

## SYMBIOTIC PRODUCTIVITY OF PHYTO-BACTERIAL SYSTEMS UNDER THE ACTION OF N-ACETYL-D-GLUCOSAMINE ON DIAZOTROPHIC MICROORGANISMS

O. V. Kyrychenko

Institute of Plant Physiology and Genetics  
of the National Academy of Sciences of Ukraine, Kyiv

E-mail: [azoleki@ukr.net](mailto:azoleki@ukr.net)

Received 11.10.2019

Revised 12.02.2020

Accepted 28.02.2020

The purpose of the work was to evaluate the symbiotic productivity of soybean–rhizobium and wheat–azotobacter phyto-bacterial systems under the action of N-acetyl-D-glucosamine (0.01 M; 0.1 M) *in vitro* on the cultures of nitrogen-fixing microorganisms *Bradyrhizobium japonicum* 634b and *Azotobacter chroococcum* T79. We have used such indicators as plants biological and seed productivity, rhizobia nodulation ability and nitrogenase activity of soybean symbioses as well as wheat rhizosphere microbiota. It was shown that the biological activity of N-acetyl-D-glucosamine during incubation with soybean nodule bacteria had a higher level of realization of the rhizobia nodulation ability: plants were more actively infected (by 12%), a greater number of nodules were formed (1.2–2.3 times) with their greater total mass per plant (1.4–2.1 times) and the mass of each root nodule (1.2 times), as well as the nitrogen-fixing activity of the symbiosis (1.7 times) and the functional capacity of each morpho-structural symbiotic unit (1.4 times). It provided higher (by 14–29%) seed productivity of this system as compared with the symbiosis formed by the rhizobia monoculture. Activation of basic physiological processes in wheat such as nitrogen fixation (the activity of the rhizosphere microbiota was increased by 1.1–1.4 times) and photosynthesis (the content of chlorophylls in leaves was increased by 1.1–1.2 times) with N-acetyl-D-glucosamine-modified azotobacter provided a higher level of realization of the productive potential of this system in comparison with both non-infected plants (by 15%) and the variant of seed inoculation with bacteria only (by 7%). While inoculants bacteria + glucosamine had positive effect on seed productivity of the symbiotic and associative systems, it was shown no significant change in the biological productivity of soybean and wheat plants.

So, the use of N-acetyl-D-glucosamine as an additional agent of carbohydrate nature in inoculants with soybean nodule bacteria and soil diazotrophs of *Azotobacter* genus led to a more complete realization of the symbiotic and productive potential of phyto-bacterial symbiosis and association when compared to using a diazotrophs only.

Our results indicated the possibility of practical use of acetylated glucose-containing aminosaccharide in the creation of complex inoculants based on nitrogen-fixing bacteria.

**Key words:** soybean-rhizobium symbiosis, wheat–azotobacter association, N-acetyl-D-glucosamine, nodulation, nitrogen fixation, rhizosphere microbiota, chlorophyll, harvest.

Pre-sowing inoculation of legume and grain seeds is an environmentally safe biotechnological technique that promotes the formation of higher yields and the production of environmentally friendly crop [1–4]. Beneficial microorganisms, introduction to the seeds or soil by specially developed

microbiological preparations [3–5] on the bases of selected bacterial strains help to improve the growth and development of agricultural crops and increase their yield. It occurs due to stimulation of seed germination, improvement of mineral, in particular, nitrogen and phosphorus nutrition of plants,

activation of their photosynthetic activity, increase of resistance to diseases and pests, as well as active formation and functioning of symbiotic and associative systems [2, 3, 6–9]. The results of recent studies indicated the prospect and effectiveness of the use of complex microbiological preparations for the bacterization of legumes and non-legumes, which provided stable yields, including under the influence of stressors of different nature [3, 10, 11]. Mono-inoculants are more sensitive to the effects of such factors, so the stabilization and optimization of agronomically useful effects of bacterial preparations in seed inoculation is achieved due to the complex action of biological agents — components of the preparations, which may be bacteria with different agronomically useful ecological functions as well as biologically active substances of natural origin [5].

The search of new biological agents that are positive exogenous regulators of the symbiotic and productive potential realization of phyto-bacterial symbioses and associations is of great interest. Today, there are a sufficient number of works indicating the activating effect of bacterial carbohydrate-containing substances (exopolysaccharides, capsular polysaccharides, glucans, lipopolysaccharides, exometabolites) on the formation and functioning of legume-rhizobial symbioses [2, 5, 12–14]. Monosaccharides that are the component of bacterial polysaccharides may be promising to be considered from this point of view, since obtaining the latter for practical use is a rather labor-intensive process. In addition, monosaccharides (hexoses, pentoses, ketoses, acetylated aminosaccharides, etc.), as products of plant metabolism, are excreted with root exudates into the rhizosphere zone [15], affecting the development and functional activity of the rhizospheric microbiota *in situ*, in particular, the bacterial population reproduction to reach the required concentration level at which the infection of the plants occurs; chemotaxis of bacterial cells to the root of the plant; growth-activating and nitrogen-fixing ability of microorganisms [16–18]. Monosaccharides, as components of nutrient media during bacterial cultivation under pure culture conditions, are actively catabolized by microorganisms as energy substrates [19]. Carbohydrates exogenously introduced into the growth medium of microorganisms, in particular galactose- and glucose-containing, as additional biologically active substances provide active reproduction of bacterial culture, which increases the

inoculant titer [20, 21] and regulate the physiological activity of bacteria *in vitro*, ensuring their survival under stressful conditions [20].

The works of recent years testify to the high biological activity of glucose-containing aminosaccharide N-acetyl-D-glucosamine, as well as natural biological substances (glucans, lipochitoooligosaccharides, chitin, chitosan) containing it in its composition, relative to bacteria, mushrooms, plants and animals [18, 21–24]. This allowed the development of biological preparations, one of the components of which is a glucosamine-containing compound for practical use in plant growing [5, 21, 23] and medicine [22]. Examples of such preparations for plant growing are the preparation of “Agrinos” company (Norway) Agrinos A+B, which contains chitin, chitosan and glucosamine in addition to nitrogen-fixing bacteria, as well as a preparation of protective action, developed at the Zabolotny Institute of Microbiology and Virology of the National Academy of Sciences of Ukraine, Averkomnova which contains 0.01M chitosan along with streptomycetes, producing the antibiotic avermectin.

Previously, we showed [24] that in the presence of 0.01M glucosamine in the culture medium of soybean nodule bacteria, the optical density of the suspension (at  $\lambda$  540 and  $\lambda$  560, respectively) in the 13- and 20-day culture, when compared with the 6-day culture, changed 1.8–1.9 and 1.6–1.7 times, respectively, while in the control variant (without the introduction of carbohydrates) — 1.6 times and 1.3–1.6 times, respectively. Comparison of control and experimental variants showed that the optical density of the culture variant with glucosamine exceeded the control by 14–16% and 12–20% for 13- and 20-day cultures, respectively, which indicates the activating effect of this carbohydrate on bacterial culture *in vitro*. It was also found [25] that N-acetyl-D-glucosamine is a substance of carbohydrate nature, which is, among other monosaccharides, in plant root exudates [26], *in vitro* up to 50% of it is bound to *Azotobacter chroococcum* T79 cells. As a result, the physiological activity of the bacteria in seed inoculation could be probably changed. Therefore, the basis of our work was the hypothesis that acetylated amino saccharide N-acetyl-D-glucosamine as a signal (regulatory) molecule can be used to modify a bacterial inoculant in order to increase the level of realization of the symbiotic and productive potential of phyto-bacterial

symbioses (soybean — *Bradyrhizobium*) and associations (spring wheat — *Azotobacter*).

Therefore, the aim of the study was to evaluate the symbiotic productivity of phyto-bacterial systems soybean — *Bradyrhizobium japonicum* 634b and wheat — *Azotobacter chroococcum* T79 under the action of N-acetyl-D-glucosamine (0.01M; 0.1M) *in vitro* on diazotrophic microorganism cultures.

### Materials and Methods

The degree of realization of the symbiotic and productive potential of phyto-bacterial symbioses and associations under seed bacterization by inoculants based on nitrogen-fixing bacteria and glucosamine was studied in pot experiments. Soybean-rhizobial symbioses formed by plants of early-ripened soybeans of foreign (Lisabon) and domestic (Almaz) breeding, as well as spring wheat variety Rannyaya 93 of domestic breeding [27] were the object of research.

