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PHENYLALANINE AMMONIA-LYASE ENZYME ACTIVITY IN THE SYMBIOTIC SYSTEM *GLYCINE MAX – BRADYRHIZOBIUM JAPONICUM* BY SEED INOCULATION DIFFERENT IN ACTIVITY AND VIRULATION STRAIN AND TREATMENT WITH FUNGICIDES

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Phenylalanine ammonia-lyase (PAL) is a key enzyme of the phenylpropanoid pathway and provides precursors for the synthesis of many secondary metabolites, which are necessary for the development and protection of plants from external factors of various natures, in particular plays an important role in the formation and development of their symbiosis with microorganisms. ***Aim****. To study the activity of PAL in soybean plants in the early stages of legume-rhizobial symbiosis under the influence of seed inoculation with Bradyrhizobium japonicum strains with different symbiotic properties on the background of fungicide treatment.* ***Methods.*** *Microbiology (bacterial culture growing, seeds inoculation), physiological (pot experiment), biochemical (determining the PAL enzyme activity).* ***Results.*** *Inoculation of soybean seeds with active virulent rhizobia induces a significant decrease in PAL activity in the roots at the primordial leaf stage and a significant increase in its activity level at the first true leaf stage, compared to inactive symbiosis. At the stage of third true leaf, the activity of PAL increased more significantly in soybean root nodules formed by inactive rhizobia, compared to active symbiosis. However, at the stage of third true leaves, the activity of PAL in soybean root nodules formed by inactive rhizobia increased significantly compared to active symbiosis. The use of fungicides for pre-sowing treatment of soybean seeds induces changes in the level of PAL activity in roots and nodules, which do not affect the overall dynamics of enzyme activity in different effective symbiotic systems Glycine max-Bradyrhizobium japonicum.* ***Conclusions.*** *The activity of PAL in the roots and especially in the root nodules of soybeans in the early stage of plant development in the case of fungicides using and bacterization is primarily due to the action of the inoculation factor, and is determined by the symbiotic properties of rhizobia strains, in particular, their virulence and nitrogen fixation activity.*

Keywords: soybean (Glycine max (L.) Merr.), nodule bacteria, symbiotic system, virulence, nitrogen fixation activity, strain, Maxim XL, Standak Top.

The formation of protective reactions of legumes to the invasion of rhizobia has significant similarities with pathogenic pathways [1]. There is a cross-interaction between the transduction pathways of ethylene, jasmonic and salicylic acids, which modulates the response of plants to infection and leads to the formation of systemically acquired resistance [2].

Phenylalanine ammonium lyase (PAL) (EC 4.3.1.5) is a key enzyme in the response of plants to biotic stress, including rhizobacteria-induced systemic resistance (ISR) [3]. It has been shown that PAL gene expression is activated by the ethylene/jasmonate signaling pathway, which leads to the development of ISR. Gene expression and PAL enzyme activity can be considered as markers of this protective reaction [4]. This enzyme is important in the biosynthesis of a wide range of secondary metabolites: polyphenols, phenylpropanoids, lignin monomers, salicylic acid (SA) and other compounds that are necessary for plant development and their protection from external factors [5]. Although the biochemical function of PAL is well established, the functional significance of its genes in symbiotic processes has not been sufficiently studied.

It has been proven that the PAL gene in *Lotus japonicus* (LjPAL1) plays a different role in the

establishment of symbiosis with *Mesorhizobium loti*, influencing the development of rhizobial infection and nodules structure by modifying the lignin content, as well as regulating the endogenous biosynthesis of SA and modulating SA-dependent signaling [6]. Transgenic plants overexpressing the LjPAL1 gene (LjPAL1-OE) showed a delay in the infection process and a decrease in the number of infection threads and nodules after *M. loti* inoculation. Conversely, in plants with inhibition of the LjPAL1 (LjPAL1i) gene function, an increase in the number of infectious threads and nodules, as well as the induced regulation of nodulin gene expression after *M. loti* infection occurs [7]. Compared to wild-type, LjENOD40 and LjNIN nodulin gene transcripts were more abundant in rhizobia-infected roots of LjPAL1i plants and fewer in LjPAL1-OE lines [7]. These results indicate that LjPAL1 may act as a regulator of rhizobial infection in symbiosis with legumes.

It is investigated that the activity of PAL in most cases is associated with the content of phenolic compounds and lignin, which play an important role in inducing resistance of plants to stress factors [8]. Thus, with the use of specific inhibitors of this enzyme in plants, inhibition of the formation of phenols and lignin precursors has been shown, which has led to increased phytopathogenic damage to plants, even genetically resistant forms [9]. Therefore, it is believed that this enzyme may serve as a marker of induced resistance to plant diseases caused by phytopathogens.

