to clarify the role of JA in the establishment of symbiotic relationships.

SA is one of the key molecules involved in the formation of systemically acquired resistance, which is due to the accumulation of a group of PR proteins (pathogenesis-related) and has been studied in detail in plant responses to infection with phytopathogens [15, 18, 19]. The negative role of SA in nodulation, as well as the possible need for compatible rhizobia and/or signal of their Nod-factor to inhibit SA-dependent defense mechanism to facilitate the penetration of bacteria into the plant and the successful establishment of symbiotic relationships with legumes, was shown in [15, 20].

In particular, treatment of roots with a 0.1 mM SA solution completely inhibited the formation of nondeterministic nodules and mitogenic effect induced by Nod factors in peas (*Pisum sativum*), alfalfa (*Medicago truncatula*), and creeping clover (*Trifolium repens*), but did not affect the formation of determined nodules in beans (*Phaseolus vulgaris*), soybeans (*Glycine max*), and Lotus japonicus [14, 16, 19]. However, the molecular mechanisms that mediate SA-induced protective signaling during nodulation are definitively unclear.

In this aspect, the study of the influence of SA and JA when used in combination with nodule bacteria of different activity and virulence on the activation of prooxidant-antioxidant systems will reveal special aspects of their participation in the formation of different-efficiency symbiotic systems. Such studies are promising for establishing the possibility of regulating the prooxidant-antioxidant balance through the use of growth regulators as components of the rhizobia cultivation environment to create effective symbiotic systems *Glycine max — Bradyrhizobium japonicum*.

The aim of the study was to investigate the effect of SA (50 μM) and MJ (0.75 μM) as components of the cultivation medium of different activity and virulence of rhizobia 604k and B1-20 for inoculation of soybean seeds on the peroxide production and catalase activity, as well as nodulation and nitrogen fixation in the formation of different-efficiency symbiotic systems.

Materials and methods. The objects of the study were selected symbiotic systems formed with the participation of soybeans (*Glycine max* (L.) Merr.) Almaz variety and different in efficiency rhizobia (*Bradyrhizobium japonicum*) of the Tn5-mutant B1-20 (active, virulent) and 604k (inactive, highly virulent). **Strains**. The authors used nodule bacteria from the museum collection of nitrogen-fixing microorganisms of the Institute of Plant Physiology and Genetics of the National Academy of Sciences of Ukraine (IPPG).

The active virulent Tn5-mutant B1-20 was created at the IPPG by transposon mutagenesis with *Esherichia coli* S17-1 with plasmid pSUP5011 containing transposon Tn5 (pSUP5011 :: Tn-5mob). It was deposited at the Zabolotny Institute of Microbiology and Virology of the National Academy of Sciences of Ukraine under registration number B-7538 [21].

The inactive highly virulent strain of *B. japonicum* 604k is a mutant that has lost the ability to effective symbiosis and is isolated from the nodule during the passage of strain 604 through soybean plants in the area of high radiation — «cesium spot» [22].

Almaz soybean variety — early ripening, recommended for cultivation in the Forest-Steppe of Ukraine, created by hybridization of Molodavo line 3/86 and the Swedish variety Fiskeby 840-5-3 on the basis of the Poltava State Agrarian Academy [23].

Before sowing, soybean seeds were inoculated with rhizobia cultures of inactive strain 604k and active Tn5 mutant B1-20 strain to which 50 μM SA (Bingospa, Poland) or 0.75 μM MJ (Fluorochem Ltd, UK) were added to the culture medium at experimental concentrations. Some of the soybean seeds were inoculated with cultures of rhizobia 604k and B1-20 without adding growth regulators to the cultivation medium. The inoculation load was 200—300 thousand rhizobium cells per seed. The other part of the seed treated with growth regulators was not subjected to bacterization with rhizobia.

Cultivation of nodule bacteria was carried out in flasks (750 ml) on a rocking chair (220 rpm) at 26—28 °C on liquid mannitol-yeast medium (MD) of the following composition (g/L): KH_2PO_4 — 0.5; $MgSO_4$ — 0.2; NaCl — 0.1; yeast extract — 1.0; mannitol — 10.0 [24]. B. *japonicum* cultures in the exponential growth phase (92—96 h) were used as seed material. The number of seeds added to the flasks was 2% of the medium volume. The number of rhizobia in the suspension made up to 200 mL of nutrient medium was 10^8 cells/mL. The purity of bacterial cultures was checked by seeding on meat-peptone agar.