Diazotrophic bacteria *B. japonicum* 634b and *A. chroococcum* T79 (strains from the collection of symbiotic and associative nitrogen-fixing microorganisms of the Institute of Plant Physiology and Genetics of the National Academy of Sciences of Ukraine) were grown at 28 °C on mannitol-yeast and Ashby nitrogen-free solid mediums respectively. The cultures were washed of with sterile water, mixed to a homogeneous suspension. The number of viable bacteria (colony-forming units) was determined by serial dilutions, seeding and counting of grown colonies [19]. The titer of rhizobia in suspension was  $10^{10}$  cells/ml, the titre of azotobacter was  $10^7$  cells/ml. The nitrogen-fixing microorganisms were modified with N-acetyl-D-glucosamine, conducting a joint (daily) incubation of the bacteria respectively with 0.01M or 0.1M carbohydrate solution (*v:v* — 1:1) at 28 °C.

Bacterization of soybean and wheat seeds was carried out on the day of sowing, keeping the sowing seeds in the inoculum (1 ml/100 seeds in the variant) for one hour. In control, bacteria were incubated with water, thus obtaining a similar inoculation titer of bacterial cells in all variants. An absolute control (a.c.) was a variant without seed inoculation (seed treatment with water).

Pot experiments were carried out at the Institute of Plant Physiology and Genetics of the National Academy of Sciences of Ukraine under natural light and air temperature in 10 kg Wagner pots vessels on sand (Lisabon

soybean variety, 2017) and soil (soil : sand — 3:1, Almaz soybean variety, 2018) substrate with Gerrigel's nutrient [2] (0.25 norms of mineral nitrogen), and also on a soil substrate with mineral salts according to Pryanishnikov [2] (with 0.5 norm of nitrogen) for wheat spring (2010) according to the following scheme:

1. Without inoculation (water treatment of seeds — absolute control, a. c.).

2. Seed inoculation with bacteria (strain — control).

3. Seed inoculation with the composition bacteria + glucosamine.

The effectiveness of the symbiotic soybean system, when seeds were inoculated with rhizobia + glucosamine composition, was evaluated by vegetative and seed productivity of plants. There were also analyzed:

- the formation of symbiosis by nodulation ability of rhizobia (the activity of root nodule formation, their mass per plant);

- functional (nitrogenase) activity — by acetylene reductase method according to Hardy et al. [28] on a Agilent GC System 6850 gas chromatograph (USA) with a flame ionization detector. The amount of ethylene formed from acetylene per 1 hour of incubation, when the nitrogenase of incubated sample is operated, was expressed in molar units of ethylene formed:

- in micromoles of  $C_2H_4$ /(plant · hour) — the actual nitrogenase activity and in micromoles of  $C_2H_4$  (g of nodules · hour) — the specific nitrogenase activity of symbiotic systems.

- in nanomoles of  $C_2H_4$ /(1 nodule · hour) and nanomoles of  $C_2H_4$ /(mass of 1 nodule · hour) — the nitrogenase activity of the morpho-structural symbiotic unit (root nodule).

- in nanomoles of  $C_2H_4$ /(1 g of soil · 2 hours) or in nanomoles of  $C_2H_4$ /(1 plant with soil · 2 hours, in the absence of root nodules on the plant) — nitrogenase activity of the rhizospheric microbiota. Nitrogenase activity was determined in 4–6 biological replicates. When evaluating the symbiotic characteristics of rhizobia — nodulation ability and nitrogenase activity, the control was the variant with seed inoculation of nodule bacteria only (No 2).

Soybean plants and rhizospheric soil were sampled under soil culture conditions during one (V1, 20-day-old plants), two (V2, 27-day-old plants) and three (V3, 33-day-old plants) trifoliolate leaves, under sandy culture conditions — during the development phases of two trifoliolate leaves (V2, 26-day-old plants),

full flowering (R2, 43-day-old plants) and full pod (R4, 62-day-old plants).

To assess the seed productivity of soybeans (Lisabon variety), under inoculation of seeds with (0.1M) glucosamine-modified rhizobia, a small-scale field experiment (accounting area of one plot was 0.6 m<sup>2</sup>) was conducted in 2016 at the field of the Institute of Plant Physiology and Genetics of the National Academy of Sciences of Ukraine in 4-fold replicates by randomized sowing. We carried out the 2.5-fold seed-sowing rate. The crop was harvested by hand and its structure was analyzed.

The sampling of spring wheat plants was carried out in the phases of seedling development, tillering, start of beginning of the heading and ripening stage. The dynamics of vegetative mass accumulation (above-ground part, root), chlorophyll content in leaves (according to Arnon after extraction of plant material in dimethyl sulfoxide [29] and expressed in mg/g of fresh tissue) and grain productivity of wheat (crop structure) were evaluated. The nitrogen-fixing activity of the rhizospheric microbiota was determined by the acetylene reductase method according to Hardy et al. [28] on a "Chromatograf 504" device (Poland, "Mera Elwro") and expressed in ethanol nanomoles per plant per hour (nmol of C<sub>2</sub>H<sub>4</sub>/(plant · h)).

The results were statistically processed (*Statgraphyc Plus*) and presented as mean values and their errors ( $M \pm m$ ), as well as the significance level  $P$  ( $P \leq 0.05$ ) calculated by Dosphehov [30].

## Results and Discussion

A significant increase in the degree of realization of the nodulation (nodule-forming) ability of soybean rhizobia under the influence of glucosamine on microorganisms under the conditions of sand culture was established (Table 1): on the roots of the soybean Lisabon variety the nodules were formed by 2.2; 2.3 and 1.4 times more than in the control variant (one-component rhizobia inoculant) respectively in the studied phases of vegetation. The mass of these nodules on the plant was greater than in the control by 2.1 and 1.9 times in the phases of two trifoliolate leaves and flowering, whereas in the phase of active beans formation it did not differ from the control.

By weight of one nodule, in the experimental variant, in the phase of two trifoliolate soybean leaves, a positive difference in 1.2 times from the control was established, whereas in the subsequent phases

of plant growth this index was the same or slightly below of the control one. The results of studies obtained under soil culture conditions (Table 1) also indicate an increase in the degree of nodulation capacity of soybean rhizobia under the influence of glucosamine. Bacteria actively entered into symbiosis with plants Almaz variety at the earliest stage of ontogeny (the development phase of a trifoliolate leaf). The number of rhizobia-nodulated plants (NOD<sup>+</sup> plants) in the variant with glucosamine was 76.5%, which was by 12% more than in rhizobia-only seed inoculation (68.6% NOD<sup>+</sup> plants). In the variant without seed inoculation, the root nodules were absent, indicating the absence of the native microbiota of the soybean nodule bacteria in the sand and soil used as a substrate for plant growth. Rhizobia under the influence of glucosamine formed on the plants of root nodules by 24% more with their total mass by 41% more than in the variant with one-component inoculant in the phase of three trifoliolate leaves. Glucosamine also contributed to an increase by 22% of the weight of each nodule formed (Table 1).

An evaluation of the functional (nitrogenase) activity of the symbiotic apparatus of the Lisabon soybean variety under the conditions of sand culture showed (Table 2) that in the flowering phase of soybean the rhizobia under the influence of glucosamine formed a symbiosis with a functional activity exceeding the control value by 1.7 times. However, already in the phase of full pod, the ability of this symbiotic system to fix nitrogen was at the level of control.

Under soil culture conditions, in developmental phase of three trifoliolate leaves the symbiotic system of the soybean Almaz variety of this variant was also characterized by 1.7-fold increased ability to fix nitrogen than the bacteria not treated with glucosamine (Table 2). Simultaneously the functional activity of the morpho-structural symbiotic unit (root nodule) increased by 1.4 times (NGA/1 nodule · hour) and 1.5 times (NGA/1 nodule mass · hour). The results obtained indicate an increase in the level of actual nitrogenase activity of symbiosis (NGA/plant · hour) not only by increasing the number of symbiotic structures on plant roots (Table 1), but also due to enhancing their functional capacity (Table 2).