Phenolic compounds act as signaling compounds or inducers of virulence genes at the time of interaction of micro- and macrosymbionts in the formation of legume-rhizobial symbiosis [10]. They play an important role in legume-rhizobial symbiosis, as they not only induce the onset of symbiosis, but can also act as signals – mediators of *Nod*-factors on the redistribution of auxin and thus be regulators of the physiological state of the symbiotic system and control the formation of nodules, as in the stages active nitrogen fixation and aging [11].

Studies have now shown that drugs with fungicidal activity disrupt the regulatory signaling system between macro- and microsymbionts by blocking the activity of nodulation genes and reducing the level of rhizobial *Nod*-factor [12]. It is known that legumes with an active symbiotic apparatus are resistant to a wide range of diseases, and a clear combination of all measures aimed at optimizing the process of symbiosis contributes to the realization of their productive potential [13]. Active research is being conducted on the possibility of using fungicides to regulate the metabolism of legumes in symbiosis with nodule bacteria and increase the tolerance of the formed symbiotic systems to the action of biotic factors [12, 13].

The study of the functioning of PAL in the early stages of legume-rhizobial interaction is of theoretical importance to establish its participation in the induction of protective reactions of legume plants in response to inoculation of rhizobia with different symbiotic properties under appropriate growing conditions. And conducting such studies in combination with the formation of the symbiotic apparatus in legumes using fungicide seed treatment will be important in practice to find effective symbiotic systems that will be able to maximize the symbiotic potential and at the same time have high tolerance to external factors.

The aim was to study the activity of PAL in soybean plants in the early stages of legume-rhizobial symbiosis under the influence of seed inoculation with different symbiotic properties of *Bradyrhizobium japonicum* strains on the background of fungicide treatment.

Materials and method. The objects of study were symbiotic systems formed with the participation of soybean plants (*Glycine max* (L.) Merr.) Almaz cultivar, strains of *B. japonicum* – 634b (active, virulent) and 604k (inactive, highly virulent) fungicides Maxim XL 035 PS (Syngenta, Switzerland) and Standak Top (BASF, Germany). We used *B. japonicum* strains from the museum collection of symbiotic nitrogen fixation department of the Institute of Plant Physiology and Genetics of the NAS of Ukraine.

Inactive highly virulent strain *B. japonicum* 604k is a mutant that has lost the ability to efficient symbiosis. It is isolated from the nodule during the straining of strain 604k through its plants in the zone of high radiation – «cesium spot» [14]. The active virulent strain of *B. japonicum* 634b was isolated from soybean nodules by analytical selection [a.s. USSR No. 922104, 1982].

Almaz soybean cultivar – early ripening, recommended for cultivation in the Forest-Steppe of Ukraine, created by hybridization of the Molodavo line 3/86 and the Swedish variety Fiskeby 840-5-3 on the basis of Poltava State Agrarian Academy [certificate No. 07020, patent No. 07105].

Fungicides that differed in the spectrum of action of the active substances were used for the

research. The composition of the fungicide Standak Top includes active substances (fipronil, 250 g/L, thiophanate-methyl, 225 g/L, pyraclostrobin, 25 g/L), which combine fungicidal and insecticidal action. The action of fipronil is to block gamma-aminobutyric acid, which regulates the passage of nerve impulses through chlorine channels in the membranes of insect nerve cells. Pyraclostrobin inhibits mitochondrial respiration by blocking electron transfer and disrupting energy metabolism in pathogen cells. Thiophanate-methyl inhibits the formation of ergosterol, as well as the biosynthesis of nucleic acids in fungal cells [https://www.pesticidy.ru]. The composition of the fungicide Maxim XL includes two active substances (fludioxonil, 25 g/L, metalaxyl, 10 g/L), one of which, fludioxonil, is an analogue of a natural antibiotic released by soil bacteria *Pseudomonas pyrocinia*. The mechanism of action of this drug is associated with processes that induce disruption of membrane transport functions in the cells of the pathogen, thus inhibiting the growth and reproduction of the pathogen [https://www.pesticidy.ru].

Before sowing, soybeans were treated with solutions of fungicides, calculated on the basis of one rate of expenditure of the active substance of each preparation indicated by the producer per ton of seeds. One portion of the fungicide-treated seeds was inoculated with rhizobia suspension for one hour. The other part of seeds treated with fungicides was sown without inoculation with rhizobia. A separate variant of the experiments was soybean seeds, not treated with fungicides, but inoculated with nodule bacteria.

The culture of rhizobium was grown on solid mannitol-yeast medium for 9 days at 26–28 °C (the titer of bacteria was 10^8 cells/mL). The inoculation load was 200–300 thousand rhizobia cells per seed. The control was non-inoculated plants, as well as inoculated with rhizobia without the use of fungicide seed treatment.

Plants were grown under strictly controlled conditions of a growing experiment in pots with a sterile substrate (sand) with the addition of Herligel nutrient medium (0.25 nitrogen norms) according to natural light and optimal water supply. For research, soybean roots were selected in the early stages of ontogenesis − seedlings leaves, primordial leaves, first true leaf, second and third true leaves, and as well as root nodules in the stage of third true leaf.

