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PECULIARITIES OF FORMATION AND FUNCTIONING OF THE SOYBEAN–*BRADYRHIZOBIUM JAPONICUM* SYMBIOTIC APPARATUS IN RELATION TO PHOTOSYNTHETIC ACTIVITY UNDER THE INFLUENCE OF SEED PROTECTANTS

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ABSTRACT

The aim of work was to investigate the influence of seed treatment with two preparations of fungicidal action, differing in the active substances composition, on the formation and functioning of legume-rhizobial symbiosis in interaction with the photosynthetic apparatus of soybean plants. It was established that in plants treated with preparation Fever, the nitrogen-fixing activity (NFA) difference with the control was insignificant, but under the impact of the preparation Standak Top NFA was by 63% less at the stage of three true leaves, by 48% – at the stage of budding, and by 34% – at the stage of budding–flowering. The net photosynthetic rate, calculated per leaf area unit, was lower than control at all investigated stages of soybean plants development in the variants with the seed protectants applying. A fairly close positive correlations (R^2 = 0.79) were observed between the average weight of one nodule and its NFA, and between the last and total NFA of the whole plant (R^2 = 0.78). Close correlations between plant NFA, chlorophyll content in leaves, and net photosynthetic rate were revealed. It was concluded that seed protectants significantly influenced on nitrogen fixation and photosynthesis.
The strength of manifestation, and the sign of this influence in comparison with the relevant parameters of the control plants depended on the preparation, the stage of plant development, as well as on the index (nodules formation, nitrogen-fixing activity, photosynthetic, chlorophyll content, chlorophyll index, biological productivity of soybean plants) under consideration.

Keywords: *Glycine max* (L.) Merr., *Bradyrhizobium japonicum,* fungicides, nitrogen-fixing activity, net assimilation rate