Thus, the functioning of soybean symbiotic apparatus, when the seeds are inoculated with rhizobia, under the influence of glucosamine occurs more intensively, which is due to

**Table 1. Degree of nodulation ability of soybean rhizobia by the action of N-acetyl-D-glucosamine (0.01 M) on bacteria**

| No                                                                         | Variant                        | Number of nodules per plant, pcs. | Weight of nodules per plant, mg | Weight of 1 root nodule, mg |
|----------------------------------------------------------------------------|--------------------------------|-----------------------------------|---------------------------------|-----------------------------|
| <i>Lisabon variety, sand culture</i>                                       |                                |                                   |                                 |                             |
| <i>Development phase of two trifoliolate leaves, 26-day-old plants</i>     |                                |                                   |                                 |                             |
| 2                                                                          | Rhizobia + water               | 15.8 ± 3.9                        | 24.77 ± 8.55                    | 1.34 ± 0.33                 |
| 3                                                                          | Rhizobia + GlcNAc <sup>1</sup> | 34.0 ± 3.8* <sup>2</sup>          | 51.88 ± 7.36*                   | 1.57 ± 0.22                 |
| <i>Flowering phase, 43-day-old plants</i>                                  |                                |                                   |                                 |                             |
| 2                                                                          | Rhizobia + water               | 12.3 ± 3.5                        | 155.83 ± 46.90                  | 13.24 ± 1.38                |
| 3                                                                          | Rhizobia + GlcNAc              | 28.8 ± 4.3*                       | 293.27 ± 39.50*                 | 10.88 ± 1.45                |
| <i>Full pod phase, 62-day-old plants</i>                                   |                                |                                   |                                 |                             |
| 2                                                                          | Rhizobia + water               | 29.2 ± 3.0                        | 720.93 ± 65.88                  | 25.41 ± 2.56                |
| 3                                                                          | Rhizobia + GlcNAc              | 40.5 ± 2.7*                       | 723.82 ± 25.83                  | 18.15 ± 0.96                |
| <i>Almaz variety, soil culture</i>                                         |                                |                                   |                                 |                             |
| <i>Development phase of one trifoliolate leaf, 20-day plants</i>           |                                |                                   |                                 |                             |
| 2                                                                          | Rhizobia + water               | 2.7 ± 0.4                         | — <sup>3</sup>                  | —                           |
| 3                                                                          | Rhizobia + GlcNAc              | 3.1 ± 0.5                         | —                               | —                           |
| <i>Developmental phase of three trifoliolate leaves, 33-day-old plants</i> |                                |                                   |                                 |                             |
| 2                                                                          | Rhizobia + water               | 13.3 ± 1.6                        | 31.50 ± 5.30                    | 2.37 ± 0.31                 |
| 3                                                                          | Rhizobia + GlcNAc              | 16.5 ± 2.6                        | 44.33 ± 5.93*                   | 2.89 ± 0.38                 |

*Notes.* See here and in the Tables 2, 3, 5, 8: 1 — GlcNAc — N-acetyl-D-glucosamine; 2\* — positively reliable ( $P \leq 0.05$ , where  $P$  is the level of significance calculated by Dosepov [30]) to the variant with inoculation of seeds with bacterial strain (No. 2); 3 — “—” — was not defined.

both an increase in the number of symbiotic structures on the roots of plants and to an increase in the level of functional capacity of each symbiotic unit.

Analysis of the nitrogen-fixing activity of the soil rhizospheric microbiota of soybean Almaz variety showed (Table 3) a significant difference between the variants without inoculation of seeds (No 1) and with pre-sowing inoculation of seeds (No 2, 3), which is due to the introduction of soybean nodule bacteria on seeds and in the soil when they are seeding. Differences in the ability between the rhizospheric microbiota of variants with bacterization of seeds by rhizobia only and the bacteria treated with glucosamine were insignificant (with a positive trend) and were at the level of control (inoculation with rhizobia only, variant No 2).

Thus, the regulatory effect of glucosamine on rhizobial culture is manifested in their joint incubation, resulting in an increased level of realization of the symbiotic potential of soybean rhizobial symbiosis (nodule-forming and nitrogen fixing activity), whereas on the functional (nitrogenase)

activity of rhizospheric diazotrophic microbiota there was no significant activating effect.

It was found (Table 4) that bacterized soybean seeds characterized more intensive germination process unlike non-bacterized seeds: by 1.5–3.6 times (sand culture) and 1.7–2.0 times (soil culture). On the 10–11<sup>th</sup> day after sowing, the difference in the number of seedlings was 10–14% for plants in the sand culture and 43–56% — in the soil culture. The activating effect of inoculant with aminosaccharide on the energy of germination of soybean seeds was noted: the number of seedlings of the Lisabon variety (sand culture) significantly exceeded the control 2.0 times (the 7<sup>th</sup> day after sowing), 1.9 times (the 8<sup>th</sup> day after sowing) and was practically equal to the indicators of the variant of seed inoculation with a rhizobial strain only on the 11<sup>th</sup> day after sowing.

A similar regularity was observed in soil culture during cultivation of the Almaz variety. However, no significant difference in the studied parameters was established (Table 4).

**Table 2. Nitrogenase activity (NGA) of the symbiotic apparatus of soybean by inoculation of seeds with rhizobia, modified by glucosamine (0.01 M)**

| No | Variant           | <i>Lisabon variety, sand culture</i>                       |                                                                   |                                                                    |                                                                         |
|----|-------------------|------------------------------------------------------------|-------------------------------------------------------------------|--------------------------------------------------------------------|-------------------------------------------------------------------------|
|    |                   | <i>Flowering phase</i>                                     |                                                                   | <i>Full pod phase</i>                                              |                                                                         |
|    |                   | NGA of symbiosis                                           |                                                                   |                                                                    |                                                                         |
|    |                   | $\mu\text{mol of C}_2\text{H}_4 /$<br>(plant $\cdot$ hour) | $\mu\text{mol of C}_2\text{H}_4 /$<br>(g of nodules $\cdot$ hour) | $\mu\text{mol of C}_2\text{H}_4 /$<br>(plant $\cdot$ hour)         | $\mu\text{mol of C}_2\text{H}_4 /$<br>(g of nodules $\cdot$ hour)       |
| 2  | Rhizobia + water  | 1.190 $\pm$ 0.265                                          | 8.397 $\pm$ 0.792                                                 | 5.870 $\pm$ 0.792                                                  | 8.059 $\pm$ 0.694                                                       |
| 3  | Rhizobia + GlcNAc | 1.996 $\pm$ 0.274*                                         | 6.880 $\pm$ 0.636                                                 | 6.278 $\pm$ 0.537                                                  | 8.656 $\pm$ 0.634                                                       |
| No | Variant           | <i>Almaz variety, soil culture</i>                         |                                                                   |                                                                    |                                                                         |
|    |                   | <i>Developmental phase of three trifoliolate leaves</i>    |                                                                   |                                                                    |                                                                         |
|    |                   | NGA of symbiotic system                                    |                                                                   | NGA of morpho-structural symbiotic unit                            |                                                                         |
|    |                   | $\mu\text{mol of C}_2\text{H}_4 /$<br>(plant $\cdot$ hour) | $\mu\text{mol of C}_2\text{H}_4 /$<br>(g of nodules $\cdot$ hour) | nmol of C <sub>2</sub> H <sub>4</sub> /<br>(1 nodule $\cdot$ hour) | nmol of C <sub>2</sub> H <sub>4</sub> /<br>(1 nodule mass $\cdot$ hour) |
| 2  | Rhizobia + water  | 0.119 $\pm$ 0.022                                          | 3.795 $\pm$ 0.377                                                 | 8.716 $\pm$ 1.130                                                  | 51.522 $\pm$ 8.136                                                      |
| 3  | Rhizobia + GlcNAc | 0.198 $\pm$ 0.046*                                         | 4.414 $\pm$ 0.899                                                 | 12.320 $\pm$ 2.745                                                 | 74.824 $\pm$ 17.537                                                     |

**Table 3. Nitrogenase activity of the rhizospheric microbiota of soybean Almaz variety under the influence of inoculant based on nodule bacteria and glucose-containing aminosaccharide (0.01 M)**

| No | Variant             | Nitrogenase activity,<br>$\text{nmol of C}_2\text{H}_4 / (20 \text{ g of soil} \cdot 2 \text{ hours})$ |                          |                            |
|----|---------------------|--------------------------------------------------------------------------------------------------------|--------------------------|----------------------------|
|    |                     | <i>Phase of trifoliolate leaves</i>                                                                    |                          |                            |
|    |                     | <i>one leaf (roots + soil)</i>                                                                         | <i>two leaves (soil)</i> | <i>three leaves (soil)</i> |
| 1  | Without inoculation | $12.650 \pm 0.502$                                                                                     | $4.187 \pm 0.459$        | $11.490 \pm 0.734$         |
| 2  | Rhizobia + water    | $19.773 \pm 4.086$                                                                                     | $6.352 \pm 0.230$        | $11.774 \pm 0.617$         |
| 3  | Rhizobia + GlcNAc   | $15.133 \pm 0.925$                                                                                     | $6.841 \pm 0.230^*$      | $12.175 \pm 0.211$         |

Inoculation of seeds with rhizobia activated with glucosamine had no significant effect on the formation of soybean plants vegetative mass (Table 5). At the same time, in the phase of one trifoliolate leaf (soil culture), no significant differences in the formation of vegetative mass of plants were observed, which is due to the same level of their nitrogen nutrition (the nutrient mix of Gelrigel's contained 0.25 norms of mineral nitrogen) in the absence of functioning of the symbiotic apparatus (Table 2). However, in bacterized plants, rooting was much more intense (by 1.4–1.8 times) (Table 5).

Glucosamine-activated nodule bacteria stimulated the formation of the soybean root system compared to rhizobia inoculation by 32%. In the phase of three trifoliolate leaves the symbiotic soybean system fixed nitrogen (Table 2), which was accompanied by as more active formation of plant green mass (1.3 times) and root (1.2–1.3 times). The difference between the weight of soybean

plants in the variants with seed inoculation with rhizobia and the rhizobia + glucosamine composition in the presence of a positive trend was not significant. There is also no significant difference in the accumulation of vegetative mass of soybean Lisabon variety of these variants in the conditions of sand culture.