Determination of PAL activity was performed according to the modified method of Zuk [15]. To obtain the enzyme extract, the plant material was homogenized with a 0.2 M solution of borate buffer (pH 8.8) in a ratio of 1:2 (weight/volume), which contained 1 mM ethylenediaminetetraacetic acid, 5 mM β-mercaptoethanol and 1 % polyvinylpyrrolidone. The homogenate was centrifuged at 12,000 rpm for 20 min at 4 °C. The supernatant was used to determine the activity of PAL using the UV-1900 scanning two-beam spectrophotometer (Shimadzu, Japan) at 290 nm for the formation of trans-cinnamic acid in 0.1 M borate buffer (pH 8.8) in the presence of 50 mm of L-phenylalanine. Incubation of the reaction mixture was performed for 1 h at 40 °C. The results are presented in units of activity of the enzyme on the concentration of protein (mg) in the supernatant. The content of total soluble protein in the enzyme extract was determined by Bradford method [16].

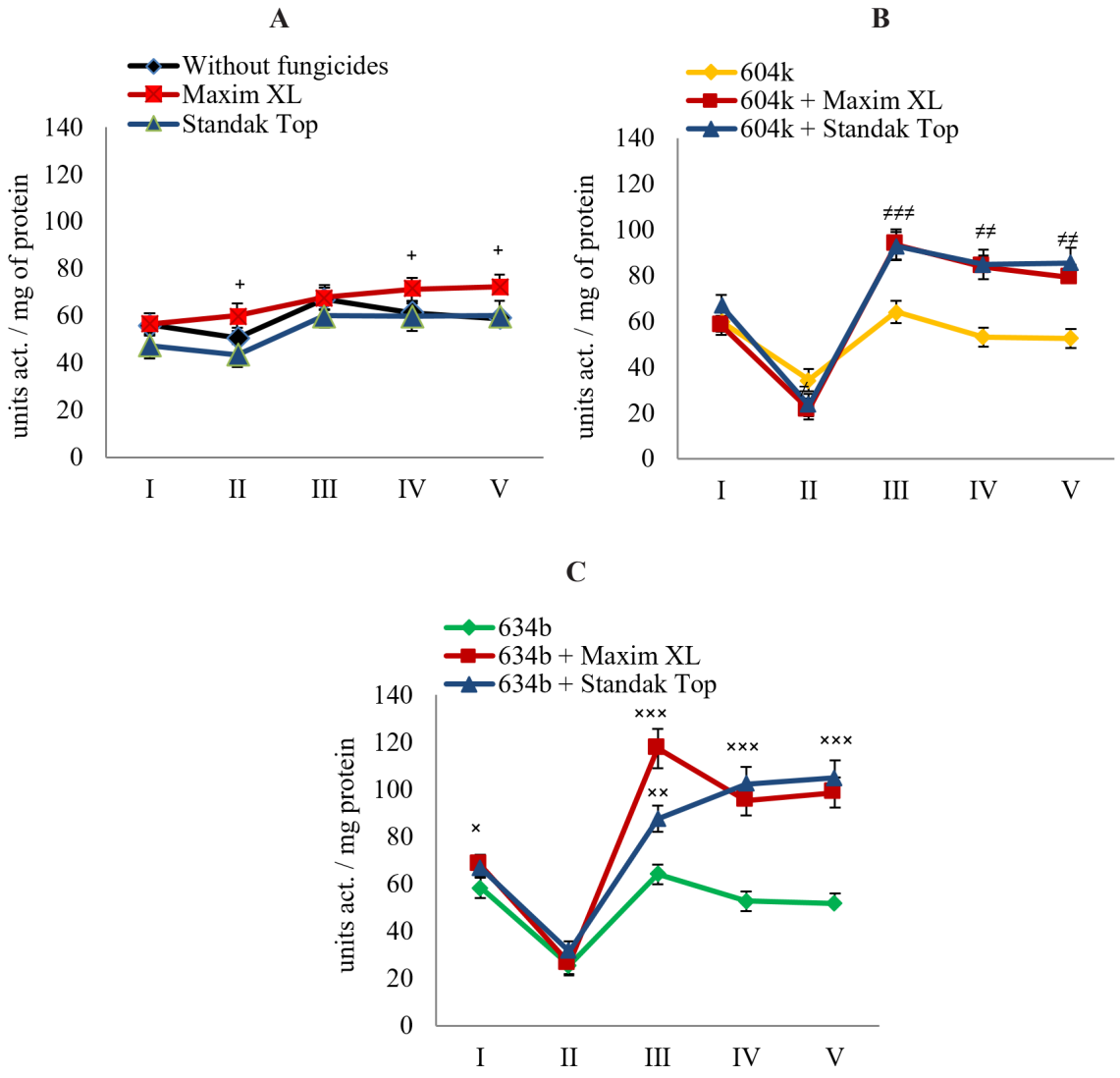
The figures show the arithmetic mean values and their standard errors ($x \pm SE$). The reliability of the differences between the samples was evaluated using the single-factor dispersion analysis (ANOVA). Differences were considered to be significant at $P < 0.05$.

