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# RELATION OF SOYBEAN PRODUCTIVITY TO THE FUNCTIONING OF THE SYMBIOTIC AND PHOTOSYNTHETIC APPARATUSES

***Nadiya Vorobey*, *Kateryna Kukol*, *Petro Pukhtaievych*, *Sergii Kots*, *Dmytro Kiriziy***

*Institute of Plant Physiology and Genetics, NAS of Ukraine*
*31/17 Vasylkivska St., Kyiv 03022, Ukraine*

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**Background.** Increasing the yield of soybean necessitates the maintenance of a high protein level in seeds, and therefore the process of fixing atmospheric $N_2$. Seed inoculation with soybean nitrogen-fixing bacteria is known to improve $N_2$-fixation and soybean grain yield. At the same time, the introduction of new nodule bacteria strains into preparations for soybean inoculation requires the study of their influence on the main interconnected physiological processes that form the basis of leguminous plants productivity – $N_2$-fixation and photosynthesis. The aim of the work was to study the relationship of vegetative growth and grain productivity of soybean inoculated with new nodule bacteria *Bradyrhizobium japonicum* strains of different functional activity with the plants' symbiotic and photosynthetic apparatuses functioning.

**Materials and Methods.** The research was carried out on symbiotic systems created with soybean plants (*Glycine max* (L.) Merr.) of the Almaz variety and nodule bacteria *B. japonicum* strains: analytically selected PC09, and recombinant strains B157, B201, D45, D52 (pSUP5011::Tn5*mob*) and C30 (pSUP2021::Tn5) from the $N_2$-fixing microorganisms museum collection of the Institute of Plant Physiology and Genetics NAS of Ukraine. Research methods – microbiological, biochemical and physiological, statistical analysis.

**Results.** It was found that the $N_2$-fixing activity (NFA) of nodules formed by *B. japonicum* PC09, D45, D52, B157 and B201 strains at the stage of 3 true leaves exceeded the NFA of nodules formed by Tn5-mutant C30 by 1.6–4.0 times, and at the stage of budding–beginning of flowering – by 4.2–6.2 times. Highly active strains also differed from each other in NFA, although to a lesser extent than with strain C30. On the basis of a comparative analysis of the physiological indices of soybean inoculated with *B. japonicum* strains of different activity, close positive linear correlations were found between NFA, photosynthetic rate, and the biological and grain productivity of plants.

**Conclusions**. The results obtained indicate that the higher the nodulating and NFA of rhizobia in the symbiotic system soybean–*Bradyrhizobium japonicum*, the higher the functional activity of photosynthetic apparatus formed by plants. This provides a more complete genetic potential release of soybean crop productivity.

***Keywords***: *Glycine max* (L.) Merr., *Bradyrhizobium japonicum*, symbiosis, nodulation, $N_2$-fixing activity, photosynthesis, productivity

## INTRODUCTION

Soybean belongs to the most important crops of world agriculture, and is the most common among legumes and oil crops. It is grown in more than 60 countries because it plays a crucial role in grain, food and feed balances and improves soil fertility (Vanlauwe *et al.*, 2019; Hanhur *et al.*, 2020; Omari *et al.*, 2022). The main soybean producing countries are the United States of America, Brazil, Argentina, China and India.

Along with the increase in soybean yields in recent decades (Vogel *et al.*, 2021; Umburanas *et al.*, 2022), the need to maintain a high level of protein in seeds has increased, which in turn has increased the plants' need for nitrogen through the process of atmospheric $N_2$ fixation (Ciampitti & Salvagiotti, 2018). It is known that due to the biological fixation of $N_2$, soybean plants provide 36–69 % of the necessary nitrogen (Kots, 2021; Tellesa *et al.*, 2023), and the rest – from available mineral nitrogen in the soil (Ohyama *et al.*, 2017).

The practice of seed inoculation with soybean $N_2$-fixing bacteria has long been successfully implemented worldwide as it improves $N_2$ fixation and soybean grain yield (Yuan *et al.*, 2020; Nakei *et al.*, 2022). In Brazil, 100 % of the areas currently under soybean cultivation have been inoculated at least once, and about 60 % of these areas are re-inoculated each year (Tellesa *et al.*, 2023). It is an affordable, environmentally friendly, and highly efficient source of nitrogen in agricultural soils (Ohyama *et al.*, 2017; Halwani *et al.*, 2021).

There is sufficient evidence for the effectiveness of soybean inoculation with industrial rhizobia strains. The use of preparations based on nodule bacteria, selected on the basis of competitiveness, $N_2$ assimilation activity, complementarity to the plants species and variety, tolerance to environmental stress factors contributes to increasing the $N_2$ fixation activity (NFA) in root nodules during the plants growing season, as well as increasing the photosynthetic rate, increasing the yield and protein content in grain (Omari *et al.*, 2022; Nakei *et al.*, 2022).

At the same time, the introduction of new nodule bacteria strains into preparations for soybean inoculation requires the study of their influence on the main interconnected physiological processes that form the basis of the leguminous plants productivity – $N_2$ fixation and photosynthesis.