INTRODUCTION

Soybean is one of the leading crops in world agriculture, due to the uniqueness of the seeds biochemical composition and the versatility of their use **(Iliash *et al.*, 2019; Gale *et al.*, 2019)**. Inoculation of soybean seeds by nodule bacteria highly active strains is an important element of cultivation technology, which promotes the intensification of growth, plant development, and realization of the yield genetic potential (**Kots *et al.*, 2011; Flajšman *et al.*, 2019; Jarecki *et al.*, 2020**).
Damage to plants by phytopathogenic organisms leads to significant economic losses, which is appeared in a decrease in grain yield and product quality. About fifty diseases affect soybeans in different phases of plant growth and development. The most common among diseases of fungal etiology are – fusarium wilt (*Fusarium oxysporum* Schl.), downy mildew (*Peronospora manshurica* Sydow.), septoria (*Septoria glycines* T. Hemmi), anthracnose (*Ascochyta sojaecola* Abramov), cercosporiose (*Cercospora sojina* Hara), root rot etc. (**Gaweda, 2020, Lavilla, 2021**). In order to avoid this problem, or to minimize the impact of diseases on crops, chemical plant protectants are used in farming industry (**Araujo *et al*., 2017; Baibakova *et al*., 2020**). Their range is permanently changing: preparations that cause long-term environmental consequences are excluded, and the list is supplemented with effective combinations of new, safer formulations with different action mechanisms (**Khodakivska *et al*., 2017**). It has been shown that amongst modern fungicides the largest share is made up by mixed preparations (26.3–39.3%) (**Vavrinevych *et al*., 2013; Haq *et al*., 2020**). When conducting social-hygienic monitoring, preparations of groups of strobilurins, triazoles, benzimidazoles, imidazoles, derivatives of inorganic compounds are priority. To date, the use of fungicides with high activity at low doses is urgent (**Vavrinevych *et al*., 2013**).
However, it should be taken into account that fungicides, as physiologically active substances, in addition to their direct action – the protection of crops from pathogens – also affect the plant itself, influencing on the course of physiological and biochemical processes (**Kuryata *et al.*, 2017; Srivastava *et al.*, 2020; Amaro *et al.*, 2020**). The scientific literature contains data on their negative effect on the symbiotic processes in legumes, which leads to a decrease in the proportion of biological nitrogen in the yield (**Saenko *et al*., 2018**). Thus, when using Maxim Star 025FS fungicide, there was a tendency to decrease the nodules actual nitrogenase activity, and under Kinto Duo treatment a decrease of this index by 1.8 times compared with the control was observed (**Vozniuk *et al*., 2015**).
It has been found that during the symbiotic process, fungicides can inhibit the flavonoid NodD receptor as well as the expression of nodulation genes (**Fox *et al.*, 2007**). Another way in which fungicides indirectly inhibit the rhizobia activity is the reduction of phytohormones and siderophores synthesis. It has been shown that thiram in concentrations of 10 to 100 μg/ml contributes to the stimulation of legume-rhizobial symbiosis nitrogenase activity, and further concentration increase inhibits nitrogen fixation (**Bikrol *et al*., 2005**). One of the mechanisms of fungicides negative effect on legume-rhizobial symbiosis is also the inhibition of phytoestrogens production by plant, that is important for attracting symbiotic bacteria to the macrosymbiont.
Carbon dioxide assimilation in the process of photosynthesis is the source of plant organism productivity, since herewith about 90% of the dry weight is formed. However, photosynthesis is quite sensitive to the influence of environmental factors, and in legumes it works in close interaction with the symbiotic apparatus (**Kiriziy *et al*., 2007**).
There is evidence in the literature that, depending on the main active substance, benzimidazole-class fungicides are capable of inhibiting photosynthesis, reducing chlorophyll content, and contributing to an increase in carotenoids to activate the mechanism of photoprotection (**Petit *et al*., 2012**). It has been shown that azole fungicides, having different active substances in their composition, can both suppress and stimulate the photosynthetic rate (**Xia *et al*., 2006**). They are capable of inducing stomata closure and opening, increasing chlorophyll content and intracellular CO_2 concentration. Azoles reduce the expression of genes encoding the small and large Rubisco subunit as well as the speed of electron transport. It has been found that photosynthetic light reactions are very sensitive to the chemical agents impact. Thus, a number of systemic and contact fungicides significantly reduced the electron transport rate in chloroplasts, the quantum yield of photosystem I, the maximum quantum efficiency of photosystem II, which was accompanied by a decrease in fluorescence photochemical quenching (qP) (**Kaplaushenko *et al*., 2016**).

The literature data indicate that fungicides, depending on the active substances, growing conditions, methods of application can act as a stress factors and affect both the plant organism and the formation and functioning of legume-rhizobial symbiosis. As seed treatment is a necessary agrotechnical technique for the cultivation of legumes, it becomes urgent to study the plants chemical protectants effect on the course of the most important processes that determine the productivity of legumes – nitrogen fixation and photosynthesis.

The aim of our work was to investigate the influence of seed treatment with two preparations of fungicidal action, differing in the composition of active substances, on the formation and functioning of legume-rhizobial symbiosis in interaction with the photosynthetic apparatus of soybean plants.

MATERIALS AND METHODS

Pot experiments conditions

The soybean variety Almaz and nodule bacteria *Bradyrhizobium japonicum* 634b strain were used. Variety Almaz obtained by hybridization of the varieties Beal'tsi 3/86-x and Fiskebv-840-5-3, significantly exceeded (by 6–8 c/ha) the harvest of the parent varieties. The owners of the patent for this variety (N 07105) are Poltava State Agrarian Academy and Biliavska L.G. Since 2007 it has been included in the State Register of Plant Varieties of Ukraine. Variety Almaz is a grain, early ripening, drought-resistant, resistant to diseases, provides stable growing season lasting 100–105 days. The protein content in the seeds is 38–39%, oil – 24–26% (**Biliyavska, 2007**).

Bradyrhizobium japonicum 634b (certificate of authorship № SU1034358A) is an active, production strain-standard from the museum collection of strains of symbiotic and associative nitrogen-fixing microorganisms of the Institute of Plant Physiology and Genetics of the National Academy of Sciences of Ukraine.