Results. It is shown that the pre-sowing treatment of soybean seeds with the fungicide Standak Top shows a slight decrease in the activity of PAL in the plant roots in the initial stages of ontogenesis – seedlings leaves, primordial leaves and the first true leaf, respectively, by 16.1; 14.4 and 10.5 %, compared with untreated plants (Fig. 1A). In the stage of the second and third true leaves, no significant differences were found in the activity of the enzyme in soybean roots during treatment with Standak Top and without its use. Under the action of the fungicide Maxim XL, an increase in the activity of PAL in the roots by 18.3 % in the stage of primordial leaves was recorded. Whereas in the stage of the first true leaf its activity was at the level of untreated plants, and in the stage of the second and third true leaves – increased by 16.5 and 22.9 %, respectively.

Inoculation of soybean seeds with rhizobia of inactive strain 604k did not induce significant changes in PAL activity in soybean roots in the seedlings leaves stage and led to a decrease in enzyme activity by 32.1 % in the stage of primordial leaves, compared with non-inoculated plants (see Fig. 1A, Fig. 1B). In the stage of the first true leaf, the activity of the enzyme approached the level of non-inoculated plants, and in the stages of the second and third true leaves observed a slight decrease in the level of its activity.

Pre-treatment of soybean seeds with Standak Top fungicide followed by inoculation with rhizobia of inactive strain 604k showed a slight increase in PAL activity in the roots by 11.8 % in the seedlings leaves stage. Significant changes in the activity of the enzyme in the roots with the use of Maxim XL, in comparison with inoculated plants without the use of fungicides were not detected (Fig. 1B). In the stage of primordial leaves, a decrease in the activity of the enzyme in the roots of soybeans inoculated with inactive rhizobia by 37.1 and 32.1 %, respectively, under the action of fungicides Maxim XL and Standak Top. In the following stages of ontogenesis: the first, second and third true leaves, in an inefficient symbiotic system, there was an increase in the activity of PAL in the roots, respectively, by 45.6, 57.5 % and 50.9 % under the actions of Maxim XL and 44.9, 59.9 and 63.1 % under the action of Standak Top, compared with inoculated plants without the use of fungicide treatment.

In an effective symbiotic system formed with the participation of soybean plants and the active strain of *B. japonicum* 634b, no significant changes in the activity of PAL in the roots in the seedlings leaves stage were recorded, compared with non-inoculated plants (see Fig. 1C). However, there was a decrease in its activity by 49.7 % in the stage of primordial leaves. In the stage of the first true leaf, the activity of the enzyme was at the level of



F i g. 1. PAL activity in soybean roots with non-cultivated rhizobia (A) and by inoculation with strains of *B. japonicum* 604k (B) and 634b (C) on the background of seed treatment with fungicides. Stage of ontogenesis: I – seedlings leaves, II – primordial leaves, III – first true leaf, IV – second true leaf, V – third true leaf (x ± SE, n = 6); data compared to control are reliable at $^{+\neq\times}$ – P <0.05; $^{++\#\,\times\times}$ – P <0.01; $^{+++\,\#\#\,\times\times\times}$ – P <0.001 ($^{+}$ – relative to variants without fungicides; $^{\neq}$ and $^{\times}$ – relative to variants with inoculation of strains 604k and 634b, respectively).

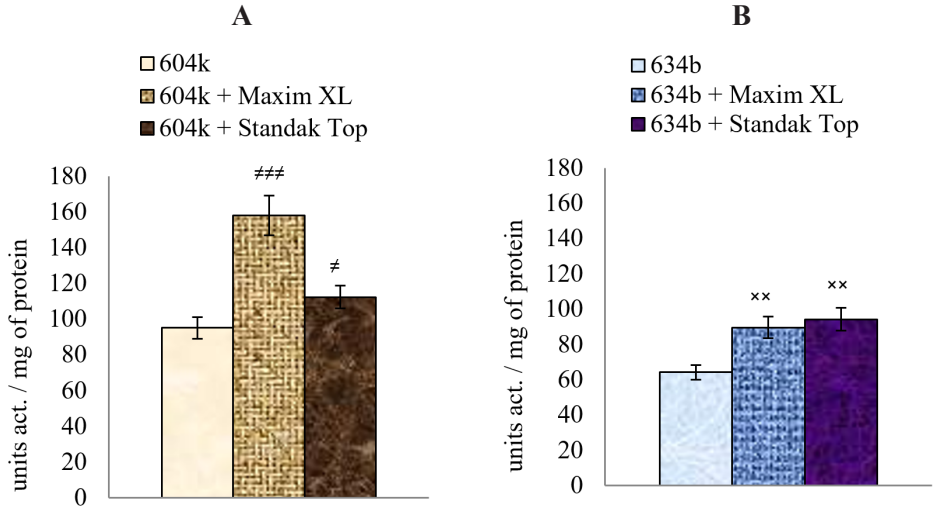
non-inoculated plants, whereas in the stages of the second and third true leaves it decreased slightly, in comparison with non-inoculated plants.