Therefore, the aim of the work was to study the relationship of vegetative growth and grain productivity of soybean inoculated with nodule bacteria *Bradyrhizobium japonicum* strains of different functional activity, created by us, with the plants' symbiotic and photosynthetic apparatuses functioning.

## MATERIALS AND METHODS

The research was carried out on symbiotic systems created with soybean plants (*Glycine max* (L.) Merr.) of the Almaz variety (originator – Poltava State Agrarian Academy, Ukraine, included in the Register of plant varieties of Ukraine since 2007), and different *Bradyrhizobium japonicum* strains.

Nodule bacteria strains PC09, D45, D52, B157, B201, C30 were obtained from the $N_2$-fixing microorganisms museum collection of the Department of Symbiotic Nitrogen Fixation of the Institute of Plant Physiology and Genetics NAS of Ukraine. The *B. japonicum* PC09 strain was isolated from nodules of the soybean variety Kyivska 27, which grew on dark gray podzolized soil in the Uman district, Cherkasy region of Ukraine. *B. japonicum* strains B157 and B201 are the Tn5-mutants (pSUP5011::Tn5*mob*) of *B. japonicum* strain 646, strains D45, D52 are the Tn5 mutants (pSUP5011::Tn5*mob*) of *B. japonicum* strain 634b, strain C30 is the Tn5-mutant (pSUP2021::Tn5) of *B. japonicum* strain 634b. The rhizobia cultures were grown at 26–28 °C on the Yeast Extract Mannitol (YEM) media ((g/L): $KH_2PO_4$ – 0.5, $MgSO_4$ – 0.2, NaCl – 0.1, yeast extract – 1.0, mannitol – 10.0) for 7 days, and inoculants were prepared the bacterial titer of which was $10^9$ cells/mL.

Soybean seeds were sterilized for 15 min with 70% ethanol, washed under running water for 1 h, inoculated with a bacterial suspension for 60 min (which provided an inoculation load of 480–500 thousand bacterial cells per 1 seed), and sown in the substrate. Soybean plants were grown in pots pre-sterilized with a 20% $H_2O_2$ solution, on washed river sand (10 kg per pot, 12 plants per pot), under natural lighting and optimal moisture (60 % FC). The source of mineral nutrition was Hellriegel's mixture with 0.25 of nitrogen rate (the full nitrogen rate is 0.708 g of $Ca(NO_3)_2$ per 1 kg of substrate), to which microelements were added. The treatments were replicated seven times.

Indices of symbiotic effectiveness and growth parameters were determined at the stages of 3 true leaves and budding–beginning of flowering. The number and weight of nodules on soybean roots as well as aboveground and root fresh weight of soybean plants inoculated with different strains of *B. japonicum* were determined. NFA was determined in four replications. The total chlorophyll content and parameters of the photosynthetic apparatus activity were determined at the budding–beginning of flowering stage in 3 replications.

Grain productivity was determined at full maturity. Biometric indices (the aboveground part, roots, nodules, and grain weight) were determined in 10 replications.

NFA was determined by the level of nodules acetylene-reducing activity by the acetylene method (Hardy *et al.*, 1968), and was expressed as the amount of ethylene (µmol) produced by the nodules of an individual plant within a one-hour incubation period. The gas mixture was analyzed on an Agilent Technologies 6850 Network GC System gas chromatograph (USA) with a flame ionization detector, on a column (Supelco Porapak N) at a thermostat temperature of +55 °C and a detector temperature of +150 °C. The carrier gas was helium (20 mL/min). The sample volume of the gas mixture was 1 $cm^3$. Pure ethylene (Sigma-Aldrich, USA) was used as a standard.

The total chlorophyll content (*a*+*b*) in plant leaves was determined by the maceration-free method by extraction with dimethyl sulfoxide (Wellburn, 1994). For determinations, leaf samples were taken from the middle tiers of five randomized plants of the same treatment. The optical density of the solutions was determined on a Shimadzu UV-1900 spectrophotometer (Japan) at 649 and 665 nm.

The net photosynthetic, photorespiration and transpiration rates were recorded under controlled conditions with the EGM-5 gas analyzer (PP Systems, USA) at the stage of budding–beginning of flowering. The middle part of the third from the top intact leaf was placed in a temperature-controlled chamber (25 °C) and illuminated by a TA-11 LED spotlight (50 W, color temperature 5200 K). The illumination at the chamber level was 1500 μmol/($m^2$·s) of photosynthetically active radiation. Conditioned atmospheric air with humidity about 10 mbar was blown through the chamber at a rate of 1 L/min. The photorespiration rate was estimated by the postillumination $CO_2$ burst from a leaf for 1 min after the light was turned off. Gas exchange parameters were calculated according to conventional methods (Busch *et al.*, 2024). Measurements were performed in three biological replications.

All statistical analyses were performed using STATISTICA ver. 7.0 software package. Significance of differences between samples was evaluated by one-way analysis of variance (ANOVA), where differences were considered significant at $P < 0.05$ using Tukey's test. In the tables and graphs arithmetic means and their standard errors (x±SE) are presented.