Thus, glucosamine-modified rhizobia did not have a reliably pronounced positive effect on the vegetative mass formation by the plants during the soybean vegetation, but activated the seeds output from the resting state and their germination energy at the initial stages of ontogenesis.

When assessing the physiological indicators of wheat development, a positive effect of azotobacter modified with glucosamine (0.1 M) was found in comparison with the variant of seed inoculation with azotobacter (variant No 3 compared to variant No 2, Table 6, 7). It was shown (Table 6) that bacterization of wheat seeds with strain *A. chroococcum* T79 promoted the accumulation of green photosynthetic

Table 4. Dynamics of soybean seeds germination under pot experiments by the action of the inoculant based on *Bradyrhizobium japonicum* 634b and N-acetyl-D-glucosamine (0.01 M)

| No                  | Variant                        | Number of seedlings per pot                                  |                | Seeds germination   | Number of seedlings per pot |                     | Seeds germination | Number of seedlings per pot |                      | Seeds germination |  |
|---------------------|--------------------------------|--------------------------------------------------------------|----------------|---------------------|-----------------------------|---------------------|-------------------|-----------------------------|----------------------|-------------------|--|
|                     |                                | pieces                                                       | % <sup>2</sup> | %                   | pieces                      | %                   | pieces            | %                           | pieces               | %                 |  |
|                     |                                | Lisabon variety, sand culture, sowing 18.05.17, 20 seeds/pot |                |                     |                             |                     |                   |                             |                      |                   |  |
| Day after sowing    |                                |                                                              |                |                     |                             |                     |                   |                             |                      |                   |  |
| the 7 <sup>th</sup> |                                |                                                              |                | the 8 <sup>th</sup> |                             |                     |                   | the 11 <sup>th</sup>        |                      |                   |  |
| 1                   | Without inoculation            | 3.4 ± 1.0                                                    | 100            | 17 ± 5.0            | 4.8 ± 1.2                   | 100                 | 24 ± 6.1          | 12.6 ± 1.5                  | 100                  | 63 ± 7.3          |  |
| 2                   | Rhizobia + water               | 5.2 ± 1.3                                                    | 153/100        | 26 ± 6.3            | 7.4 ± 1.6                   | 154/100             | 37 ± 7.9          | 13.8 ± 1.2                  | 110/100              | 69 ± 5.9          |  |
| 3                   | Rhizobia + GlcNAc <sup>1</sup> | 10.4 ± 1.1 <sup>*3</sup>                                     | 306/200        | 52 ± 5.7*           | 13.8 ± 1.0*                 | 288/186             | 68 ± 4.7          | 14.4 ± 0.8                  | 114/104              | 72 ± 4.1          |  |
| No                  | Variant                        | Almaz variety, soil culture, sowing 11.05.18, 20 seeds/pot   |                |                     |                             |                     |                   |                             |                      |                   |  |
|                     |                                | Day after sowing                                             |                |                     |                             |                     |                   |                             |                      |                   |  |
|                     |                                | the 6 <sup>th</sup>                                          |                |                     |                             | the 7 <sup>th</sup> |                   |                             | the 10 <sup>th</sup> |                   |  |
| 1                   | Without inoculation            | 2.9 ± 0.7                                                    | 100            | 14.4 ± 3.8          | 4.6 ± 0.8                   | 100                 | 23.1 ± 3.9        | 7.3 ± 0.6                   | 100                  | 36.3 ± 3.0        |  |
| 2                   | Rhizobia + water               | 4.9 ± 1.4                                                    | 169/100        | 24.4 ± 7.2          | 7.6 ± 1.2                   | 165/100             | 38.1 ± 5.8        | 11.5 ± 0.9                  | 156/100              | 57.4 ± 4.5        |  |
| 3                   | Rhizobia + GlcNAc              | 5.9 ± 1.1                                                    | 203/120        | 29.4 ± 5.5          | 8.6 ± 1.1                   | 187/113             | 43.1 ± 5.6        | 10.4 ± 0.9                  | 143/90               | 51.9 ± 4.7        |  |

Notes: 1 — GlcNAc — N-acetyl-D-glucosamine (0.01M); 2 — % — relative to control; before the line — absolute (without inoculation, variant No 1), behind the line — the rhizobia strain (variant No 2); 3 — \* — positively significant ( $P \leq 0.05$ ) for strain control (variant No 2).

chlorophyll pigments in plant leaves, which may indicate the activation of photosynthetic processes in plants. At the beginning of wheat vegetation (seedling development phase) and during the heading phase, the level of chlorophyll in the leaves of the plants of this variant significantly (by 10%) exceeded the indicator of unbacterized plants. The significant difference between the variants with inoculation of seeds with azotobacter and composition of azotobacter with glucosamine in the content of chlorophyll in wheat leaves was 13, 11 and 4% respectively to the investigated phases of vegetation. The difference with control (the variant of seed treatment with water) was the largest (from 11 to 24%). It was noted that the composition manifested the maximal activating effect (13%) at early stage of plant ontogenesis (the stage of seedling development).

Thus, one of the manifestations of the activating effect of glucosamine on bacterial cells in association with plants formation was the intense accumulation of chlorophyll in the leaves of wheat. At the same time, an increase in the nitrogen-fixing ability of rhizospheric diazotrophic microorganisms (Table 6) was also associated with it. The nitrogen-fixing activity of the microbiota in the variant azotobacter + glucosamine significantly exceeded (by 10 and 38%, respectively, in the phases of tillering and heading of wheat) the indicators in the variant with inoculation of seeds with bacterial strain.

Wheat plants in associations with glucosamine-modified bacteria more actively formed a vegetative mass, however, only a positive tendency in the change of this indicator was observed (Table 6). As with the inoculation of soybean seeds with the rhizobia + glucosamine composition

Table 5. Formation of soybean plants vegetative mass by seed inoculation with rhizobia modified by glucosamine

| No | Variant             | Vegetative mass of plants, g<br>(aboveground part — AGP, root — R) |              |                                                       |             |
|----|---------------------|--------------------------------------------------------------------|--------------|-------------------------------------------------------|-------------|
|    |                     | AGP                                                                | R            | AGP                                                   | R           |
|    |                     | <i>Lisabon variety, sand culture</i>                               |              |                                                       |             |
|    |                     | <i>Flowering phase</i>                                             |              | <i>Full pod phase</i>                                 |             |
| 1  | Without inoculation | 3.92 ± 0.64                                                        | 3.32 ± 0.46  | 5.68 ± 0.32                                           | 4.11 ± 0.31 |
| 2  | Rhizobia + water    | 4.33 ± 0.63                                                        | 3.31 ± 0.25  | 10.26 ± 0.89                                          | 5.60 ± 0.74 |
| 3  | Rhizobia + GlcNAc   | 4.60 ± 0.37                                                        | 2.30 ± 0.18  | 10.68 ± 0.85                                          | 5.46 ± 0.49 |
| No | Variant             | <i>Almaz variety, soil culture</i>                                 |              |                                                       |             |
|    |                     | <i>Development phase of one trifoliolate leaf</i>                  |              | <i>Development phase of three trifoliolate leaves</i> |             |
|    |                     |                                                                    |              |                                                       |             |
| 1  | Without inoculation | 1.46 ± 0.23                                                        | 0.14 ± 0.03  | 4.60 ± 0.98                                           | 2.01 ± 0.27 |
| 2  | Rhizobia + water    | 1.57 ± 0.11                                                        | 0.19 ± 0.03  | 5.82 ± 0.16                                           | 2.36 ± 0.35 |
| 3  | Rhizobia + GlcNAc   | 1.45 ± 0.16                                                        | 0.25 ± 0.05* | 6.05 ± 0.36                                           | 2.56 ± 0.28 |