The experiments were carried out at the vegetation bench of Institute of Plant Physiology and Genetics NAS of Ukraine. Soybeans were grown per 7 plants in pots filled with washed river sand, under natural light and temperature, with optimal water supply (70% FC). The source of mineral nutrition was Helrigel nutrient mixture, enriched with trace microelements (g/kg sand): molybdenum ($(NH_4)_6Mo_7O_{24} \times 4H_2O$ – 0.006), boron (H_3BO_3 – 0.007), manganese ($MnSO_4 \times H_2O$ – 0.0014) and copper ($CuSO_4 \times 5H_2O$ – 0.0039) and depleted in nitrogen – 0.25 norm (1 norm of nitrogen corresponds to 708 mg $Ca(NO_3)_2 \times 4H_2O$ per 1 kg of substrate). The substrate humidity was maintained by controlled irrigation.

Seeds treatment

Soybean seeds were treated on the day of sowing with modern fungicides: Standak Top containing active substances of phenylpyrazoles, benzimidazoles and strobilurins chemical classes, with a rate of 1.5 L/t (BASF, Germany), and Fever – of triazoles class, with a rate of 0.3 L/t (Bayer CropScience AG, Germany).

Before sowing, soybean seeds were inoculated with a suspension of *B. japonicum* 634b – an active industrial strain-standard from the museum collection of symbiotic and associative nitrogen-fixing microorganisms strains (Institute of Plant Physiology and Genetics NAS of Ukraine). To prepare the inoculum, nodule bacteria culture was grown on mannitol-yeast agar for 7-8 days at 26-28°C, then was washed with saline (0.9% NaCl). The final bacterial concentration was 10^8 cells in 1 ml of suspension. The seeds inoculation was performed by moistening them for 1 hour. Controls were soybean plants which seeds were inoculated with *B. japonicum* 634b without fungicides.

Plant material analysis

The fresh weight of soybean plants organs was determined by scales. To determine the dry weight of the samples, they were fixed in an oven at 105°C for 4 h and dried to constant weight at 70°C.

The roots of plants were washed from the substrate and nodules number and weight were determined. The nitrogen-fixing activity (NFA) of the nodules formed on the roots of one plant was determined by the acetylene method (**Hardy *et al.*, 1968**) on the gas chromatograph Agilent GC system 6850 (Agilent, USA).

Determination of photosynthetic apparatus activity

The total chlorophyll content in the second trifoliate leaves was determined by extraction of pigments from fresh tissue with dimethyl sulfoxide, followed by determination of the resulting solution density on the spectrophotometer Smart Spec Plus (Bio-Rad, USA). The calculations were performed using the formulas given in (**Wellburn, 1994**).

The net assimilation rate was recorded under controlled conditions by an infrared gas analyzer GIAM-5M (Russian Federation). For measurements, it was used the middle lobe of the third from the top leaf not separated from the plant, which was placed in a temperature-controlled (+25°C) chamber and illuminated (1800 μmol/(m^2 · s) PAR) by the KG-2000 incandescent lamp through a water filter to eliminate excess infrared radiation in its spectrum. The natural air was blown through the chamber. The CO_2 assimilation rate was measured in 30–40 min after placing the leaf in the chamber on reaching a stationary level, and calculated using the formula $A_N = (\Delta C \cdot V)/S$, where ΔC – is the difference in CO_2 concentrations in the air at the inlet and outlet from the chamber (μmol/l), V – the air flow rate through the chamber (L/s), S – the area of the chamber (m^2).

Statistical data analysis

Samples were taken at stages of three true leaves, budding and flowering. Biometric parameters were determined four to ten times, physiological – three times. The results were statistically processed. The reliability of the differences between the samples was evaluated by the single-factor dispersion analysis ANOVA using the standard formulas of "Microsoft Excel 2010" computer program. The tables and figures show the arithmetic mean values and their standard errors (x±SE). Differences between controls and treatments were considered significant at $P < 0.05$.