## RESULTS AND DISCUSSION

The primary indicator of the beginning of interaction between leguminous plants and nodule bacteria is the formation of active nodules (symbiotic organs) on the roots, which determines the ability of leguminous–rhizobial systems to fix atmospheric $N_2$ (Kots, 2021). Positive dynamics of nodule formation on the roots of inoculated soybean plants were revealed in all treatments. The highly active *B. japonicum* strains PC09 and B201 were ahead of other microsymbionts in the number and weight of nodules initiated and formed with their participation (**Table 1**). At the same time, the infectivity of D45, D52 and B157 strains in symbiosis was somewhat inferior to the previous ones. The nodule-forming ability of C30 strain when establishing a symbiosis with soybean was the lowest – at the period of budding–beginning of flowering, the weight of nodules was by 4.6–5.5 times less compared to plants in other treatments (see **Table 1**).

Differences in inoculant strains in terms of nodulating activity led to different functioning of *Glycine max*–*B. japonicum* symbiotic complexes, that is, the symbiotic apparatus formed by different strains of nodule bacteria was characterized by different NFA. At the stage of 3 true leaves, the NFA of root nodules formed by C30 strain was inferior to this index of other microsymbionts by 1.6–4.0 times, and at the budding–beginning of flowering stage – by 4.2–6.2 times (see **Table 1**). Since the plants were grown on sand with Hellriegel's mixture with 0.25 of nitrogen rate, this element was actually used by them completely in the early period of their growth and development. Obviously, further $N_2$ fixation by nodules was the dominant source of nitrogen-containing compounds for soybean plants.

At the stage of budding–beginning of flowering, the highest NFA was under inoculation with strain PC09, and the lowest – with strain C30. This index was lower in strains D52 and B201 (without a significant difference between them) compared with PC09, and even lower for strains D45 and B157 (again, without a significant difference in this pair). Interestingly, these pairs were formed by Tn-5-mutants of two different strains, but obtained using the same plasmid (Vorobey *et al.*, 2013).

It should be noted that nodules formed with the involvement of B157 and B201 strains provided the highest level of $N_2$ fixation at the stage of 3 true leaves, and were somewhat inferior in functional activity to other active microsymbionts at the stage of budding–beginning of flowering. In contrast, at this stage, the NFA of soybean in symbiosis with *B. japonicum* PC09 and D52 strains increased significantly. This indicates the probably different NFA dynamics of soybean–rhizobial systems, which can be caused by a number of factors from both the micro- and macrosymbiont side. In particular, variations in NFA during the soybean growth cycle are explained by differences in the expression of genes that control nodule development (Mergaert *et al.*, 2020).

*Table 1.* **Number, fresh weight and $N_2$-fixing activity (NFA) of nodules on roots of soybean plants inoculated with different strains of *B. japonicum* and grown in pots on washed river sand with Hellriegel's mixture (0.25 of nitrogen rate) under natural lighting and optimal moisture (60 % FC)**

| Treatment (strain) | Nodule number, pcs/plant | Nodule weight, mg/plant | NFA, μmol $C_2H_4$/(plant · h) |
|---|---|---|---|
| Developmental stage of 3 true leaves | | | |
| C30 | 3.3±0.1[d] | 8.0±0.3[e] | 0.32±0.02[d] |
| PC09 | 17.3±1.5[b] | 48.0±0.1[a] | 0.50±0.02[c] |
| D45 | 10.0±0.5[c] | 25.0±0.2[d] | 0.54±0.01[c] |
| D52 | 12.8±1.5[c] | 30.0±0.1[c] | 0.53±0.02[c] |
| B157 | 13.8±0.5[c] | 34.0±0.2[b] | 1.30±0.01[a] |
| B201 | 22.0±0.5[a] | 49.0±0.2[a] | 1.14±0.03[b] |
| Developmental stage of budding-beginning of flowering | | | |
| C30 | 3.5±0.2[c] | 43.4±2.7[b] | 1.03±0.12[c] |
| PC09 | 28.3±1.5[a] | 238.7±14.2[a] | 6.43±0.32[a] |
| D45 | 23.8±1.3[b] | 198.0±13.1[a] | 4.80±0.23[b] |
| D52 | 26.8±1.8[ab] | 207.3±13.7[a] | 5.96±0.31[ab] |
| B157 | 27.3±1.4[ab] | 211.2±8.7[a] | 4.90±0.13[b] |
| B201 | 24.3±1.8[ab] | 227.0±7.5[a] | 5.75±0.12[ab] |

**Note:** different superscript letters indicate significant differences among treatments at the same developmental stage, as determined by Tukey's post-hoc test following a one-way ANOVA ($P < 0.05$)

The microsymbiont *B. japonicum* C30 formed the inefficient symbiosis with soybean, which significantly affected the growth of the aboveground part of plants under this treatment. The root system of plants inoculated with C30 strain was less developed and significantly inferior in fresh weight to plants of other treatments. Inoculation with active rhizobia stimulated growth processes in soybean, in particular, rhizogenesis and the growth of the aboveground part (**Table 2**).