In the seedlings leaves stage, no significant difference in PAL activity in soybean roots was observed between variants of the experiment with seed inoculation with inactive and active rhizobia (see Fig. 1B, Fig 1C). However, in the stage of primordial leaves, the activity of PAL in soybean roots inoculated with the active strain of rhizobia 634b was 25.8 % lower compared to the activity of the enzyme in the roots of soybeans inoculated with an inactive strain of rhizobia 604k. In the following stages of ontogenesis before the formation of the third true leaf, no significant difference was found in the activity of the enzyme in soybean roots by seed inoculation with active and inactive rhizobia.

When using soybean seed treatment with fungicides in an effective symbiotic system, an increase in the activity of the enzyme in the seedlings leaves stage by 16.5 % (Maxim XL) and 14.4 % (Standak Top), compared with inoculated plants without fungicides (see Fig. 1C). In the primordial leaf stage, PAL activity increased in soybean roots inoculated with active rhizobia by 23.5 % during seed treatment with Standak Top fungicide, whereas under Maxim XL the enzyme activity was at the level of inoculated plants without fungicides. In the following stages of ontogenesis, the first, second and third true leaves, the activity of PAL increased significantly in soybean roots in the formation of an effective symbiotic system using pre-sowing seed treatment with Maxim XL fungicide by 83.1, 80.6 and 90.3 %, and under the influence Standak Top – by 36.7, 93.8 and 102.3 %, respectively.

It was investigated that in the stage of third true leaves, the activity of PAL in the root nodules of soybeans inoculated with the inactive rhizobia strain 604k was 1.5 times higher compared to the active rhizobia strain 634b (Fig. 2).

The use of pre-sowing treatment of soybean seeds with fungicides by Maxim XL and Standak Top led to an increase in PAL activity in soybean root nodules in the stage of third true leaves, regardless of the efficiency of the symbiotic system (see Fig. 2). In particular, in soybean plants treated with different fungicides simultaneously with rhizobia of inactive strain 604k, the activity of PAL in root nodules increased by 66.2 and 18.1 %, respectively, under the action of Maxim XL and Standak Top. Whereas in the complex treatment of seeds with fungicides and rhizobia of the active strain 634b, the activity of PAL in the root nodules increased by 39.6 and 46.8 %, respectively, under the action of Maxim XL and Standak Top.



F i g. 2. PAL in soybean root nodules in the stage of the third true leaf by inoculation seeds with *B. japonicum* 604k (A) and 634b (B) and fungicide treatment. (x ± SE, n = 6); data compared with control are reliable at # ×× – P <0.01; ## ××× – P <0.001 (≠ and × – relative to variants with inoculation of strains 604k and 634b, respectively). In non-inoculated soybean rhizobia, root nodules were not created.

Discussion. Rhizobial and pathogenic bacteria have similar strategies for colonizing, penetrating, and establishing effective infection of host plant cells [17]. The PAL gene has been shown to act as a positive regulator of the SA-dependent protective signal to control phytopathogens due to its enzymatic activity in the phenylpropanoid pathway [18]. Similarly, transgenic plants of *L. japonicus*

with overexpression of the PAL gene (LjPAL1-OE) were observed to increase the accumulation of SA levels and less susceptibility to *M. loti* infection. And in plants with loss of PAL gene function (LjPAL1i) – a decrease in the level of endogenous SA and the expression of SA-dependent marker gene [6].

It is known about the negative role of SA in nodulation, as well as the possible need for complementary rhizobia and (or) their *Nod*-factor signal to inhibit SA-dependent defense mechanism to facilitate bacterial penetration into the plant and successfully establish a symbiotic relationship with legumes [19]. In particular, when inoculating *Medicago. sativa* complementary strain of *Rhizobium meliloti*, the level of SA in the roots of plants either decreased or did not change. Inoculation of *M. sativa* with a strain of *R. leguminosarum* or a mutant of *R. meliloti*, defective in the biosynthesis of Nod-factors, led to the accumulation of SA in the roots [20]. Thus, *Nod*-factors synthesized by complementary rhizobia were involved in the suppression of SA-mediated protection of legumes.

It was shown that SA-dependent protective signaling mediated by PAL genes is rapidly activated at the early stages of symbiotic interaction, which is subsequently suppressed, which contributes to the growth of an infectious thread in the root cortex and the formation of nodule primordia [21]. These results indicate that PAL can affect the early stages of legume-rhizobial symbiosis by regulating the SA-dependent protective signal.