Plants were grown under strictly controlled conditions of a vegetation experiment in pots with a sterile substrate (sand) including the introduction of a nutrient mixture of Herligel (0.25 nitrogen norms) under natural light and optimal moisture (60% of the total moisture content). For research, soybean roots were selected at the early stages of ontogenesis, i.e., etiolated seedlings, cotyledon leaves, primordial leaves, and the first true leaf. The control was symbiotic soybean systems with different in activity and virulence rhizobia 604k or B1-20 without adding growth regulators to their cultivation media, as well as a model soybean system including seed treatment with growth regulators in the absence of rhizobia bacterization.

Biological Nitrogen Fixation (BNF) was measured on an Agilent GC system 6850 gas chromatograph (USA) with a flame ionization detector [25]. Separation of gases was performed on a column (Supelco Porapak N) at 55 °C and a detector — at 150 °C. The carrier gas was helium (20 mL/min). The volume of the analyzed sample of the gas mixture was 1 cm^3. Pure ethylene was of Sigma-Aldrich, No 536164 (USA).

The total nitrogen fixation activity is presented in molar units of ethylene formed (μmol C_2H_4) per plant per hour. Its specific value was calculated per unit mass of root nodules from one plant and is presented in molar units of ethylene formed in one hour (μmol C_2H_4 / g of nodules · h).

The content of peroxides was determined by the ferrothiocyanite method [26]. Extraction of plant material was performed with a cooled 5% solution of trichloroacetic acid (TCA) in a ratio of 1:3 (weight: volume). Supernatant was obtained by centrifugation at 14,000 rpm for 5 min at 4 °C. The hydrogen peroxide concentration was determined by a color reaction with potassium thiocyanate spectrophotometrically at 480 nm and calculated using a calibration curve with known concentrations of hydrogen peroxide. The results are presented in μmol per g of dry weight. The dry weight was obtained by drying the samples at 105 °C to a constant value of weight.

To obtain an enzyme extract, a portion of the plant material was ground in a ratio of 1:2 (weight/volume) with a cooled 0.5 M Tris-HCl buffer (pH 7.8), which contained 5 mM β-mercaptoethanol and 0.1% solution of polyvinylpyrrolidone. The homogenate was centrifuged at 10,000 rpm for 20 min at 4 °C. The supernatant was used to determine the activity of catalase (CAT) (EC 1.11.1.6). The activity of the enzyme was determined by the development of the color reaction with ammonium molybdate at 410 nm according to a modified method of Koroliuk et al. [27] using a scanning two-beam spectrophotometer «UV-1900» (Shimadzu, Japan). The results are presented in mmol of hydrogen peroxide in terms of the content (mg) of total soluble protein per minute, which was determined by Bradford [28]. Determination of BNF was repeated 10 times, peroxides and CAT — 6 times.

Data analysis was performed using Statistica 6.0 (StatSoft Inc., USA). The data are presented in figures as $x \pm SD$ ($x \pm$ standard deviation). Differences between values in the control and experimental groups were determined using the Tuki test, where the differences are considered significant at $P < 0.05$ (taking into account the Bonferroni correction).

Results. It was shown that inoculation of seeds with different activity and virulence of rhizobia (604k and B1-20) led to a decrease in peroxide production in soybean roots in the early stages of formation of different-efficiency symbiotic systems, compared with their accumulation in soybean roots without seed bacterization (Figs. 1, *a*; 1, *b*; 1, *c*). At the same time, a significant decrease in the peroxide content (from 20.0 to 38.4%) was recorded in soybean roots during the formation of effective symbiosis with rhizobia of active virulent Tn5-mutant B1-20, compared with ineffective symbiosis formed with *B. japonicum* 604k.