RESULTS AND DISCUSSION

Soybean-rhizobial symbiosis formation and functioning

The study of the chemical seed protectants effect on soybean-rhizobial symbiosis under the conditions of model pot experiment showed that the nodules number under the Fever treatment was greater relative to control, especially at flowering stage. Nodules formation was most inhibited by Standak Top at the stage of three true leaves, when their number was by 39% less than in the control (Tab 1). A similar tendency for fungicides impact was observed when forming the total weight of root nodules. However, the calculations of the one nodule average weight revealed that the difference between the control plants and Fever treated ones in this index at the stages of three true leaves and budding was insignificant, and at the flowering stage the one nodule weight under fungicides treatment was significantly less.

The highest nitrogen-fixing activity (NFA) of the control plants was observed at the budding stage (Tab 1). In Fever treated plants, the difference of this index with the control was insignificant, but under the action of the preparation Standak Top, NFA at the stage of three true leaves was less by 63%, at the budding stage – by 48%, and at the flowering stage – by 34% relative to control.

NFA per one nodule showed a trend similar to that indicated for total NFA – at the stages of three true leaves and budding the difference between control and variant with Fever treatment was insignificant, and only at the flowering stage did this negative effect appear. On the other hand, under the Standak Top influence, at all investigated stages of soybean plant development, there was a decrease in the nodule average NFA compared to the control.

Table 1 Soybean–*B. japonicum* symbiosis formation and functioning under the influence of seed protectants, (x±SE)

Treatment	Nodules weight, g/plant	Nodules number, pcs./plant	Nodule average weight, mg	Total NFA, μmol C_2H_4/(plant · h)	NFA, nmol C_2H_4/(nodule · h)	Specific NFA, nmol C_2H_4/(g nodules · h)
Stage of three true leaves						
Control	0.24±0.02	31.75±2.46	7.6±0.2	1.30±0.06	40.9±1.2	5.42±0.16
Fever	0.30±0.02*	36.75±1.25	8.2±0.2	1.43±0.10	38.9±1.2	4.77±0.14*
Standak Top	0.09±0.01*	19.5±0.96*	4.6±0.1*	0.48±0.03*	24.6±0.7*	5.33±0.16
Budding stage						
Control	0.30±0.01	26.75±1.93	11.2±0.3	1.71±0.07	63.9±1.9	5.70±0.17
Fever	0.34±0.01*	28.75±1.65	11.8±0.4	1.59±0.09	55.3±1.7	4.68±0.14*
Standak Top	0.16±0.01*	34.55±2.17*	4.6±0.1*	0.89±0.07*	25.8±0.8*	5.56±0.17
Flowering stage						
Control	0.36±0.02	27.00±0.91	13.3±0.4	1.50±0.12	55.6±1.7	4.17±0.13
Fever	0.38±0.02	40.25±3.25*	9.4±0.3*	1.46±0.09	36.3±1.1*	3.84±0.12*
Standak Top	0.25±0.02*	24.75±2.06*	10.1±0.3*	0.99±0.07*	40.0±1.2*	3.96±0.12*

Note: * – significant difference compared to the control ($P < 0.05$).

Calculations of specific NFA per nodule weight unit revealed that at the stages of three true leaves and budding the difference between the control and the variant with Standard Top was insignificant, but under Fever treatment this index was much smaller (Tab 1). At the flowering stage, there was a tendency for a decrease in the specific NFA under the action of the two studied seed protectants.

Photosynthetic apparatus capacity and plant productivity

With regard to the fungicides effect on another component of the soybean plant production process – the photosynthetic apparatus, the total chlorophyll content at the stages of three true leaves and flowering under the Fever treatment was higher than the control and slightly lower at the budding stage (Tab 2). Under the Standak Top treatment, this index was significantly lower at the first two investigated stages of plant development, and higher at the flowering stage, compared to the control.