It was studied that PAL genes regulate not only the biosynthesis of SA, but also affect the root architecture and the process of rhizobial infection through modification of lignin synthesis [9]. Lignin, which is synthesized from phenylpropanoid compounds, is the main structural component of the secondary thickening of the cell wall [22]. It adds stiffness to cell walls and mechanical stability and thus influences plant tolerance to stressors [23]. It has been suggested that accumulations of phenylpropanoid compounds in the cells of some plants, especially lignin, may form a mechanical barrier to infection by microorganisms [24]. This was observed in transgenic plants LjPAL1 (*L. japonicus*), which was accompanied by a change in the number of infectious filaments and nodules, and could lead to accelerated or slowed down the organogenesis of nodules [18]. It has been shown that in the model legume *Medicago sativa* the decrease in lignin content correlates with the increase in the number of nodules [25]. It is reported that the accumulation of endogenous SA levels correlates with the lignin content in a number of transgenic alfalfa lines [26]. In transgenic plants *L. japonicus* LjPAL1i, a decrease in lignin and SA [6] was observed, although it is not yet clear how this is related to symbiotic processes.

In our studies was found that inoculation of soybean seeds with rhizobia of different efficiency reduces the activity of PAL in soybean roots in the stage of primordial leaves. The level of enzyme activity decreased significantly in soybean roots in symbiosis with active rhizobia, in comparison with inactive symbiosis. We assume that changes in the activity of PAL in the roots could affect various processes that determine the peculiarities of the formation of nodules, including the above-mentioned. Obviously, this is necessary to change the structure of the cell wall of the root, due to the active formation of symbiotic structures – the formation of nodular primordia.

Compared to the stage of primordial leaves, in the stage of the first true leaf the activity of PAL increases almost 2 times in soybean roots in symbiosis with inactive rhizobia and 2.5 times in active soybean-rhizobial symbiosis. The dynamics of enzyme activity in the roots in the stages of the second and third true leaves remains almost at the same level and differs insignificantly in both symbiotic systems, as well as the level of enzyme activity in the roots of non-inoculated plants.

Earlier we showed that in the stage of primordial leaves and in the stage of the first true leaf significantly increase the intensity of ethylene release by soybean plants by inoculation with both active and inactive rhizobia [27]. It is known that in soybean plants, which form nodules of the deterministic type, the phytohormone ethylene does not induce negative regulation of nodulation processes, as in other legumes [28]. Ethylene controls the site of initiation of nodules around the stele, the number of sites of initiation of nodules and the growth of nodule primordia [29]. It is believed that ethylene may also act through the induction of flavonoid synthesis, which then regulates the transport of indole-acetic acid [30]. Due to the fact that the expression of PAL genes is activated by the signaling pathways of ethylene and jasmonate, as described above, it is possible that this has led to increased synthesis of the enzyme at this stage of ontogenesis.

Experiments with transcription and translation inhibitors have shown that increased PAL activity depends on RNA and protein biosynthesis, and its synthesis and repression occur in all induced

systems [31]. Whereas inactivation of the enzyme is not a universal mechanism, as its synthesis precedes the synthesis of a protein inhibitor induced by trans-cinnamic acid. Regulation of the enzyme/inhibitor ratio has evolved as an important means of regulating the mechanisms of a living cell and its peculiar response to a stressor [32].

In the stage of third true leaf, the level of PAL activity in soybean root nodules formed by inactive highly virulent rhizobia of strain 604k was almost 1.5 times higher compared to the activity of the enzyme in soybean nodules in symbiosis with active virulent rhizobia of strain 634b. In an inefficient symbiotic system formed with the participation of inactive highly virulent rhizobia of strain 604k, a higher activity of the enzyme in soybean root nodules was determined, in comparison with roots. For an effective symbiotic system formed with the participation of active virulent rhizobia of strain 634b, no significant differences in the activity of the enzyme in the soybean roots and nodules were recorded. In soybeans, the seeds of which were not inoculated with rhizobia, no nodules were formed on the roots (see Fig. 3). In our opinion, this was due to the controlled conditions of the growing experiment (specified in the method), which excluded the possibility of bacterization of seeds with rhizobia.

An inefficient symbiotic system formed with the participation of soybean plants and inactive highly virulent rhizobia of strain 604k is not able to fully realize its symbiotic properties, as it forms a large number of nodules on the roots, which are unable to perform nitrogen-fixation function. This can induce changes in the course of biochemical processes, including increased activity of PAL, as a response in the formation of a stress-protective state of plants to the insufficient supply of nitrogen in plants. Other researchers have already demonstrated the high mobility of PAL in response to various stressors and its key role in the synthesis of protective secondary metabolites [33, 34]. Thus, PAL is an extremely sensitive enzyme to changes in the physiological state of plants, the level of activity of which can change significantly in a short period of time depending on the age and stage of development of plants, organs and tissues, as well as external factors.