The increased $N_2$ assimilation rate in root nodules formed by *B. japonicum* B157 and B201 strains improved the nitrogen nutrition of soybean, which contributed to an increase in the plants aboveground fresh weight by 16.3–25.0 % compared to the use of the less active strain C30. Aboveground biomass accumulation largely determines the level of crop productivity because it enhances the production and storage of assimilate reserves, which are subsequently utilized for the development of reproductive structures and grain formation (Vogel *et al.*, 2021).

*Table 2.* **Aboveground and root fresh weight of soybean plants inoculated with different strains of *B. japonicum* and grown in pots on washed river sand with Hellriegel's mixture (0.25 of nitrogen rate) under natural lighting and optimal moisture (60 % FC)**

| Treatment (strain) | Aboveground weight, g/plant | Root weight, g/plant |
|---|---|---|
| Developmental stage of 3 true leaves | | |
| C30 | 2.56±0.17[b] | 1.35±0.12[b] |
| PC09 | 3.27±0.22[a] | 1.85±0.09[a] |
| D45 | 2.95±0.18[a] | 1.52±0.11[ab] |
| D52 | 3.35±0.20[a] | 1.86±0.08[a] |
| B157 | 3.20±0.12[a] | 1.49±0.10[ab] |
| B201 | 3.45±0.18[a] | 1.74±0.11[a] |
| Developmental stage of budding-beginning of flowering | | |
| C30 | 3.92±0.13[b] | 2.54±0.04[a] |
| PC09 | 4.70±0.23[a] | 2.96±0.18[a] |
| D45 | 4.50±0.21[a] | 2.67±0.20[a] |
| D52 | 4.73±0.18[a] | 2.93±0.17[a] |
| B157 | 4.56±0.16[a] | 2.56±0.16[a] |
| B201 | 4.90±0.22[a] | 2.62±0.08[a] |

**Note:** different superscript letters indicate significant differences among treatments at the same developmental stage, as determined by Tukey's post-hoc test following a one-way ANOVA ($P < 0.05$)

The root system of plants inoculated with *B. japonicum* PC09, D52 strains was well developed (see **Table 2**), which obviously increased the area of water and nutrients absorption from the substrate. At the same time, at the stage of budding–beginning of flowering, the aboveground fresh weight had increased by 19.9–20.7 %, and the roots fresh weight increased by 15.3–16.5 % compared to the index of plants that developed under a low-active symbiosis with *B. japonicum* C30 strain.

A number of authors report that bean number and yield of soybean were positively correlated with the number and weight of nodules at the growth stages of 3 true leaves and budding (Thilakarathna & Raizada, 2017; Moretti *et al.*, 2018). Our observations and literature data show that the active formation of $N_2$-fixing nodules on soybean roots is important for increasing the grain yield (Kots, 2021; Nakei *et al.*, 2022).

It should be noted that under conditions of the pot experiment, equivalent external conditions for the formation and functioning of legume–rhizobial symbiosis (plants growing substrate, mineral nitrogen starting dose, water supply, light regime, etc.) were created. The only variable factor in the plant–bacteria system was the *B. japonicum* genotypes, as they possessed distinct genetic origins and varied in their potential for atmospheric $N_2$ fixation through the action of the nitrogenase enzyme. This enabled the exploration of the interconnections between symbiotic $N_2$ fixation, photosynthesis, and grain productivity in Almaz variety soybean plants as influenced by the activity of *B. japonicum* strains.

It is known that in annual plants, including soybean, physiological and biochemical processes reach their maximum at the flowering period (Hamawi *et al.*, 2023). After the appearance of new sink organs (fruits), they acquire the highest priority in the source-sink system of the plant. This is accompanied by the outflow to them of a larger share of assimilates newly synthesized by the photosynthetic apparatus, and temporarily deposited before the end of flowering, as well as by remobilization of nitrogen-containing compounds from vegetative organs, especially from leaves (Havé *et al*., 2017). Therefore, during the soybean ripening period, the activities of the symbiotic and photosynthetic apparatuses decrease: the former – due to a decrease in the proportion of the assimilates incoming it to meet energy and biosynthetic needs, and the latter – due to the outflow of nitrogen-containing compounds, which are mainly the degradation products of structural and enzymatic proteins of chloroplasts, to fruits.

Based on this assumption, in order to find relationships between the activities of the symbiotic and photosynthetic apparatuses, and the soybean plants productivity under inoculation with different nodule bacteria strains, we used physiological indices at the stage of budding–beginning of flowering (**Table 3**). The grain productivity of plants is given when seed reach full maturity. As already mentioned, since the plants were grown in sand culture under a four-fold reduced dose of nitrogen in the nutrient solution, it is arguable that the symbiotic apparatus was the main source of this element for plants.