The addition of SA (50 μM) or MF (0.75 μM) to the culture medium of inactive high-virulence strain 604k did not affect changes in peroxide pro-

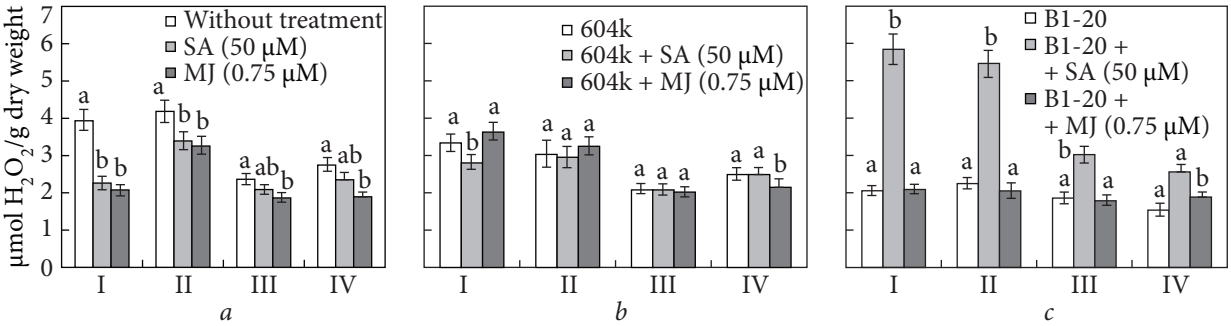

Fig. 1. Production of peroxides in soybean roots upon seed treatment with SA (50 μM) and MJ (0.75 μM) without bacterization (*a*) and inoculation with rhizobia 604k (*b*) and B1-20 (*c*) during their cultivation with growth regulators. Stages of ontogenesis: I — etiolated seedlings; II — cotyledon leaves; III — primordial leaves; IV — the first true leaf; (x ± SD, n = 6). Here and in Figs. 2—4, different letters indicate the mean values of indicators, which differ significantly within the column of the chart compared to the Tukey test, P <0.05 (adjusted for Bonferroni correction)

duction levels in soybean roots during the formation of an inefficient symbiotic system (Fig. 1, *b*).

When using SA as a component of the culture medium of active rhizobia Tn5-mutant B1-20, there was observed an increase in the peroxide content in soybean roots in the formation of an effective symbiotic system by 143.2% (etiolated seedlings), 184.2% (cotyledon leaves), 62.7 and 66.6% (primordial and first true leaves), compared with their concentrations in soybean roots inoculated with rhizobia B1-20 without adding a growth regulator during their cultivation (Fig. 1, *c*). At the same time, the use of MJ (0.75 μM) to modify the culture medium of active rhizobia B1-20 did not cause significant changes in the levels of peroxide production in soybean roots in the early stages of effective symbiosis (Fig. 1, *c*).

Inoculation of soybean seeds with different-activity and different-virulence rhizobia 604k and B1-20 did not affect the change in the activity of CAT in soybean roots in the stage of cotyledon leaves, compared with the activity of the enzyme in soybean roots without bacterization (Figs. 2, *a*, 2, *b*, 2, *c*). Differences in the functioning of the soybean catalase enzymatic complex in symbiotic systems formed with the participation of *B. japonicum* 604k and B1-20 were observed during the period of primordial initiation and nodule formation (Figs. 2, *b* and 2, *c*). In the formation of an ineffective symbiotic system with rhizobia strain 604k, there was observed a decrease in the activity of CAT in soybean roots at the stages of primordial (62.8%) and the first true leaves (29.0%), compared with enzyme activity in soybean roots without seed bacterization (Figs. 2, *a*, 2, *b*). For effective soybean-rhizobial symbiosis with the participation of virulent Tn5-mutant B1-20, an increase in the CAT levels in soybean roots was recorded during the laying of primordial (by 153.9%) and first true leaves (by 164.5%), compared with the enzyme in soybean roots inoculated with ineffective rhizobia of highly virulent strain 604k (Figs. 2, *b*, 2, *c*).

Using growth regulators (SA or MJ) as components of the culture medium of rhizobia strain 604k revealed a significant increase in the CAT levels in the roots, compared with the activity of the enzyme in soybeans without the addition of growth regulators, in particular, by 4.7 times (etiolated seedlings) and 2.5 times (cotyledon leaves) for SA and by 2.3 times (etiolated seedlings) and 3.6 times (cotyledon leaves) for MJ (Fig. 2, *b*).