The use of pre-sowing treatment of soybean seeds with the fungicide Standak Top in the absence of inoculation with rhizobia led to a decrease in the activity of the enzyme in the roots and increases its activity level under the fungicide Maxim XL during the early stages of ontogenesis. It is obvious that such changes in the activity of the enzyme in soybean roots were due to the influence of active substances in the composition of fungicides (description in the «materials and method»). The fungicide Standak Top contains a complex of chemical compounds that combine fungicidal and insecticidal action, which can be quite physiologically active for soybeans as a very sensitive crop to certain growing conditions, as shown earlier [35]. At the same time, this was not observed under the action of Maxim XL fungicide.

The use of pre-sowing treatment of seeds with fungicides in an inefficient symbiotic system formed with the participation of inactive highly virulent rhizobia strain 604k is an even more significant reduction in the activity of PAL in the roots in the stage of primordial leaves. In this stage of ontogenesis in an effective symbiotic system formed by active virulent rhizobia of strain 634b, an increase in PAL activity in the roots was observed with pre-treatment of seeds with Standak Top fungicide, while under Maxim XL the enzyme activity in the roots was at the level of inoculated plants without the use of fungicide.

In the following stages of ontogenesis to the formation of the third true leaf, the activity of PAL in the roots increased in both symbiotic systems with the use of fungicides, compared with the activity of the enzyme in the roots of inoculated soybeans without fungicides. In the stage of the third true leaf, a significant increase in enzyme activity was observed in soybean root nodules formed by rhizobia of different efficiency during seed treatment with fungicides, compared with enzyme activity in these symbiotic systems without fungicides.

It was found that the dynamics of enzyme activity in soybean roots and root nodules in different in efficiency symbiotic systems using seed treatment with fungicides did not change significantly during the early stages of ontogenesis. Most likely, changes in enzyme activity in the roots and, especially, in soybean root nodules in symbiosis with rhizobia significantly depended on the symbiotic properties of the strain, in particular, its activity and virulence, the ability to realize its nitrogen-fixation potential. Under the action of fungicides there were only changes in the level of enzyme activity in the roots and nodules of soybeans, inoculated with rhizobia of different efficiency, which could be the result of specific changes in plant metabolism in response to treatment.

Studies have now shown that the treatment of seeds with fungicides in combination with bacterization is an important factor in protecting plants from diseases and pests, and also helps to increase their productivity [36, 37]. At the same time, there is evidence that drugs with fungicidal activity disrupt the regulatory signaling system between macro- and microsymbionts by blocking the activity of nodulation genes and reducing the level of rhizobial Nod-factor [38]. The study of the chemical nature of signaling compounds or inducers of Nod-genes secreted by the roots of the host plant showed their phenolic nature (flavonoids) [39]. Phenolic compounds play an important role in legume-rhizobial symbiosis, as they not only induce symbiosis as mediators of Nod-factors, but also affect the redistribution of auxin, thus controlling the formation of nodules and remain regulators of the physiological state of the symbiotic system in process of plant growth and development [39]. It is obvious that the increased level of PAL activity found in the roots and root nodules of soybeans, formed by different efficiency rhizobia when using fungicides, could be due to the specific effect of the latter on the phenolic metabolism of plants. We have previously shown that under the action of fungicidal substances in an inefficient symbiotic system there was an increase in the activity of guaiacol peroxidase in the roots and polyphenol oxidase in the root nodules of soybeans in the early stages of legume-rhizobial symbiosis. In an effective symbiotic system, no significant changes in the activity of these enzymes in soybean nodules and roots were observed during pre-treatment of seeds with fungicides [40]. At the same time, in an effective symbiotic system with the use of both fungicides, we recorded an increase in the synthesis of ethylene by soybean plants in the early stages of ontogenesis to the formation of the second true leaf [27]. Whereas in the inefficient soybean-rhizobial symbiosis under the action of fungicides, a decrease in the intensity of ethylene release by soybean plants in the primordial leaf stage and the absence of their significant influence on its synthesis in the subsequent stages of ontogenesis was observed. The participation of ethylene in the phenolic metabolism of legumes, in particular soybeans, we mentioned earlier in this publication. One can only testify to a complex set of biochemical processes that determine the specificity of legume-rhizobial interaction, especially under the influence of additional factors, including fungicides.

In this aspect, we have been conducting research for the past three years. Indicators of the formation and functioning of the symbiotic apparatus of soybeans for seed treatment with fungicides are not presented in this publication, as they have a similar tendency to influence, as noted in published works [27]. In an inefficient symbiotic system formed with the participation of soybean plants and inactive highly virulent rhizobia strain 604k with the use of fungicides, inhibition of nodulation processes in the stage of the first true leaf was found, which was leveled to the stage of the third true leaf [27]. Pre-treatment of seeds with fungicides in an effective symbiotic system formed with the participation of active virulent rhizobia of strain 634b increased the activity of nodulation and nitrogen fixation processes in soybean root nodules. Such differences in the formation and functioning of the symbiotic apparatus of soybeans could be related to the complex of biochemical processes identified by us (described above), which occur in the early stages of symbiotic interaction of soybeans – *B. japonicum* using seed inoculation with different activity and virulence of rhizobia and treatment of seeds with fungicides.