*Table 3.* **Parameters of the photosynthetic apparatus activity, transpiration rate, and biological productivity at the budding–beginning of flowering stage, and grain performance of soybean plants at full maturity stage under inoculation with different nodule bacteria strains**

| Treatment (strain) | Chlorophyll (*a*+*b*), mg/g FW | Photosynthetic rate, μmol $CO_2$/($m^2$·s) | Transpiration rate, mmol $H_2O$/($m^2$·s) | Whole plant fresh weight, g | Grain weight, g per plant |
|---|---|---|---|---|---|
| C30 | 1.60±0.05[c] | 15.30±0.16[c] | 2.21±0.07[c] | 6.46±0.19[b] | 3.97±0.12[c] |
| PC09 | 2.05±0.06[a] | 18.29±0.25[a] | 3.32±0.10[a] | 7.66±0.23[a] | 4.82±0.14[a] |
| D45 | 1.70±0.05[b] | 17.23±0.22[b] | 3.05±0.09[b] | 7.17±0.22[a] | 4.37±0.13[b] |
| D52 | 1.94±0.06[ab] | 18.42±0.25[a] | 3.56±0.11[a] | 7.65±0.23[a] | 4.52±0.14[ab] |
| B157 | 1.85±0.06[b] | 18.05±0.24[a] | 3.39±0.10[a] | 7.12±0.21[a] | 4.32±0.13[b] |
| B201 | 2.20±0.07[a] | 18.07±0.24[a] | 3.06±0.09[b] | 7.52±0.23[a] | 4.63±0.14[ab] |

**Note:** different superscript letters indicate significant differences among treatments at the same developmental stage, as determined by Tukey's post-hoc test following a one-way ANOVA ($P < 0.05$)

It is known that the development and functioning of the photosynthetic apparatus depends on the supply of plants with nitrogen, since without this macronutrient the synthesis of proteins, most of which are concentrated precisely in the chloroplasts of the leaves mesophyll cells, is impossible (Luo *et al.*, 2021). The main enzyme of $CO_2$

assimilation (RuBisCO) can amount to 50 % of the soluble protein in photosynthetic cell. There is evidence in the literature that parameters of biological nitrogen fixation and soybean photosynthesis are closely related, in particular, chlorophyll content in leaves at the stage of full flowering can serve as an indicator of the level of nitrogen fixation (Hamawi *et al.*, 2023).

These statements are supported by the data in **Table 3** which show that the total chlorophyll content in leaves was the highest in the plants inoculated with the PC09 strain and the lowest in those inoculated with the C30 strain. The photosynthesis and transpiration rates were also the lowest in the last treatment, and the highest – under inoculation with strain D52, in which case NFA was the second highest after PC09. It should be noted that we recorded both net photosynthesis and photorespiration. Thus, **Table 3** shows the value of gross photosynthesis, which is calculated as the sum of these gas exchange parameters. This index reflects the activity of the $CO_2$ assimilation process more accurately as it takes into account the masking effect of the emission of $CO_2$ in the light as a result of photorespiration, which is reassimilated inside the leaf.

If we calculate the ratio of photosynthesis to transpiration, we will get an index of the photosynthetic water-use efficiency (also called instantaneous water-use efficiency WUEi (Busch *et al.*, 2024)) (**Fig. 1**). On average, it was 5.75 µmol $CO_2$/mmol $H_2O$ for all treatments. This value completely coincides with the results previously obtained in other experiments with soybean, which confirms its representativeness for this crop (Kiriziy *et al.*, 2022).

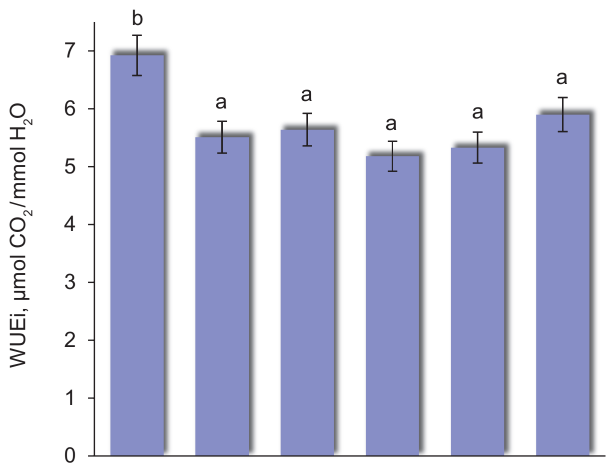


**Fig. 1.** Photosynthetic water-use efficiency (also called instantaneous water-use efficiency WUEi) in leaves of soybean plants at the budding–beginning of flowering stage inoculated with different *B. japonicum* strains

The fresh weight of the whole plant, which characterizes its biological productivity, as well as the grain weight from the plant, were the highest under inoculation with the PC09 strain, and the lowest – with the C30 strain.

Therefore, the primary analysis of experimental data revealed their variability depending on the strain of nodule bacteria used for inoculation, which is the basis for finding correlational relationships between them. Since the symbiotic apparatus is the main source of nitrogen-containing compounds in the plant, without which the functioning of the photosynthetic apparatus is impossible, firstly the relationships between NFA and the total chlorophyll content in leaves, and their $CO_2$ assimilation rate were investigated (**Fig. 2**).