At the stages of primordial and the first true leaves, in comparison with the previous stages of ontogenesis, a decrease in the overall CAT level in soybean roots in symbiosis with ineffective rhizobia of strain 604k was recorded. With the use of ineffective rhizobia 604k modified

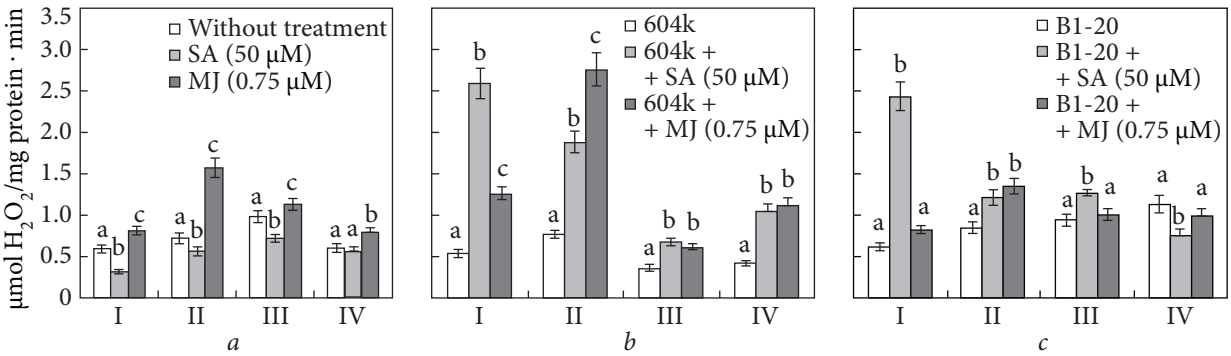

Fig. 2. Catalase activity in soybean roots upon seed treatment with SA (50 μM) and MJ (0.75 μM) without bacterization (*a*) and for inoculation with rhizobia 604k (*b*) and B1-20 (*c*) during their cultivation with growth regulators. Stages of ontogenesis: I — etiolated seedlings; II — cotyledon leaves; III — primordial leaves; IV — the first true leaf; (x ± SD, n = 6)

with growth regulators (SA or MJ), the CAT increased in the roots by 84.4% (SA) and 68.5% (MJ) in the stage of primordial leaves and by 146.9% (SA) and 162.2% (MJ) in the stage of the first true leaf, compared with the enzyme activity in the soybean roots without adding growth regulators to the culture medium of nitrogen-fixing microorganisms (Fig. 2, *b*).

The formation of effective symbiotic systems involving active rhizobia of virulent Tn5-mutant B1-20 and adding SA (50 μM) to their culture medium revealed a significant CAT intensification in the roots of etiolated soybean seedlings by 3.9 times compared to that in the absence of a growth regulator. At the following stages of ontogenesis, i.e., cotyledon and primordial leaves, an increase in the enzyme activity in soybean roots was observed by 43.7 and 33.3%, whereas during the appearance of the first true leaf, there was revealed a decrease by 39.2% in the variant with inoculation of seeds with effective rhizobia B1-20, modified with SA during their cultivation (Fig. 2, *c*).

The use of MJ as an additional component to the culture medium of the effective rhizobiavirulent Tn5-mutant B1-20 showed a slight increase in CAT in soybeanroots by 33.4% at etiolated seedlings and by 59.6% in the cotyledon leaves, compared with the CAT level in the soybean roots for the variant without using a growth regulator (Fig. 2, *c*). In the stage of primordial and first true leaves, there were no significant differences in the CAT in soybean roots between the experiment's variants with adding MJ to the culture medium of effective rhizobia B1-20 and without using this growth regulator. Significant activation of the nodulation process in the stage of the first true leaf was observed due to the formation of symbiotic systems of different efficiency with the participation of growth regulators. Thus, adding SA (50 μM) to the culture medium of rhizobia of the inactive high-virulence strain 604k caused an increase in the number of root nodules by 29.1%, and in the case of using MJ — by 41.8%, as well as an increase in nodules mass by 23.4% (SA) and 33.2% (MJ), compared with the same indicators for soybean plants grown without those growth regulators (Fig. 3).