Table 6. Chlorophyll content in spring wheat leaves, vegetative mass formation by plants, and nitrogen-fixing ability (NFA) of the rhizospheric microbiota by the seed inoculation with 0.1 M glucosamine-modified azotobacter

| No <sup>1</sup> | Phase of plant development                   |                 |                                |                                                             |                                |                                                          |                             |                           |                |                 |                  |
|-----------------|----------------------------------------------|-----------------|--------------------------------|-------------------------------------------------------------|--------------------------------|----------------------------------------------------------|-----------------------------|---------------------------|----------------|-----------------|------------------|
|                 | seedling                                     | tillering       | beginning<br>of the<br>heading | tillering                                                   | beginning<br>of the<br>heading | tillering                                                | beginning of<br>the heading | full ripeness of<br>seeds |                |                 |                  |
|                 | Chlorophyll content,<br>mg/g of fresh leaves |                 |                                | NFA, nmol of C <sub>2</sub> H <sub>4</sub><br>/ (plant · h) |                                | Vegetative mass, g<br>(aboveground part — AGP, root — R) |                             |                           |                |                 |                  |
|                 |                                              |                 |                                |                                                             |                                | AGP                                                      | R                           | AGP                       | R              | AGP             | R                |
| 1               | 1.15 ±<br>0.01                               | 1.78 ±<br>0.02  | 2.08 ±<br>0.05                 | 2.00 ±<br>0.15                                              | 1.03 ±<br>0.12                 | 1.90 ±<br>0.09                                           | 0.57 ±<br>0.02              | 7.07 ±<br>0.34            | 2.28 ±<br>0.11 | 4.11 ±<br>0.04  | 1.45 ±<br>0.04   |
| 2               | 1.27 ±<br>0.01^                              | 1.78 ±<br>0.04  | 2.28 ±<br>0.04^                | 4.24 ±<br>0.22^                                             | 2.56 ±<br>0.38^                | 2.09 ±<br>0.12^                                          | 0.62 ±<br>0.03              | 7.96 ±<br>0.40            | 2.26 ±<br>0.13 | 4.42 ±<br>0.07^ | 1.52 ±<br>0.07   |
| 3               | 1.43 ±<br>0.01^* <sup>2</sup>                | 1.98 ±<br>0.07* | 2.37 ±<br>0.02^*               | 4.68 ±<br>0.29^                                             | 3.53 ±<br>0.27^*               | 2.14 ±<br>0.08^                                          | 0.69 ±<br>0.04^             | 7.99 ±<br>0.30^           | 2.59 ±<br>0.23 | 4.51 ±<br>0.09^ | 1.70 ±<br>0.08^* |

Notes. Hereinafter:

1. No — variant number: 1 — without inoculation, seed treatment with water (absolute control); 2 — inoculation seeds with *A. chroococcum* T79 (T79, control strain); 3 — inoculation seeds with *A. chroococcum* T79, preincubated with N-acetyl-D-glucosamine 0.1M (T79 + GlcNAc, composition);

2. <sup>^</sup> — positively significant ( $P \leq 0.05$ ) compared to variant No 1 (absolute control); \* — positively significant ( $P \leq 0.05$ ) compared to variant No 2 (control strain).

**Table 7. Formation of spring wheat crop during inoculation of seeds with glucosamine(0.1 M)-modified azotobacter**

| Crop structure parameters  |      | Variant (seed inoculation) |                           |                           |
|----------------------------|------|----------------------------|---------------------------|---------------------------|
|                            |      | Without inoculation        | T79                       | T79+GlcNAc                |
| Spike length               | cm   | $7.0 \pm 0.0$              | $7.1 \pm 0.0^{\wedge}$    | $7.2 \pm 0.0^{*}$         |
| Spike weight               | g    | $2.53 \pm 0.04$            | $2.74 \pm 0.06^{\wedge}$  | $2.78 \pm 0.04^{\wedge}$  |
| Grains number in the spike | psc. | $31.4 \pm 1.3$             | $34.0 \pm 0.7^{\wedge}$   | $33.6 \pm 0.6^{\wedge}$   |
| Grains mass in the spike   | g    | $1.06 \pm 0.03$            | $1.19 \pm 0.04^{\wedge}$  | $1.22 \pm 0.04^{\wedge}$  |
| Grain yield from a pot     | g    | $15.84 \pm 0.47$           | $17.20 \pm 0.56^{\wedge}$ | $18.19 \pm 0.28^{*}$      |
| Weight of 1000 grains      | g    | $39.31 \pm 0.18$           | $40.79 \pm 0.52^{\wedge}$ | $42.06 \pm 0.77^{\wedge}$ |
| Index harvest              | ih   | $0.26 \pm 0.01$            | $0.27 \pm 0.00$           | $0.28 \pm 0.01$           |

**Table 8. Structure of soybean crop during bacterization seeds by composition of rhizobia and glucosamine**

| Soybean crop structure                           |           | Variant (presowing seed inoculation) |                                 |                             |                                 |                                          |                                |
|--------------------------------------------------|-----------|--------------------------------------|---------------------------------|-----------------------------|---------------------------------|------------------------------------------|--------------------------------|
|                                                  |           | Rhizobia (control)                   | Rhizobia + glucosamine (0,01 M) | Rhizobia (control)          | Rhizobia + glucosamine (0,01 M) | Rhizobia (control)                       | Rhizobia + glucosamine (0,1 M) |
|                                                  |           | Pot experiments                      |                                 |                             |                                 | Field conditions, small-scale experiment |                                |
|                                                  |           | Lisabon variety, sand culture        |                                 | Almaz variety, soil culture |                                 | Lisabon variety, soil culture            |                                |
|                                                  |           |                                      |                                 |                             |                                 |                                          |                                |
| Number of beans, pcs.                            | per plant | $6.8 \pm 0.3$                        | $7.9 \pm 0.5^{*}$               | $15.0 \pm 0.6$              | $14.7 \pm 0.5$                  | $5.2 \pm 0.3$                            | $5,9 \pm 0,3^{*}$              |
| Number of internodes, pcs.                       |           | $5.8 \pm 0.3$                        | $6.6 \pm 0.3^{*}$               | $9.5 \pm 0.7$               | $8.4 \pm 0.4$                   | $4.5 \pm 0.2$                            | $4,9 \pm 0,2$                  |
| Weight of beans, g                               |           | $4.04 \pm 0.03$                      | $4.56 \pm 0.35^{*}$             | — <sup>1</sup>              | —                               | —                                        | —                              |
| Number of seeds, pcs.                            |           | $16.0 \pm 0.5$                       | $17.2 \pm 1.4$                  | $29.5 \pm 1.1$              | $29.3 \pm 1.3$                  | $9.8 \pm 0.8$                            | $11,6 \pm 0,8$                 |
| Weight of seeds, g                               |           | $2.96 \pm 0.05$                      | $3.38 \pm 0.25^{*}$             | $5.54 \pm 0.10$             | $5.67 \pm 0.19$                 | $1.73 \pm 0.13$                          | $2,07 \pm 0,14^{*}$            |
| Weight of seeds/pot, g<br>Harvest, kg/ha (field) |           | $17,80 \pm 0,31$                     | $20.25 \pm 1.50^{*}$            | $33.21 \pm 0.61$            | $34.09 \pm 1.08$                | $33.4 \pm 1.0$                           | $43.0 \pm 2.2^{*}$             |
| Weight of 1000 seeds, g                          |           | $187.17 \pm 4.88$                    | $199.20 \pm 0.80^{*}$           | $199.4 \pm 4.3$             | $205.0 \pm 5.1$                 | $183.1 \pm 8.4$                          | $191.6 \pm 4.7$                |
| Plant weight, g                                  |           | $6.80 \pm 0.10$                      | $7.53 \pm 0.85$                 | $10.09 \pm 0.18$            | $10.68 \pm 0.19$                | —                                        | —                              |
| Index harvest, ih                                |           | $0.44 \pm 0.01$                      | $0.45 \pm 0.02$                 | $0.55 \pm 0.01$             | $0.53 \pm 0.02$                 | —                                        | —                              |

Notes: “—<sup>1</sup>” — not defined.

(Table 5) as well as the bacterization of wheat seeds with the azotobacter + glucosamine composition (Table 6), a more pronounced effect of the composition on the development of the root system of plants — the habitat and life place of rhizospheric microbiota [15].

Thus, in the formation and functioning of associative systems of wheat plants with bacteria *A. chroococcum* T79, the manifestation of glucosamine regulatory activity toward bacterial cells *in situ* was the activation of the functional capacity of phyto-bacterial system — the nitrogenase activity of the microbiota and the growth-activating ability of the soil [18], as well as the increase in the level of chlorophyll in wheat leaves (Table 6).

Wheat plants in association with bacteria *A. chroococcum* T79 were characterized by a higher level of productive potential realization in comparison with unbacterized plants (Table 7), which was manifested in an increase in the indicators of spike mass (by 8%), the number and weight of grains in the spike (by 8 and 12%, respectively), grain yield from the pot (by 9%) and mass of 1000 grains (by 4%). Azotobacter, in combination with glucosamine, formed an association with wheat plants, with significantly higher productivity than unbacterized plants: by 3 and 10% (length and weight of the spike), by 7 and 15% (number and weight of grains in the spike), by 15% (grain yield from the pot), by 7% (weight of 1000 grains). The comparing variants when seeds are inoculated with azotobacter only and glucosamine-modified azotobacter, the latter showed a positive tendency to increase the indexes of the wheat yield structure, whereas a significant difference was obtained only in the index “grain yield from the pot”. In this case, the maximal value of the crop index was noted in the variant with the use the composition azotobacter + glucosamine for the inoculation of seeds (Table 7).