Table 2 Photosynthetic apparatus capacity and biological productivity of soybean plants under the influence of seed protectants, (x±SE)

Treatment	Total chlorophyll content, mg/g	Chlorophyll index, mg chl./plant	Net assimilation rate, µmol CO_2/(m^2 · s)	Plant total dry weight, g
		Stage of three true leaves		
Control	4.34±0.13	7.73±0.23	14.1±0.4	1.00±0.03
Fever	5.16±0.15*	8.93±0.27*	13.9±0.4	0.97±0.03
Standak Top	2.33±0.07*	3.75±0.11*	6.1±0.2*	0.82±0.02*
		Budding stage		
Control	4.96±0.15	10.47±0.31	12.2±0.4	1.31±0.04
Fever	4.15±0.12*	8.01±0.24*	10.5±0.3*	1.27±0.04
Standak Top	2.49±0.07*	4.51±0.14*	8.0±0.2*	1.12±0.03*
		Flowering stage		
Control	3.49±0.10	9.32±0.28	15.4±0.5	1.54±0.05
Fever	4.93±0.15*	15.78±0.47*	14.8±0.4	2.05±0.06*
Standak Top	4.70±0.14*	13.21±0.40*	12.1±0.4*	1.50±0.05

Note: * – significant difference compared to the control (P < 0.05).

Calculations of soybean plants chlorophyll index (the product of the chlorophyll content on the weight of all leaves on the plant) revealed that, under the influence of Fever, this parameter exceeded the control at the stage of three true leaves and flowering by 16 and 69%, respectively, and at the budding stage was less by 23%. In the variant with Standak Top seed treatment, the chlorophyll index at the three true leaf and budding stages was less than the control by 51 and 57%, respectively, and exceeded control by 42% only at the flowering stage.

The net photosynthetic rate, calculated per leaf area unit, in variants with the applying of seed protectants was lower than control at all investigated stages of soybean plant development (Tab 2). It should be noted that the negative effect of Standak Top was markedly stronger than that of Fever.

On the biological productivity, which we estimated by the whole plant dry matter accumulation (the total weight of leaves, stems and roots), treated plants slightly conceded to the control at the stages of three true leaves and budding (Tab 2). Only at the flowering stage, plants of the variant with the Standak Top treatment matched with control in their weight, and under the Fever treatment even exceeded it by 33%.

The results obtained revealed that the negative effect of Standak Top on the formation and functioning of soybean plants symbiotic and photosynthetic apparatus was more pronounced than that of Fever. The latter in some cases positively influenced on some of the studied indices, possibly due to the lower application rate. In addition, there is information in the literature about the positive effect of triazole class substances on the crops production process (**Kuryata *et al.*, 2017**).

It should also be noted that fungicides negative effect was more pronounced at the stages of three true leaves and budding, while at the flowering stage it was leveled. This can be explained by the decrease in the toxic effect of the applied to the seeds fungicide as the plant biomass increases, as well as by the activation of protective systems against the action of xenobiotics, and the positive role of fungicides in the protection against fungal phytopathogens. The latter, in particular, is indirectly confirmed by a significant increase of the chlorophyll index at the flowering stage of plants, whose seeds were treated with fungicides, compared with control (Tab 2), as well as by the literature data concerning the use of fungicides on soybean under field conditions (**Mostoviak *et al*., 2018**).

These results are consistent with our previous studies, which found that fungicides inhibit the formation and functioning of soybean symbiotic systems at the early stages of plant development, and can shift the peak of nitrogen-fixing activity to later stages of ontogenesis (**Pavlyshche *et al.*, 2017**). This development of events is likely to be related to the inhibitory effect of the fungicides on the formation of legume-rhizobial symbiosis, in particular at the stage of micro- and macro-symbiont recognition with disturbance of the regulatory signaling system, blocking the activity of nodulation genes and decreasing the level of rhizobial *Nod*-factor (**Fox *et al.*, 2007**).

Relationships between symbiotic and photosynthetic apparatus parameters

In addition to identifying patterns that directly related to the fungicides effects on legume-rhizobial symbiosis, our data set allowed us to analyze more deeply the relationships between individual parameters of symbiotic and photosynthetic apparatus for a better understanding of their role in soybean production process. It turned out that there is a negative correlation between the nodules weight and the specific NFA per nodules weight unit (Fig 1). Moreover, this tendency was observed both for the total nodules weight per plant (Fig 1, A) and for the average weight of one nodule (Fig 1, B). It can be assumed that with the increase of nodule weight, the proportion of inert parenchymal tissues increase in them, the bark thicken, as well as the molecular nitrogen diffusion from the environment to bacteroids impede.