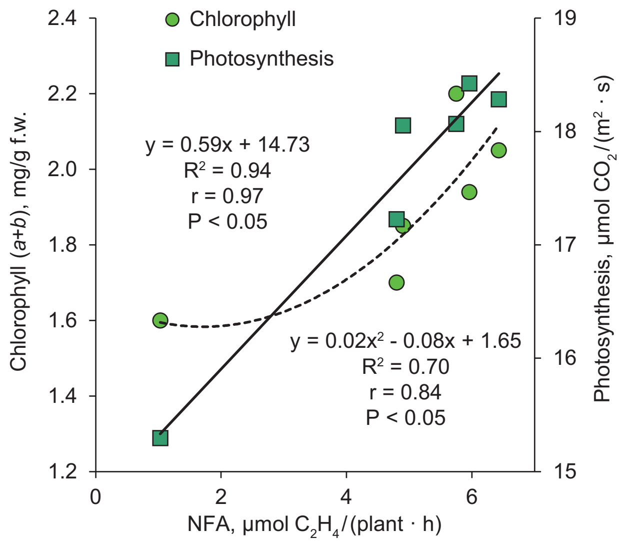


**Fig. 2.** Correlations between the $N_2$-fixing activity (NFA), chlorophyll content, and the photosynthetic rate in soybean plants at the budding–beginning of flowering stage inoculated with different *B. japonicum* strains (f.w. – fresh weight)

The first relationship was approximated by a quadratic equation with a lag phase in the NFA range of 1–3.5 μmol $C_2H_4$/(plant·h). This phenomenon can be explained by very low NFA in plants inoculated with strain C30. Obviously, in this treatment, the formation of the photosynthetic apparatus took place mainly due to the starting dose of mineral nitrogen, which was insufficient to fully unlock the genetic potential of soybean plants. This is confirmed by the lowest value of the photosynthetic rate under this treatment.

It should also be noted that chlorophyll content in the plant leaves under inoculation with the D45 strain practically did not differ from C30, but their NFA and photosynthetic rate were significantly higher. It can be speculated that nitrogen-containing compounds coming from the nodules of plants inoculated with D45 strain have directed mainly to the formation of structural and enzymatic proteins in chloroplasts, which are involved in $CO_2$ assimilation processes, while the amount of photosynthetic pigments was quite sufficient for their energy supply. A stimulating effect on the photosynthesis of the increased demand for assimilates from the nodules formed by this strain, compared to strain C30, is also not excluded. It is known that increasing the attractive power of the sink stimulates the activity of the source of assimilates, in this case – the photosynthetic apparatus (Glanz-Idan *et al.*, 2020). All these factors led to an almost linear relationship between NFA and the photosynthetic rate with a rather high close correlation (see **Fig. 2**).

Usually, there is a correlation between the chlorophyll content in leaves and $CO_2$ assimilation rate, but this statement is valid for a certain range of these indices. In **Fig. 3** can be seen that in our experiments a close linear correlation of the photosynthetic rate with the chlorophyll content was observed for the values of the latter in the range from

1.6 to 2.0 mg/g of fresh weight. With a further increase in the chlorophyll content, the photosynthetic rate remained almost at the same level (even with some tendency to decrease), and there was no correlation between these indices.

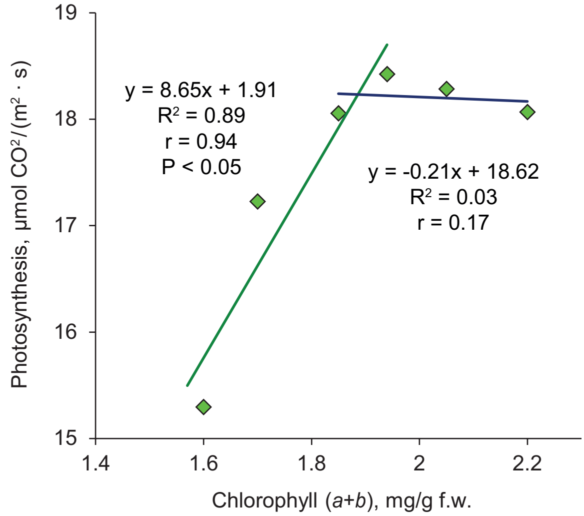


**Fig. 3.** Correlations between the chlorophyll content and photosynthetic rate in leaves of soybean plants at the budding–beginning of flowering stage inoculated with different *B. japonicum* strains

As a rule, a high chlorophyll content in leaves is characteristic of plants well supplied with nitrogen (Vorobey *et al.*, 2021). Under such conditions, especially during the flowering period, a certain excess of chemically bound nitrogen may occur, which the plant deposits and later uses for fruit growth. The main nitrogen-containing compounds in which it is stored are non-structural proteins. These are mainly proteins of the leaf photosynthetic apparatus. At the same time, there is information that due to the high need for nitrogen in leguminous plants for seed filling, the lipoxygenase enzyme also plays a significant role in its deposition. It accumulates in large quantities in the vacuoles of paraveinal mesophyll cells (Fischer *et al*., 1999).

Among nitrogen-storage compounds, RuBisCO, which at the same time is the main enzyme of $CO_2$ assimilation, occupies a special place (Feller *et al*., 2008; Luo *et al.*, 2021). According to some data, it can accumulate in chloroplasts in amounts close to the transition to the crystalline form, while its overall activity decreases (Sakoda *et al.*, 2019). This can explain the lack of relationship between the photosynthesis and chlorophyll content at its high values when the mesophyll cells in leaves during the flowering period have filled with a large number of chloroplasts, the photosynthetic activity of which was inhibited by an excess of deposited nitrogen-containing compounds. However, in the further process of ripening, these compounds are remobilized into the grain, while the photosynthetic apparatus activity remains at a high level for longer, since the excess of reserve proteins is initially remobilized. All this facilitates an increase in the grain productivity of soybean plants.