Analysis of the soybean yield structure also confirmed the positive effect of the glucosamine-containing inoculant on the formation of generative organs (beans) and seeds by plants (Table 8), which was particularly pronounced (significant difference with control) in the conditions of soybean cultivation in sand culture. In the seed inoculation with rhizobia + glucosamine (0.01M) variant, the plants formed internodes and beans by 14% and 16% more than in the seed inoculation with glucosamine-unmodified rhizobia variant. The mass of beans thus exceeded the control by 13%. Indicators of the

number and weight of seeds per plant were by 8 and 14% higher than in the control when the mass of 1 000 seeds was significantly higher (by 6%) than the control index.

The seed yield was significantly (by 14%) higher than the crop formed by the plants when seeds were inoculated only with nodule bacteria, which indicates a more complete realization of the seed potential of soybean-rhizobial symbiosis formed by soybean plants and glucosamine-modified rhizobia.

In soil culture, no significant differences in the parameters of soybean crop structure of the Almaz variety were obtained, and only a positive trend was observed in terms of “seed mass from plant”, “seed mass from pot”, “mass of 1000 seeds” and “plant mass” (Table 8). A reliable yield increase (29%) of Lisabon soybean seeds in the variant with the use of rhizobia + glucosamine (0.1M) composition was obtained in the small-scale field experiment with manual harvesting and 2.5 norms of seed sowing. At the same time, the number of beans per plant (by 13%) and the weight of seeds per plant (by 20%) changed positively, whereas for the indicators of “number of internodes per plant”, “number of seeds per plant” and “mass of 1000 seeds”, only a positive trend of 9, 18 and 5%, respectively, was noted (Table 8).

The results obtained by us (Tables 1, 2, 6) indicate the regulatory (activating) action of N-acetyl-D-glucosamine by co-incubation with cells of the nitrogen-fixing bacteria *B. japonicum* 634b and *A. chroococcum* T79. This was manifested in a significant and reliable increase of the level of the symbiotic potential realization (nodulation and nitrogenase activity of rhizobia in the formation of soybean-rhizobial symbiosis and nitrogen-fixing ability of the rhizospheric microbiota of wheat in the formation of wheat-azotobacter association). The increase in the level of nitrogen-fixing ability of diazotrophic microorganisms may be explained by the activating effect of carbohydrate substances on the functioning of bacterial nitrogenase. Thus, it was established in nodules bacteria of lupine *in vitro* [31], that the carbohydrate fraction of metabolites of root nodules increased the nitrogen-fixing activity of rhizobia. Under *in situ* conditions, when studying the effect of additives of artificial root exudates and individual saccharides as carbon sources on the activity and qualitative composition of nonsymbiotic diazotrophs, it was shown [17] that only those substances that contained a carbohydrate component induced nitrogen fixation in rhizosphere. The

ability of bacterial carbohydrate-containing exometabolites and exopolysaccharides, which are specific for rhizobia culture used as inoculant [2], as well as lipopolysaccharides and glucans [12, 13], when acting on cells of nodule bacteria, increase the level of symbiotic potential realization of legume-rhizobial systems. The ability to enhance the functioning of the nitrogenase complex of alfalfa root nodules during incubation of alfalfa seedlings with glucan of specific rhizobia and their further inoculation with these bacteria was established [12].

Thus, the results obtained by us (Tables 2, 6 [2, 18]) as well as the works of other researchers [12, 13, 17] indicate the inductive activity of carbohydrate substances with respect to the functional activity of soil diazotrophic symbiotic and free-living microorganisms, as in symbiosis with plants and in plant-microbial associations.

Our findings showed (Table 2) in the phase of full pod of soybean, the reduction to the control value of nitrogen fixation ability of the symbiotic system formed by glucosamine-modified rhizobia may be due to the small (at the control level) mass of root nodules in plants of this variant, since for soybean a direct positive relationship between the intensity of nitrogen fixation and the mass of root nodules on the plant was established [32].

Thus, more active process of nitrogen fixation in both soybean root nodules (Table 2) and in the rhizosphere of wheat (Table 6) in the variant with seed inoculation with the composition of diazotrophic bacteria modified by glucosamine also provides a higher level of nitrogen nutrition of plants, which in turn intensifies the synthesis of green pigments chlorophylls (Table 6) and photosynthetic activity of plants, resulting in an increase in the amount of photoassimilates required for plant metabolism, formation of vegetative (Tables 5, 6) and grain (Tables 7, 8) productivity of crops.

One of the manifestations of the activating effect of glucosamine on bacterial cells of *azotobacter* during the formation of association with wheat plants was the intensive accumulation of chlorophyll in the leaves (Table 6), which may be associated not only with the improvement of nitrogen nutrition of plants due to symbiotrophic nitrogen of phyto-bacterial system, but also, probably, with a change in the level and balance of endogenous hormones in plant leaves under the influence of exogenous glucosamine or glucose-containing regulatory substances [12, 33]. Due to the fact

that the level of hormones of the cytokinin nature in plants controls chlorophyll content and photosynthetic activity of plants [34], it can be assumed that activation of the process of chlorophyll accumulation in the leaves of wheat, the variant with seed inoculation with the composition, is related to the additional effect of glucosamine on the hormone of cytokinin nature content. We showed [33] that, when treating wheat seeds with glucosamine (0.1 M), the level of cytokinin nature hormones zeatin and zeatin riboside in the leaves of vegetative plants in bumping stage of wheat increased 2.0 and 2.5 times, respectively. The activating effect of seed bacterization on the level of chlorophyll in the leaves can also be explained by the ability of bacteria of the genus *Azotobacter* to synthesize hormones of cytokinin nature [35–37].

N-acetyl-D- glucosamine is a derivative of glucose as well as bacterial glucans (cyclic glucose polymers with  $\beta(1,2)$ -,  $\beta(1,3)$ -,  $\beta(1,6)$ -bonds) are a product of targeted synthesis of bacterial cells. Glucans of alfalfa nodule bacteria, when exogenous act on rhizobia, have the ability to regulate positively the synthesis of hormones by bacteria. It was shown [12] that under the influence of a specific glucan on alfalfa nodule bacteria with subsequent inoculation of alfalfa seedlings with them, there is a significant increase in the level of peroxidase activity in leaves and roots of plants (by 27 and 22%, respectively). On this basis, the authors suggest that glucan activates the hormones synthesis by bacterial cells, in particular indolyl-acetic acid (IAA), as well as their ability to stimulate plant growth through this mechanism.

More active development of the root system of plants, both soybean and wheat, under the influence of seed bacterization with diazotrophic microorganisms (Tables 5, 6) may be due to the ability of bacteria of the genera *Bradyrhizobium* and *Azotobacter* to synthesize hormones of auxin nature [35–38]. Advantages in the formation of the root system of plants of the variant with seed treatment with glucosamine-activated microorganisms, as compared to inoculation with a bacterial strain, may be caused by the additional action of aminosachharide on the content of endogenous auxins in plants. When treating wheat seeds with a solution of glucosamine (0.1M), we noted an increase in the level of IAA 3.2 times in the leaves of vegetative plants of wheat, as well as a shift in the balance of hormones of cytokinin and auxin nature in the direction of increasing the proportion of IAA

(1:2.9 in the experiment as compared with 1:2.2 in control) [33].

So, peculiarities of formation and functioning of symbiotic systems of early ripening varieties of soybean domestic (Almaz) and foreign (Lisabon) breeding under conditions of pot experiments with sandy and soil cultures by inoculation of seeds with glucosamine-modified nodule bacteria, unlike its bacterization with only rhizobia, there is a higher level of realization of the nodulation (nodule-forming) ability of rhizobia (plants were actively infected, more nodules were formed with their greater total mass per plant and the mass of one root nodule), as well as the nitrogen-fixing activity of symbiosis and the functional ability of each morpho-structural symbiotic unit. This is a prerequisite for improved nitrogen nutrition of soybean plants and the realization of their biological and seed productivity. Thus, acetylated aminosaccharide N-acetyl-D-glucosamine is an effective regulatory agent that has a positive effect on the ability of nodule bacteria to form a symbiotic apparatus on the roots of soybean plants, its active functioning, and the formation of productive symbiotic systems.

In the association of wheat plants with glucosamine-modified azotobacter, the activation of the basic physiological processes — the nitrogen-fixing ability of the rhizospheric microbiota and the synthesis

of chlorophylls in the leaves (photosynthetic activity of plants) provided a higher level of realization of the productive potential of this phito-bacterial system in comparison with both non-bacterial control and variant of seed inoculation with a strain of azotobacter.

### Conclusions

The use of glucose-containing amino-saccharide N-acetyl-D-glucosamine as an additional environmentally friendly natural agent in inoculants with diazotrophic microorganisms (nodule bacteria of the soybean and microorganisms of the genus *Azotobacter*) contributes to a fuller realization of the symbiotic and productive potential of symbioses and associations when compared to using a bacterial strain. The presented here results indicate the possibility of practical application of N-acetyl-D-glucosamine as an exogenous regulatory agent of a carbohydrate nature in the creation of complex inoculants based on nitrogen-fixing bacteria to improve the productivity of phyto-bacterial symbioses and associations.