However, according to the results of our experiments, the relationship between the total NFA of the plant and the number of root nodules was quite weak (R^2 = 0.18), and completely absent between the nodules number per plant and the average NFA of one nodule, as well as between the nodules number and the average weight of one nodule.

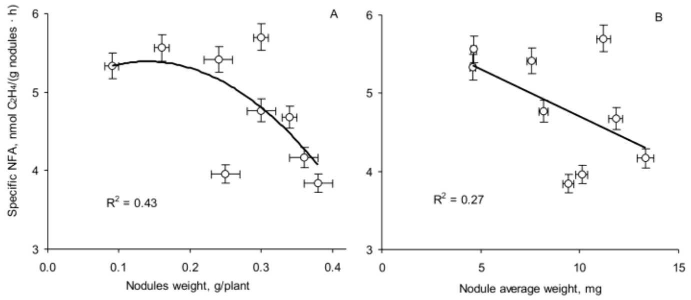

Figure 1 Relationship between specific nitrogen-fixing activity (NFA) per nodule weight unit and the total nodules weight per soybean plant (A), and average weight of one nodule (B)

This is probably due to the fact that an index such as the nodules number is more relevant for the evaluation of a nodule bacteria strain virulence, and in the process of nitrogen fixation the size of bacteria-infected tissue, in which molecular nitrogen fixation occurs, and the presence of leghemoglobin in this tissue are crucial (**Jarecki *et al.*, 2020**). It is known that nitrogen fixation also depends on the nodules size – it is more intensive in large nodules, i.e. their effectiveness is related to the infectious tissues volume, the bacteroids number, the duration of their functioning, and the activity of the corresponding enzymes (**Petrychenko *et al.*, 2016**). Therefore, the nodule bacteria with the highest NFA form usually large nodules located on and around the main root.

The results of our experiments quantitatively confirm these patterns. Thus, a sufficiently close positive correlation was observed between the average weight of one nodule and its NFA (Fig 2, A), as well as between the last and total NFA of the whole plant (Fig 2, B). At the same time, it is quite obvious that the tendency of specific activity decreasing with increasing nodule weight, discussed above (Fig 1), signals about a decrease in the symbiotic apparatus efficiency. In our opinion, this is the reason for the tendency to enter the plateau of dependence between the single nodule NFA and the whole plant NFA (Fig 2, B). All this reveal that to improve the efficiency of whole plant symbiotic nitrogen fixation, there must be an optimal relationship between the number, weight and specific NFA of the nodules.

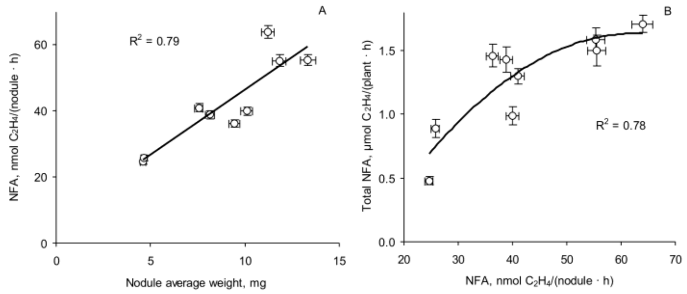

Figure 2 Relationship between average nitrogen-fixing activity (NFA) of one nodule and its weight (A), and total NFA of soybean plants (B)

Due to the plants symbiosis with rhizobia, root nodules absorb molecular nitrogen from the air, transforming it into ammonium form, and supply the plant in exchange for photosynthetic products. Thus, the photosynthetic apparatus receives the organic nitrogen-containing compounds for the synthesis of a large number of required proteins, and the nodules in turn are provided with the energy substrates required for the assimilation of molecular nitrogen. Therefore, it can be argued that photosynthesis, respiration, and molecular nitrogen assimilation by symbiotic systems are the main physiological processes in the energy and trophic supply of legumes, on which their productivity depends (**Petrychenko *et al.*, 2016; Khan *et al.*, 2019; Kirizii *et al.*, 2007**).