This is confirmed by correlations between NFA and the whole plant fresh weight at the budding–beginning of flowering stage, and grain weight at full maturity (**Fig. 4**). It is interesting that in the first case the correlation coefficient was slightly higher than in the second.

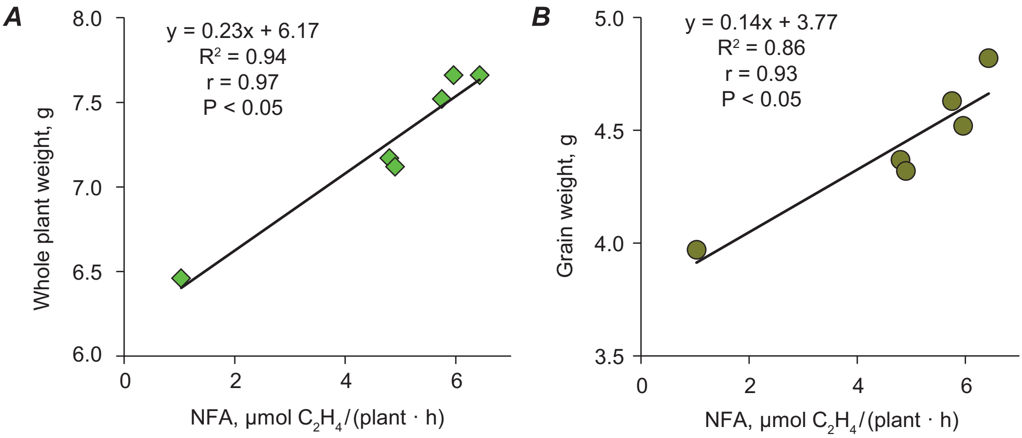


**Fig. 4.** Correlations between $N_2$-fixing activity (NFA) and the whole plant fresh weight at the budding-beginning of flowering stage (***A***), and grain (***B***) weight per plant at full maturity

Similar correlations were observed between the photosynthetic rate and the whole plant fresh weight during the flowering period, and grain weight at full maturity (**Fig. 5**). However, in this case, the correlations were quite high, but somewhat lower than for NFA. It can be assumed that not all nitrogen-containing compounds originating from nodules participate in the photosynthetic apparatus functioning. A significant part of them is used for growth processes, or is deposited for further remobilization into grain.

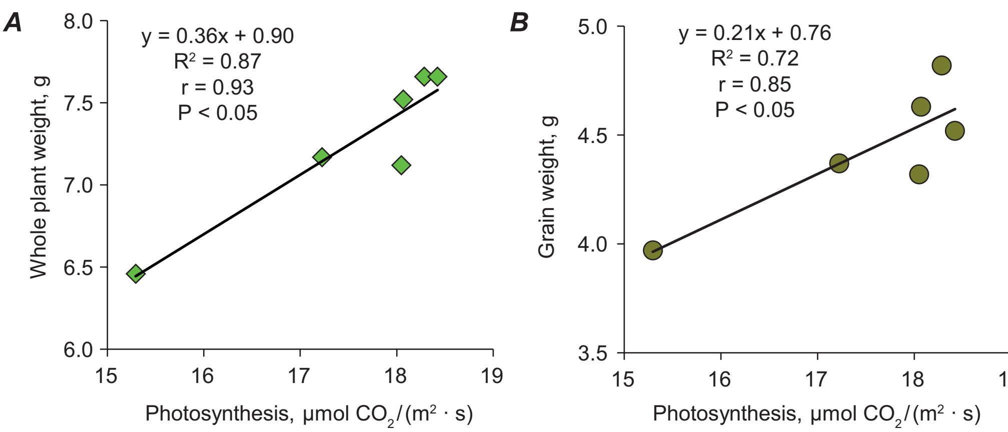


**Fig. 5.** Correlations between the photosynthetic rate and the whole plant fresh weight at the budding–beginning of flowering stage (***A***), and grain (***B***) weight per plant at full maturity

It should also be noted that the relationship between the whole plant weight and its grain productivity is mediated by the system of source-sink relations between vegetative and generative organs, including the patterns of assimilates and nitrogen-containing compounds redistribution between them. At the same time, the remobilization coefficients are usually less than 1, that is, not all assimilates from the vegetative organs get into the grain when it is filled. In addition, a lot of assimilated carbon is incorporated in the cell wall structural elements, and remains in the dry vegetative organs after ripening.

On our opinion, all these are the reasons for a certain decrease of the correlation coefficients between NFA, photosynthesis and grain weight, compared to the correlations with whole plant fresh weight.