The work was funded as the budget topic “To study the reaction of legume-rhizobial symbiosis to the action of biotic and abiotic factors” (0115U007217) (departmental topics of the National Academy of Sciences of Ukraine, 2016-2020).

### REFERENCES

1. Isobe K., Ohte N. Ecological Perspectives on Microbes Involved in N-cycling. *Microbes Environ.* 2014, 29 (1), 4–16. <https://doi.org/10.1264/jsme2.ME13159>
2. Kyrychenko E. V. Crop Biotechnology. Nikolaev: Ilion. 2014, 436 p. (In Russian).
3. Chebotar V. K., Malfanova N. V., Shcherbakov A. V., Ahtemova G. A., Borisov A. Y., Lugtenberg B., Tikhonovich I. A. Endophytic Bacteria in Microbial Preparations That Improve Plant Development (Review). *Applied Biochem. Microbiol.* 2015, 51 (3), 271–277. <https://doi.org/10.1134/S0003683815030059>
4. Shiro S., Matsuura S., Rina Saiki G., Sigue C., Yamamoto A., Umehara Y., Hayashi M., Saeki Y. Genetic Diversity and Geographical Distribution of Indigenous Soybean-Nodulating *Bradyrhizobia* in the United States. *Appl. Environ. Biol.* 2013, 79 (12), 3610–3618. <https://doi.org/10.1128/AEM.00236-13>
5. Kyrychenko O. V. Market Analysis and Microbial Biopreparations Creation for Crop Production in Ukraine. *Biotechnol. acta.* 2015, 8 (4), 40–52. <https://doi.org/10.15407/biotech8.04.040>
6. Kiriziy D. A., Vorobey N. A., Kots S. Ya. The Relationship of Nitrogen Fixation and Photosynthesis as the Main Components of the Production Process in Alfalfa. *Russ. Plant Physiol.* 2007, 54 (5), 666–671. (In Russian).
7. Beneduzi A., Ambrosini A., Passaglia L. M. P. Plant-Growth-Promoting Rhizobacteria (PGPR): Their Potential as Antagonists and Biocontrol Agents. *Genet. Mol. Biol.* 2012, 35 (Suppl. 4), 1044–1051. [www.scielo.br/pdf/gmb/v35n4s1/20.pdf](http://www.scielo.br/pdf/gmb/v35n4s1/20.pdf)
8. Trivedi P., Pandey A., Palni L. M. S. Bacterial Inoculants for Field Applications under Mountain Ecosystem: Present Initiatives and Future Prospects. In: *Bacteria in Agrobiological Plant Probiotics*; Ed. D. K. Maheshwari. Springer Berlin Heidelberg. 2012, P. 15–44. [https://doi.org/10.1007/978-3-642-27515-9\\_2](https://doi.org/10.1007/978-3-642-27515-9_2)
9. Djordjevic M., Mohd Radzman N., Imin N. Small-Peptide Signals That Control Root Nodule Number, Development and Symbiosis.

- J. Exp. Bot.* 2015, 66 (17), 5171–5181. <https://doi.org/10.1093/jxb/erv357>
10. Chebotar V. K., Raphalskiy S. V., Aritkin A. G., Esion V. V. Efficiency of Combined Application of Microbiological Means for Soybean Cultivation. *Dostizheniya nauki i tekhniki. APK.* 2013, N 8, P. 23–25. (In Russian).
  11. Jabbarova D. P., Egamberdieva D., Davranov K., Jabbarov M. P. Restoration of Growth of Soybean Under Saline Conditions Due to Inoculation with *Bradyrhizobium japonicum* and *Pseudomonas putida*. *Uzbek. Biol. J.* 2013, N 5, P. 23–26.
  12. Kosenko L. V., Mykhalkiv L. M., Krugova E. D., Mandrovskaya N. M., Zatovskaya T. V., Kots S. Ya. Biological Activity of *Sinorhizobium meliloti* Glucan. *Rus. J. Microbiol.* 2003, 72 (5), 633–638. (In Russian).
  13. Antipchuk A. F., Kosenko L. V. The Influence of Lipopolysaccharides and Glucans From Two *Rhizobium leguminosarum* bv. *viciae* Strains on the Formation and Efficiency of Their Symbioses With Pea Plants. *Rus. J. Microbiol.* 2004, 73 (1), 51–55. (In Russian).
  14. Laus M. C., Logman T. J., Lamers, G. E., Van Brussel A. A., Carlson R. W., Kijne J. W. A Novel Polar Surface Polysaccharide from *Rhizobium leguminosarum* Binds Host Plant Lectin. *Mol. Microbiol.* 2006, 59 (6), 1704–1713. <https://doi.org/10.1111/j.1365-2958.2006.05057.x>
  15. Mc Near Jr. D. H. The Rhizosphere. Roots, Soil and Everything in Between. *Nat. Educ. Knowl.* 2013, 4 (3), 1–20. <http://www.nature.com/scitable/knowledge/library/the-rhizosphere-roots-soiland-67500617>
  16. Kyrychenko O. V. Study of Chemotaxis of Nodule Bacteria of Lupin to Organic Substances. *Mikrobiol. Zh.* 2005, 67 (3), 19–26. (In Russian).
  17. Bürgmann H., Meier S., Bunge M., Widmer F., Zeyer J. Effects of Model Root Exudates on Structure and Activity of a Soil Diazotroph Community. *Environ. Microbiol.* 2005, 7 (11), 1711–1724. <https://doi.org/10.1111/j.1462-2920.2005.00818.x>
  18. Kyrychenko O. V. Biological Activity of Rhizosphere Soil Spring Wheat in Association with Bacteria *Azotobacter chroococcum* T79 Modified N-acetyl-D-glucoseamine. *Microbiol. and Biothechnol.* 2016, N 3, 30–42. (In Russian). [http://dx.doi.org/10.18524/2307-4663.2016.3\(35\).77955](http://dx.doi.org/10.18524/2307-4663.2016.3(35).77955)
  19. Antypchuk A. F., Pilyashenko-Novokhatniy A. I., Evdokimenko T. M. Practicum on Microbiology. *Kyiv: Manual. University “Ukraine”.* 2011, 156 p. (In Ukrainian).
  20. Sadovnikova Yu. N. Features of Cellular Responses of *Azospirillum brasilense* to the Interaction of Stress Factors and Wheat Lectin: *Author. dis. ... cand. biol. sciences on a specialty 03.00.04 — biochemistry, 03.00.07, microbiology, Yu. N. Sadovnikova; Institute of Biochemistry and Physiology of Plants and Microorganisms RAS, Saratov.* 2009, 23 p. (In Russian).
  21. Denarie J., Maillet F., Poinso V., Andre O., Becard G., Gueunier M., Cromer L., Haouy A., Giraudet D. Lipohitooligosaccharides Stimulating Arbuscular Mycorrhizal Symbiosis. *Pat. W0 2010/049751 A1, W0 2010/049817 A2, W0 2010/049817 A3, CN 102215669 A, EP 2358194 A2, US 2011/0301032.* France, Institute National De La Recherche Agronomique. Filing date 28.10.2009 (N CA 2740082); Pub. date 06.05.2010 (N CA 2740082 A1).
  22. Djordjevic M. A., Susanti A. B., Marmuse L., Driguez H., Samain E., Vauzeilles B., Beau J.-M., Kordbacheh F., Rolfe B. G., Schwörer R., Daines A. M., Gresshoff P. M., Parish Ch. R. Lipo-Chitin Oligosaccharides, Plant Symbiosis Signalling Molecules That Modulate Mammalian Angiogenesis *In Vitro*. *PLoS ONE (Public Library of Science)*. 2014, 9 (12), 112635. <https://doi.org/10.1371/journal.pone.0112635>
  23. Iutynska G. O., Biliavska L. O., Kozyrytska V. E. Averkom-Nova Phyto-protective Biological Preparation for Plant Treatment. *Pat. UA N 107972 C2, A 01N 63/02, A 01P 3/00, A 01P 5/00, C12N 1/20, C12 R 1/465.* Ukraine, Institute of Microbiology and Virology im. Zabolotnoho NAS of Ukraine. Filing date 07.02.2013 (N a 2013 01463); Pub. date 10.03.2015. Bull. N 5 (N 107972 C2). (In Ukrainian).
  24. Kyrychenko O. V. The Effect of Glucose- and Galactose-Containing Monosaccharides on *In Vitro* Development of *Bradyrhizobium japonicum*. *Silskohospodarska mikrobiologia.* 2017, N 25, P. 3–9. (In Ukrainian).
  25. Kyrychenko O. V. Change of the Wheat Lectin Activity and Degree of Its Interaction With Different Components of Compositions of Lectin Nature. *Ukr. Biochem. J.* 2006, 78 (6), 105–112. (In Ukrainian).
  26. Grodzinskiy A. M. Allelopatiya Rasteniy i Pochvoutomlenie: Izbr. Tr. *Kyiv: Naukova dumka.* 1991, 432 p. (In Russian).
  27. State Register of Plant Varieties Suitable for Dissemination in Ukraine in 2018. *Kyiv: Ministry of Agrarian Policy and Food of Ukraine.* 447 p. [www.sops.gov.ua/uploads/page/5aa63108e441e.pdf](http://www.sops.gov.ua/uploads/page/5aa63108e441e.pdf) (In Ukrainian).
  28. Hardy R. W. F., Burns R. C., Holsten R. D. Application of the Acetylene-Ethylene Assay for Measurement of Nitrogen Fixation. *Soil. Biol. Biochem.* 1973, 5 (1), 41–83. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1086994/>