Inoculation of soybean seeds with active rhizobia of the virulent Tn5 mutant B1-20 cultured with the addition of SA (50 μM) led to an increase in the number of root nodules in the stage of the first true leaf by 22.8%, while their weight was at the level of nodules without a growth regulator (Fig. 3). In the case of adding MJ (0.75 μM) to the culture medium of active rhizobiaB1-20, we recorded an increase in the number of root nodules by 18.9%, whereas their wight was 16.1% lower, compared with these indicators for the variant without a growth regulator (Fig. 3).

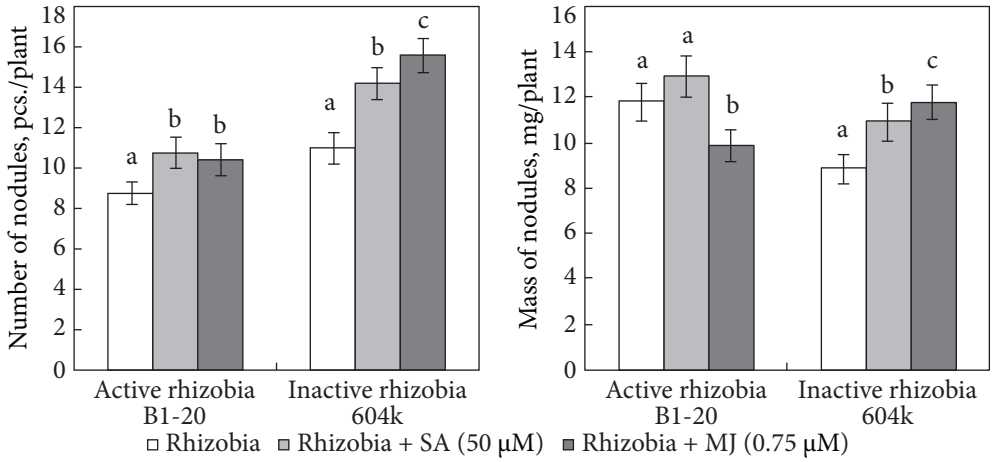

Fig. 3. Influence of SA (50 μM) and MJ (0.75 μM) as components of the cultivation medium for rhizobia of different activity and virulence on the nodulation activity of soybean root nodules at the stage of the first true leaf; (x ± SD, n = 10)

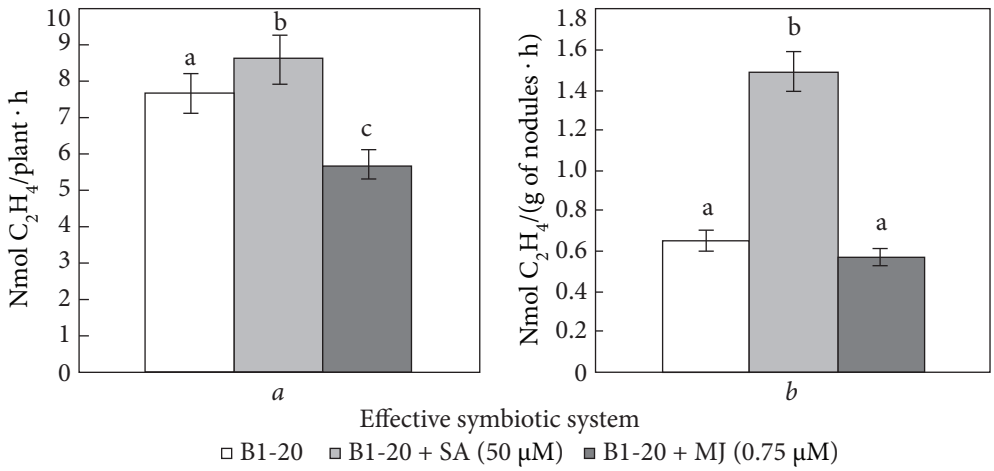

Fig. 4. Influence of SA (50 μM) and MJ (0.75 μM) as components of the cultivation medium for effective rhizobia B1-20 on the total (*a*) and specific (*b*) nitrogen-fixation activity of soybean root nodules at the stage of the first true leaf; (x ± SD, n = 10)

Modification with SA (50 μM) added to the rhizobia culture medium of active virulent Tn5-mutant B1-20 provided an insignificant increase in the total BNF of root nodules by 12.4% and a significant increase in the efficiency of fixing molecular nitrogen per unit mass of nodules by 129.2% at the stage of the first true leaf, compared with BNF of soybean root nodules for the variant without a growth regulator (Fig. 4, *a*). The use of MJ (0.75 μM) in the culture medium of active rhizobia B1-20 led to a decrease in the total BNF of root nodules by 25.5% and slight changes in its specific value, compared with the same indicators in the variant without using a growth regulator (Fig. 4, *b*).