The data obtained indicate that inoculation of soybean seeds with active, virulent rhizobia of strain 634b and treatment with fungicides did not have a negative effect on plant metabolism and can be used to increase the realization of the symbiotic potential of soybeans under appropriate growing conditions. The proposed method of pre-treatment the soybean seeds can be used in modern technologies for growing this crop to meet the needs of the plant in environmentally friendly nitrogen and at the same time increase their tolerance to external factors.

Conclusions. The activity of PAL in the roots and especially in the root nodules of soybeans in the early stage of plant development when using fungicides and bacterization is primarily due to the action of the inoculation factor, and is determined by the symbiotic properties of rhizobia strains, in particular, their virulence and nitrogen fixation activity.

АКТИВНІСТЬ ФЕНІЛАЛАНІН-АМОНІЙ-ЛІАЗИ У СИМБІОТИЧНІЙ СИСТЕМІ *GLYCINE MAX – BRADYRHIZOBIUM JAPONICUM* ПРИ ІНОКУЛЯЦІЇ НАСІННЯ РІЗНИМИ ЗА АКТИВНІСТЮ Й ВІРУЛЕНТНІСТЮ ШТАМАМИ ТА ОБРОБКИ ФУНГІЦИДАМИ

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Резюме

Фенілаланін-амоній-ліаза (ФАЛ) є ключовим ферментом фенілпропаноїдного шляху та забезпечує попередників для синтезу багатьох вторинних метаболітів, які необхідні для розвитку і захисту рослин за дії зовнішніх чинників різноманітної природи, зокрема відіграють важливу роль при становленні і розвитку їх симбіозу з мікроорганізмами. **Мета**. Вивчити активність ФАЛ у рослинах сої на ранніх етапах формування бобово-ризобіального симбіозу при інокуляції насіння різними за симбіотичними властивостями штамами *Bradyrhizobium japonicum* на фоні обробки фунгіцидними препаратами. **Методи**. Мікробіологічні (вирощування бактеріальної культури, інокуляція насіння), фізіологічні (вегетаційний експеримент), біохімічні (визначення активності ФАЛ). Об`єктами дослідження обрано симбіотичні системи, утворені за участю сої (*Glycine max* (L.) Merr.) *сорту Алмаз,* різних за ефективністю штамів *Bradyrhizobium japonicum* 634б (активний, вірулентний) і 604к (неактивний, високовірулентний) та фунгіцидів Максим XL 035 PS (Syngenta, Швейцарія) і Стандак Топ (BASF, Німеччина). **Результати**. Виявлено, що при інокуляції насіння сої ризобіями різної ефективності відбувається зниження активності ФАЛ у коренях сої у фазі примордіальних листків. При цьому рівень активності ензиму суттєвіше знижувався у коренях сої в симбіозі з активними ризобіями у порівнянні з неактивним симбіозом. У порівнянні з фазою примордіальних листків у фазі першого справжнього листка активність ФАЛ зростає майже в 2 рази у коренях сої в симбіозі з неактивними ризобіями та в 2,5 рази – у активному соєво-ризобіальному симбіозі. У наступних фазах онтогенезу до формування третього справжнього листка активність ензиму у коренях залишається практично на такому ж рівні та не суттєво відрізняється в обох симбіотичних системах, а також від рівня його активності у коренях неінокульованих рослин. У фазі третього справжнього листка рівень активності ФАЛ у кореневих бульбочках сої, утворених неактивними високовірулентними ризобіями штаму 604к був майже у 1,5 рази вище у порівнянні з активністю ензиму у бульбочках сої в симбіозі з активними вірулентними ризобіями штаму 634б. Використання фунгіцидних препаратів для передпосівної обробки насіння сої та інокуляції різними штамами ризобій спричиняє зниження активності ФАЛ у коренях у фазі примордіальних листків та підвищення рівня активності ензиму у наступних фазах онтогенезу до формування третього справжнього листка. Виявлено суттєве зростання рівня активності ензиму у кореневих бульбочках сої в обох симбіотичних системах за використання фунгіцидних препаратів. Встановлено, що використання фунгіцидних речовин індукує зміни рівня активності ФАЛ у коренях і кореневих бульбочках, які не впливають на загальну динаміку активності ферменту у різних за ефективністю симбіотичних системах *Glycine max-Bradyrhizobium japonicum*. **Висновки**. Активність фенілаланін-амоній-ліази у коренях та особливо у кореневих бульбочках сої на ранніх фазах розвитку рослин при застосуванні фунгіцидів та бактеризації обумовлена, в першу чергу, дією фактора інокуляції і визначається симбіотичними властивостями штамів ризобій, зокрема їх вірулентністю та азотфіксувальною активністю.

Ключові слова: соя (*Glycine max* (L.) Merr.), бульбочкові бактерії, симбіотична система, вірулентність, азотфіксувальна активність, штам, Максим XL, Стандак Топ.

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