There is usually a close relationship between the rates of nitrogen fixation and photosynthesis of legumes (**Friel *et al.*, 2019**). In the literature, there are numerous data on the dependence of efficiency of nodule bacteria symbiosis with leguminous plants on the influx of photoassimilates, and the process of nitrogen fixation not only depends on the assimilates flow into the nodules at that, but in turn can affect the photosynthesis and assimilates distribution between plant organs. It has been experimentally proved that the inhibition of photosynthetic rate reduces the nodules nitrogen-fixing activity (**Kots *et al*., 2011**).

Thus, theoretical considerations and literature indicate that there is a close relationship between the symbiotic and photosynthetic apparatus of legumes. The results of our experiments fully fit into this concept, which is confirmed by figure 3. Close correlations between plant NFA, chlorophyll content in leaves, and CO_2 net assimilation rate were revealed. These dependencies do not require special comment, since it is well known that chlorophyll content and photosynthetic rate depend on the nitrogen status of the plant (**Evans & Clarke, 2019**).

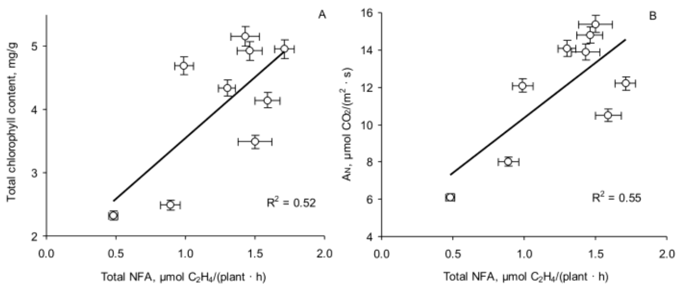

Figure 3 The relationship between total nitrogen-fixing activity (NFA) and chlorophyll content (A), and the net assimilation rate (A_N) of soybean plants leaves (B)

There was also a positive correlation between the net assimilation rate and total plant dry weight (Fig 4, A), although less close than that found in our other experiments (**Kots *et al.*, 2018**). It is obvious that the relation of the specific photosynthetic CO_2 assimilation rate, measured on a single leaf, with the whole plant productivity is mediated by other factors, first of all – the total leaf area on the plant, leaf overlap, their functional status, etc., which depend on the conditions of experiment.

In the case of the experiments discussed in this article, the chlorophyll index appeared to be a more representative index of the relationship between the photosynthetic apparatus and plant productivity (Fig 4, B). It should be noted that in other crops, especially in the field, where plants are affected by a large number of uncontrolled factors, this index also well correlates with yield (**Liu *et al*., 2019**).

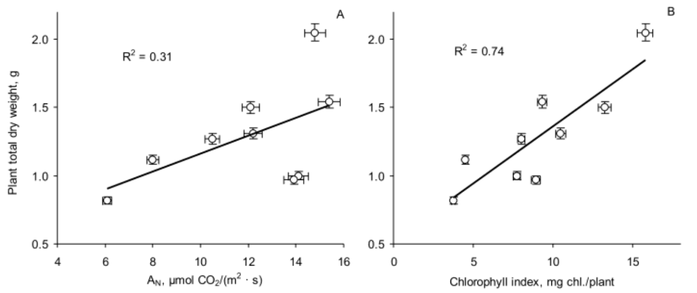

Figure 4 Relationship between soybean plant total dry weight and net assimilation (A_N) rate (A), and chlorophyll index (B)

CONCLUSION

Thus, the experimental data obtained allow us to argue that seed protectants have a significant influence on both determining components of the formation of legume-rhizobial symbiosis productivity – symbiotic and photosynthetic apparatus of the plant. The strength of manifestation and the sign of this influence in comparison with the relevant parameters of the control plants depend on the preparation, the stage of plant development, as well as on the index under consideration. There are close positive correlations between the nitrogen-fixing activity of the plant and the indices of its photosynthetic apparatus efficiency, that can be modified by substances with fungicidal activity.

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