Discussion. Regulation of the nodulation process in the interaction of macro- and microsymbionts is associated with the formation of ROS and the activity of antioxidant enzymes [4]. Invasion of rhizobia in the cells of the root hairs

of legumes, similar to the process of pathogenesis, causes an intensification of oxidative processes in plant cells, accompanied by increased generation of ROS, which induces a number of reactions in plants [29]. It is believed that in the formation of symbiotic relationships, ROS can be involved in the regulation of rhizobia root infection both due to direct antibacterial effects and by regulating the functional activity of the protective systems of the host plant [30].

Analysis of the results showed that inoculation of soybean seeds with rhizobia of different activity and virulence (604k and B1-20) leads to a decrease in peroxide production levels in soybean roots in the initial stages of soybean-rhizobial symbiosis. In an effective symbiotic system with the participation of rhizobia of virulent Tn5-mutant B1-20, a lower concentration of peroxides in the roots was revealed as compared with their content in soybean roots in the formation of ineffective symbiosis with rhizobia of highly virulent strain 604k.

Significant differences are shown in the functioning of the catalase enzymatic complex in soybean roots during the establishment of nodule primordia and the formation of nodules in rhizobia of different efficiency (604k and B1-20). At the same time, a decrease in CAT activity in soybean roots was shown during the formation of an ineffective symbiotic system with rhizobia of strain 604k and an increase in enzyme activity — during the formation of an effective soybean-rhizobial symbiosis with the participation of *B. japonicum* B1-20.

In the transduction pathways of plant cells in response to external factors of various natures, peroxides serve as a local signal for the development of hypersensitive cell response associated with cell wall strengthening by increasing regulation of phenylpropanoid genes expressing lignin proteins and fenone compounds [31]. It has been suggested that the accumulation of phenylpropanoid compounds in the cells of some legumes, especially lignin, may form a mechanical barrier to infection by microorganisms [32]. At the same time, it is believed that the activity of CAT may reflect the metabolic changes that occur in the cells of the root bark when infected with rhizobia of different activity and virulence [33, 34]. It is obvious that the changes we recorded in the levels of peroxide production and catalase activity in soybean roots, induced by inoculation of seeds with rhizobia of different activity and virulence, could be due to a complex of the above biochemical processes.

The obtained results are consistent with the literature on the possible influence of virulence and activity of the bacterial strain nodules on changes in the CAT levels in soybean roots at the early stages of symbiosis. We noted changes in the accumulation of peroxides and catalase activity in soybean roots in the early stages of the formation of different-efficiency symbiotic systems indicatinge significant changes in metabolic processes in the roots of the host plant in response to rhizobia invasion, which depends on the activity and virulence of the rhizobia.

It has been shown that the addition of SA (50 μM) or MJ (0.75 μM) to the culture medium of inactive rhizobia of the highly virulent strain of *B. japonicum* 604k induces an increase in CAT activity in the roots, however, does not lead to a significant change in peroxide accumulation in soybean roots. Their production remains at the same low level as in an inefficient symbiotic system without the use of growth regulators. Such changes in soybean metabolism in the formation of ineffective symbiosis (strain 604k) with the participation of growth-regulating compounds (SA or MJ) are accompanied by stimulation of nodule formation in soybeans during the formation of the first true leaf.

A number of studies have shown that jasmonates are involved in increasing the resistance of plants to infection by phytopathogens, reducing the CAT level and increasing the activity of oxalate oxidase, which leads to the accumulation of hydrogen peroxide [35]. Earlier we noted the

similarity of some reactions of legumes to inoculation with rhizobia, to infection with pathogenic microorganisms. However, in the formation of nodules, the result of these reactions is not the inactivation of the microorganism, but the regulation of its reproduction and metabolic activity in symbioses. It is possible that the role of jasmonates in the formation of symbiotic interaction, in contrast to their participation in pathogenesis, is to induce the opposite chain of biochemical reactions, including increasing CAT activity and reducing peroxide accumulation, which affects the stimulation of nodulation in the formation of an inefficient soybean symbiotic system.

