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

Serhii Kots, Dmytro Kiriziy, Anastasiia Pavlyshche*, Liliia Rybachenko

Address(es): PhD Anastasiia Pavlyshche
Institute of Plant Physiology and Genetics, National Academy of Sciences of Ukraine, Department of Symbiotic Nitrogen Fixation, 31/17 Vasylkivska str., 03022, Kyiv, Ukraine, phone: +38(093) 118-2427.

*Corresponding author: zapadenks2015@gmail.com

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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 (R² = 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 (R² = 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.

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; Hajieman 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 f. Soybean is fusarium wilt (Fusarium oxysporum Schw.), downy mildew (Peronospora manshurica Sydow.), septoria (Septoria glycines T. Henmi), anthracnose (Ascochyta sojae 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; Balbakova 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 mixtures of f. fusarium wilt (Fusarium oxysporum Schw.), downy mildew (Peronospora manshurica Sydow.), septoria (Septoria glycines T. Henmi), anthracnose (Ascochyta sojae 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; Balbakova 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 mixtures of...
The literature data indicate that fungicides, depending on the active substances, growing conditions, methods of application can act as 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 protec nce 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 Fiskevib-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 Poval'ska State Agrarian Academy and Bilisavska 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% (Bilyavska, 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 (NH₄)₂MoO₄·2H₂O – 0.006, boron (H₂BO₃) – 0.007, manganese (MnSO₄·H₂O – 0.0014) and copper (CuSO₄·5H₂O – 0.0039) and depleted in nitrogen – 0.25 norm (1 norm of nitrogen corresponds to 708 mg Ca(NO₃)₂ · 4H₂O 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 10⁶ 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 hours, then taken out and left in the chamber. The CO₂ 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/(m² · 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 CO₂ 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 \( A = (\Delta(C)/\Delta(t)) \times V \), where \( A \) – is the difference in CO₂ 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 (m²).

### 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 (±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 node average NFA compared to the control.

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

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Nodules weight, mg/plant</th>
<th>Nodules number, pcs/plant</th>
<th>Nodule average, mg</th>
<th>Total NFA, μmol C/H₂/(plant · h)</th>
<th>NFA, μmol C/H₂/(nodule · h)</th>
<th>Specific NFA, μmol C/H₂/g nodules · h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.24±0.02</td>
<td>31.75±2.46</td>
<td>6.7±0.2</td>
<td>1.30±0.06</td>
<td>40.9±1.2</td>
<td>5.42±0.16</td>
</tr>
<tr>
<td>Fever</td>
<td>0.30±0.02</td>
<td>36.51±1.25</td>
<td>8.2±0.2</td>
<td>1.43±0.10</td>
<td>38.9±1.2</td>
<td>4.77±0.14</td>
</tr>
<tr>
<td>Standak Top</td>
<td>0.09±0.01*</td>
<td>19.5±0.06*</td>
<td>4.6±0.1*</td>
<td>0.48±0.03*</td>
<td>24.6±0.7*</td>
<td>5.23±0.16</td>
</tr>
</tbody>
</table>

**Stage of three true leaves**

- **Budding stage**
  - Control: 0.30±0.01
  - Fever: 0.34±0.01*<br>
  - Standak Top: 0.16±0.01*<br>

- **Flowering stage**
  - Control: 0.36±0.02
  - Fever: 0.38±0.02*<br>
  - Standak Top: 0.25±0.02*<br>

*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)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Total chlorophyll content, mg/g</th>
<th>Chlorophyll index, mg/chl/plant</th>
<th>Net assimilation rate, μmol CO₂/(m²·s)</th>
<th>Plant total dry weight, g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage of three true leaves</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>4.34±0.13</td>
<td>7.73±0.23</td>
<td>14.1±0.4</td>
<td>1.00±0.03</td>
</tr>
<tr>
<td>Fever</td>
<td>5.16±0.15*</td>
<td>8.93±0.27*</td>
<td>13.9±0.4</td>
<td>0.97±0.03</td>
</tr>
<tr>
<td>Standak Top</td>
<td>2.33±0.07*</td>
<td>3.75±0.11*</td>
<td>6.1±0.2*</td>
<td>0.82±0.02*</td>
</tr>
</tbody>
</table>

Budding stage

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Total chlorophyll content, mg/g</th>
<th>Chlorophyll index, mg/chl/plant</th>
<th>Net assimilation rate, μmol CO₂/(m²·s)</th>
<th>Plant total dry weight, g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>4.96±0.15</td>
<td>10.47±0.31</td>
<td>12.2±0.4</td>
<td>1.31±0.04</td>
</tr>
<tr>
<td>Fever</td>
<td>4.15±0.12*</td>
<td>8.01±0.24*</td>
<td>10.5±0.3*</td>
<td>1.27±0.04</td>
</tr>
<tr>
<td>Standak Top</td>
<td>2.49±0.07*</td>
<td>4.51±0.14*</td>
<td>8.0±0.2*</td>
<td>1.12±0.03*</td>
</tr>
</tbody>
</table>

Flowering stage

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Total chlorophyll content, mg/g</th>
<th>Chlorophyll index, mg/chl/plant</th>
<th>Net assimilation rate, μmol CO₂/(m²·s)</th>
<th>Plant total dry weight, g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>3.49±0.10</td>
<td>9.32±0.28</td>
<td>15.4±0.5</td>
<td>1.54±0.05</td>
</tr>
<tr>
<td>Fever</td>
<td>4.93±0.15*</td>
<td>15.78±0.47*</td>
<td>14.8±0.4</td>
<td>2.05±0.06*</td>
</tr>
<tr>
<td>Standak Top</td>
<td>4.70±0.14*</td>
<td>13.21±0.40*</td>
<td>12.1±0.4*</td>
<td>1.50±0.05</td>
</tr>
</tbody>
</table>

Note: * – significant difference compared to the control (F > 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 (Pavlischev 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 impedes.

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 (R² = 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.
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; Kiziliz et al., 2007).

There is usually a close relationship between the rates of nitrogen fixation and photosynthetic activity of legumes (Friel et al., 2017). 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, there is a close positive correlation between soybean plant NFA, chlorophyll content in leaves, and CO₂ 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).

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 CO₂ 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).

REFERENCES