## CONCLUSIONS

The rhizobia strains used in our work formed symbiotic systems of different effectiveness with soybean plants of the same variety. The number and weight of nodules, as well as NFA per plant, differed significantly between inoculation treatments. Since the plants grew on a sandy substrate on a nutrient medium with a nitrogen rate reduced by 4 times, the main source of this element during the budding–beginning of flowering stages was the symbiotic apparatus. This led to the existence of close positive correlations between NFA and the leaf photosynthetic rate, as well as between these physiological indices and the biological, and grain productivity of plants.

Hence, we have identified quantitative connections between the two main components of the production process of legume-rhizobium symbiosis – $N_2$ fixation and photosynthesis. The higher the efficiency of $N_2$ fixation of a particular strain of rhizobia used for inoculation, the higher the performance of the photosynthetic apparatus of soybean plants. This, in turn, helps to improve their provision with assimilated carbon and nitrogen, which facilitates a more complete realization of the productivity genetic potential of a soybean variety. Therefore, the expediency of creating new rhizobia strains with high NFA for further increasing the yield of this strategic for mankind crop is physiologically substantiated.

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## COMPLIANCE WITH ETHICAL STANDARDS

**Conflict of Interest**: the authors declare that research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

**Human Rights**: this article does not contain any studies with human subjects performed by any of the authors.

**Animal Studies**: this article does not include animal studies.

## AUTHOR CONTRIBUTIONS

Conceptualization, [N.V.; K.K.; S.K; D.K.]; methodology, [N.V.; P.P.; S.K.]; validation, [N.V.; K.K.; P.P.; S.K.; D.K.]; formal analysis, [N.V.; K.K.; P.P.; D.K.]; investigation, [N.V.; K.K.; S.K.; D.K.]; resources, [N.V.; K.K.; P.P.; S.K.; D.K.]; data curation, [N.V.; K.K.; P.P.; S.K.; D.K.]; writing – original draft preparation, [N.V.; K.P.; P.P.; D.K.]; writing – review and editing, [N.V.; K.K.; P.P.; S.K.; D.K.]; visualization, [P.P.; S.K.; D.K.]; supervision, [N.V.; S.K.; D.K.].

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## ЗАЛЕЖНІСТЬ ПРОДУКТИВНОСТІ СОЇ ВІД ФУНКЦІОНУВАННЯ СИМБІОТИЧНОГО ТА ФОТОСИНТЕТИЧНОГО АПАРАТІВ

***Надія Воробей, Катерина Кукол,***
***Петро Пухтаєвич, Сергій Коць, Дмитро Кірізій***

*Інститут фізіології рослин і генетики НАН України*
*вул. Васильківська, 31/17, Київ 03022, Україна*

**Вступ.** Підвищення врожайності сої зумовлює необхідність підтримувати високий рівень білка в насінні, а отже, процес фіксації атмосферного $N_2$. Відомо, що інокуляція насіння азотфіксувальними бактеріями сої покращує $N_2$-фіксацію та врожайність зерна сої. Поряд із тим, введення нових штамів бульбочкових бактерій у препарати для інокуляції сої потребує вивчення їхнього впливу на основні взаємопов'язані фізіологічні процеси, які становлять основу продуктивності бобових рослин – азотфіксацію і фотосинтез. Тому метою роботи було вивчити залежність формування вегетативної маси та зернової продуктивності сої, інокульованої різними за функціональною активністю бульбочковими бактеріями *B. japonicum*, від активності функціонування симбіотичного та фотосинтетичного апарату рослин.

**Матеріали та методи.** Об'єкт дослідження – симбіотичні системи, створені за участю рослин сої (*Glycine max* (L.) Merr.) сорту Алмаз і бульбочкових бактерій *Bradyrhizobium japonicum*: аналітично селекціонованого РС09 та рекомбінантних штамів В157, В201, D45, D52 (pSUP5011::Tn5*mob*) і С30 (pSUP2021::Tn5) із музейної колекції азотфіксувальних мікроорганізмів ІФРГ НАН України. Методи дослідження – мікробіологічні, біохімічні та фізіологічні, статистичний аналіз.

**Результати.** Виявлено, що інтенсивність азотфіксації бульбочок (АФА), сформованих культурами *B. japonicum* РС09, D45, D52, В157 і В201, у фазу 3-х справжніх листків перевищувала АФА бульбочок, сформованих Tn5-мутантом С30 в 1,6–4,0 рази, а у фазу бутонізації–початку цвітіння – в 4,2–6,2 раза. На підставі порівняльного аналізу фізіологічних показників сої, інокульованої різними за активністю штамами *B. japonicum* знайдено тісні позитивні лінійні кореляційні зв'язки між АФА, інтенсивністю фотосинтезу та біологічною і зерновою продуктивністю рослин.

**Висновки.** Отримані результати свідчать, що чим більша нодуляційна й азотфіксувальна активність ризобій у симбіотичній системі соя–*B. japonicum*, тим вища функціональна активність сформованого рослинами фотосинтетичного апарату. Це забезпечує повніше розкриття генетичного потенціалу продуктивності культури.

***Ключові слова***: *Glycine max* (L.) Merr., *Bradyrhizobium japonicum*, симбіоз, нодуляція, азотфіксувальна активність, фотосинтез, продуктивність

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