It was shown that the increase in the levels of synthesis of SA and MJ occurs along with a slight decrease in the content of cytokinins and auxins, as well as the accumulation of abscisic acid, which plays an important role in triggering protective reactions of the plant [8]. In addition, it was shown that SA affects the generation of ROS, as well as the activity of antioxidant enzymes (superoxide dismutase, catalase, peroxidase), causing intracellular changes in the antioxidant system in plants [19, 13]. Our use of growth regulators (SA or MJ) as additional components of the rhizobia cultivation medium could induce changes in the functioning of the host plant metabolism due to the modification of the phytohormonal balance, in particular, an increase in the endogenous content of SA or MJ in cells, and also cause the activation of protective complexes in the host including the CAT level.

However, the effect of SA and MJ on changes in the activity of pro-antioxidant systems in legumes during inoculation with rhizobia of different activity and virulence has not been sufficiently studied. It is known about the negative role of SA in nodule formation, as well as the possible need for homologous rhizobia and (or) their Nod-factor signal to inhibit the SA-dependent defense mechanism to facilitate bacterial penetration into the plant and successfully establish a symbiotic relationship with legumes [14, 20]. Thus, when inoculated with *Medicago sativa* by a complementary strain of *Rhizobium meliloti*, the level of SA in plant roots either decreased or did not change. Inoculation of *Medicago sativa* with a strain of *Rhizobium leguminosarum* or a mutant of *Rhizobium meliloti*, defective in the biosynthesis of Nod-factor, led to the accumulation of SA in the roots [16, 18]. Consequently, Nod factors synthesized by homologous rhizobia were involved in the suppression of SA-mediated defense in leguminous plants. At the same time, it was shown that SA-dependent protective signaling is quickly activated at the early stages of symbiotic interaction (pre-infection), but is suppressed over time, which contributes to the growth of an infectious thread into the root cortex and the formation of nodule primordia [36].

We have shown that the addition of SA (50 μM) to the culture medium of rhizobia effective virulent Tn5-mutant B1-20 increases the levels of peroxide production and CAT intensification in soybean roots, accompanied by stimulation of nodulation and efficiency of molecular nitrogen fixation on root nodule's stages of symbiosis formation. This indicates the important role of SA (50 μM) in the activation of pro-antioxidant defense systems and regulation of soybean metabolism in the formation of symbiosis with active rhizobia B1-20, which leads to a more effective level of its functioning.

The addition of MJ to the cultivation medium of active rhizobia Tn5-mutant B1-20 did not affect the changes in the levels of peroxide accumulation and CAT in soybean roots, compared with the values of the same indicators in an effective symbiotic system formed without the use of a growth regulator. At the same time, an increase in the number of nodules and a decrease in their mass and nitrogen-fixing activity in soybeans were recorded when seeds were inoculated with active B1-20 rhizobia modified with MJ during their cultivation. Our studies have shown that the use of MJ (0.75 μM) in the culture medium of active rhizobia B1-20 induces a complex

of biochemical processes, which primarily affect the reduction of nitrogenase enzyme complex in soybean root nodules at the early stages of the effective symbiotic system formation, which requires further thorough studies.

The transduction of the jasmonate signal to the genetic apparatus of the cell has not yet been sufficiently clarified. It was shown that one of the key components of jasmonate signaling is specific proteins, in particular, COI1 (coronative insensitive 1) protein, which is necessary for the removal of repressor proteins of transcript factors of FJ signaling genes [35]. At the same time, there is evidence that a number of effects of MJ are realized with the participation of mechanisms dependent on ROS [13]. However, the participation of ROS and their signaling pathways in the integration with the metabolic pathways of the phytohormones studied by us, including jasmonates, in the transduction of symbiotic signals in the formation of different-efficiency legume-rhizobial symbioses remain poorly clarified.

Conclusions. SA at a concentration of 50 μM as an additional component of the culture medium of active rhizobia Tn5-mutant B1-20 induces activation of pro-antioxidant systems to a more effective level of their functioning, which has a positive effect on the regulation of soybean metabolism in the formation of effective symbiosis with B1-20.

When MJ (0.75 μM) is used to modify the cultivation medium of active rhizobia B1-20, there is no change in the peroxide content and catalase activity in the roots, and at the same time, there are induced biochemical processes that affect the decrease in the work of the nitrogenase enzymatic complex in soybean nodules during the formation of symbiosis with *B. japonicum*.

The use of SA (50 μM) or MJ (0.75 μM) as components of the cultivation medium for ineffective rhizobia of the highly virulent strain *B. japonicum* 604k does not affect the change in the peroxide content and leads to an increase in catalase activity in soybean roots with significant activation of the nodulation process.

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ВПЛИВ МЕТИЛЖАСМОНАТУ ТА САЛІЦИЛОВОЇ КИСЛОТИ ЯК КОМПОНЕНТІВ СЕРЕДОВИЩА КУЛЬТИВУВАННЯ РИЗОБІЙ НА ОСОБЛИВОСТІ ФОРМУВАННЯ РІЗНИХ ЗА ЕФЕКТИВНІСТЮ СИМБІОТИЧНИХ СИСТЕМ СОЯ—*BRADYRHIZOBIUM JAPONICUM*

В активації основних шляхів трансдукції симбіотичних сигналів між макро- і мікросимбіонтами важливе значення відіграють фітогормони, а їхня участь в інтеграції з іншими метаболічними шляхами, зокрема про-антиоксидантних систем є визначальною при формуванні різних за ефективністю симбіотичних систем. **Мета.** Дослідити вплив саліцилової кислоти (СК, 50 мкМ) та метилжасмонату (МЖ, 0,75 мкМ) як компонентів середовища культивування різних за активністю та вірулентністю ризобій 604к і В1-20 на особливості формування симбіотичних систем соя—*Bradyrhizobium japonicum*, різних за інтенсивністю продукування пероксидів та активністю каталази, а також перебігом процесів нодуляції та азотфіксації. **Методи.** Мікробіологічні (культивування азотфіксуючих мікроорганізмів, інокуляція насіння), фізіологічні (вегетаційний експеримент), біохімічні (спектрофотометрія, газова хроматографія) та статистичні. **Результати.** Використання СК (50 мкМ) як додаткового компонента середовища культивування активних ризобій Tn5-мутанту В1-20 індукує підвищення рівнів продукування пероксидів та активності каталази у коренях сої на ранніх етапах формування симбіозу, що сприяє ефективності роботи симбіотичного апарату за рахунок підвищення процесу нодуляції та ефективності фіксації молекулярного азоту на одиницю маси бульбочки. Отримані дані доводять, що СК у концентрації 50 мкМ індукує активацію роботи про-антиоксидантних систем до більш ефективного рівня їх функціонування, що позитивно впливає на регуляцію метаболізму сої при формуванні ефективного симбіозу за участю активних ризобій В1-20. Застосування МЖ (0,75 мкМ) для модифікації середовища культивування активних ризобій В1-20 не впливає на зміну вмісту пероксидів та активності каталази у коренях, однак викликає стимуляцію процесу нодуляції та зниження азотфіксації. Очевидно, що за додавання МЖ (0,75 мкМ) до середовища культивування ризобій В1-20 індукуються біохімічні процеси, які в першу чергу позначаються на зниженні роботи нітрогеназного ензиматичного комплексу сої на ранніх етапах симбіотичної взаємодії. Показано, що модифікація середовища культивування неактивних ризобій високовірулентного штаму 604к за допомогою СК (50 мкМ) або МЖ (0,75 мкМ) не спричиняє зміни вмісту пероксидів та приводить до підвищення активності каталази у коренях сої при формуванні неефективних симбіотичних систем із суттєвою активацією процесу нодуляції. **Висновки.** З використанням СК (50 мкМ) або МЖ (0,75 мкМ) як компонентів середовища культивування ризобій різної активності та вірулентності (604к і В1-20) зафіксовано відмінності у рівнях функціонування про-антиоксидантних систем, зокрема продукування пероксидів та активності каталазного ензиматичного комплексу на ранніх етапах формування симбіотичних систем соя — *Bradyrhizobium japonicum*, що позначається на інтенсивності протікання процесів нодуляції та азотфіксації.

Ключові слова: *Glycine max (L.) Merr., Bradyrhizobium japonicum, симбіотична система, вірулентність, азотфіксувальна активність, регулятори росту.*