29. Hiscox J. D., Israelstam R. J. The Method for the Extraction of Chlorophyll from Leaf Tissue Without Maceration. *Can. J. Bot.* 1979, 57 (12), 1332–1334. <https://doi.org/10.1139/b79-163>
30. Dospekhov B. A. Field experiment technique (Field experience). *Moskva: Agropromizdat.* 1985, 351 p. (In Russian).
31. Malichenko S. M., Krugova O. D., Starchenkov Yu. P. Nitrogenase of the Pure Culture *Rhizobium lupine* and her Effect on the Metabolites of the Legume Plants Nodules. *V Ukr. Biochem. Cong.* (Kyiv, 1987): Abstracts (Ch. 1, P. 102–103). (In Russian).
32. Kots S. Ya., Morgun V. V., Patyka V. Ph., Malichenko S. M., Mamenko P. N., Kiriziy D. A., Mykhalkiv L. M., Beregovenko S. K., Melnikova N. M. Biological nitrogen fixation: Legume-rhizobium symbiosis. *Kiev: Logos.* 2011, V. 2, 523 p. (In Russian).
33. Kyrychenko O. V., Volkogon N. V., Kots S. Ya. Effect of Presowing Seed Treatment with N-acetyl-D-glucosamine on Auxin and Cytokinin Contents in Leaves of Spring Wheat. *The 7<sup>th</sup> Congress of the Plant Physiology Island of Russia "Plant Physiology is the Fundamental Basis of Ecology and Innovative Biotechnologies": Reports (Part 1, P. 331), Nizhniy Novgorod: UNN im. N. I. Lobachevskiy.* 2011, Ch. 1., P. 331. (In Russian).
34. Chernyadev I. I. The Protective Action of Cytokinins on the Photosynthetic Machinery and Productivity of Plants under Stress (Review). *Appl. Biochem. Microbiol.* 2009, 45 (4), 351–362. (In Russian). <https://doi.org/10.1134/S0003683809040012>
35. Tsavkelova E. A., Klimova S. Yu., Cherdyn-tseva T. A., Netrusov A. I. Hormones and Hormone-Like Substances of Microorganisms (Review). *Appl. Biochem. Microbiol.* 2006, 42 (3), 229–235. (In Russian). <https://doi.org/10.1134/S000368380603001X>
36. Dragovoz I. V., Leonova N. O., Biliavska L. O., Yavorska V. K., Iutynska G. O. Phytohormone Production by Some Free-Living and Symbiotic Soil Microorganisms. *Dop. NAS Ukr.* 2010, No. 12, 154–159. (In Ukrainian).
37. Dragovoz I. V., Leonova N. O., Iutynska G. A. Phytohormones Synthesis by *Bradyrhizobium japonicum* Strains with Different Symbiotic Effectiveness. *Mikrobiol. Zh.* 2011, 73 (4), 29–35. (In Ukrainian).
38. Kots S. Ya., Volkogon N. V., Gryshuk E. A. Ability of Strains and Tn5-mutants of *Bradyrhizobium japonicum* to Synthesize IAA and ABA *In Vitro*. *Physiol. Biochem. Cultural Plant.* 2010, 42 (6), 491–496. (In Russian).

### СИМБІОТИЧНА ПРОДУКТИВНІСТЬ ФІТОБАКТЕРІАЛЬНИХ СИСТЕМ ЗА ДІЇ N-АЦЕТИЛ-D-ГЛЮКОЗАМІНУ НА ДІАЗОТРОФНІ МІКРООРГАНІЗМИ

О. В. Кириченко

Інститут фізіології рослин і генетики  
НАН України, Київ

E-mail: azoleki@ukr.net

Метою роботи було оцінити симбіотичну продуктивність фітобактеріальних систем соя—ризобії та пшениця—азотобактер (за біологічною та насінневою продуктивністю рослин, за нодуляційною здатністю ризобій і нітрогеназною активністю симбіозу сої, а також ризосферної мікробіоти пшениці) за дії N-ацетил-D-глюкозаміну (0,01 М; 0,1 М) *in vitro* на культури азотфіксувальних мікроорганізмів *Bradyrhizobium japonicum* 6346 й *Azotobacter chroococcum* T79. Встановлено, що біологічна активність N-ацетил-D-глюкозаміну за інкубації з бульбочковими бактеріями сої виявлялась у більш високому рівні реалізації нодуляційної здатності ризобій: рослини активніше інфікувались (на 12%), формувалась більша кількість бульбочок (в 1,2–2,3 раза) з більшою їхньою загальною

### СИМБИОТИЧЕСКАЯ ПРОДУКТИВНОСТЬ ФИТОБАКТЕРИАЛЬНЫХ СИСТЕМ ПРИ ДЕЙСТВИИ N-АЦЕТИЛ-D-ГЛЮКОЗАМИНА НА ДИАЗОТРОФНЫЕ МИКРООРГАНИЗМЫ

Е. В. Кириченко

Институт физиологии растений и генетики  
НАН Украины, Киев

E-mail: azoleki@ukr.net

Целью работы была оценка симбиотической продуктивности фитобактериальных систем соя—ризобии и пшеница—азотобактер (по биологической и семенной продуктивности растений, по нодуляционной способности ризобий и нитрогеназной активности симбиоза сои, а также ризосферной микробиоты пшеницы) при действии N-ацетил-D-глюкозамина (0,01 М; 0,1 М) *in vitro* на культуры азотфиксирующих микроорганизмов *Bradyrhizobium japonicum* 6346 и *Azotobacter chroococcum* T79. Установлено, что биологическая активность N-ацетил-D-глюкозамина при инкубации с клубеньковыми бактериями сои проявлялась в более высоком уровне реализации нодуляционной способности ризобий: растения активнее инфицировались (на 12%), формировалось большее количество клубеньков (в 1,2–2,3

масою на рослині (в 1,4–2,1 раза) і масою однієї кореневої бульбочки (в 1,2 раза), а також — азотфіксувальної активності симбіозу (в 1,7 раза) та функціональної здатності кожної морфоструктурної симбіотичної одиниці (в 1,4 раза). Це зумовило підвищення (від 14% до 29%) насінневу продуктивність цієї системи порівняно до симбіозу, утвореного немодифікованими вуглеводом ризобіями. Активізація азотобактером, модифікованим глюкозаміном, основних фізіологічних процесів пшениці — азотфіксації (активність ризосферної мікробіоти зростає в 1,1–1,4 раза) і фотосинтезу (вміст хлорофілів у листках — в 1,1–1,2 раза) забезпечила більш високий рівень реалізації продуктивного потенціалу цієї системи порівняно як до небактеризованих рослин (на 15%), так і до варіанта з інокуляцією насіння монокультурою азотобактера (7%). За позитивної дії інокулянтів бактерії + глюкозамін на насінневу продуктивність симбіотичної й асоціативної системи не встановлено достовірних відмінностей біологічної продуктивності рослин сої та пшениці. Таким чином, застосування N-ацетил-D-глюкозаміну як додаткового агента вуглеводної природи в інокулянтах із бульбочковими бактеріями сої та ґрунтовими діазотрофами роду *Azotobacter* сприяло більш повній реалізації симбіотичного і продуктивного потенціалу фітобактеріального симбіозу й асоціації порівняно з використанням лише діазотрофів, що вказує на можливість практичного використання ацетилюваного глюкозовмісного аміноцукру під час створення комплексних інокулянтів на основі азотфіксувальних мікроорганізмів.

**Ключові слова:** соєво-ризобіальний симбіоз, асоціація пшениця–азотобактер, N-ацетил-D-глюкозамін, нодуляція, азотфіксація, ризосферна мікробіота, хлорофіл, урожай.

раза) с большей их общей массой на растение (в 1,4–2,1 раза) и массой одного корневого клубенька (в 1,2 раза), а также — азотфиксирующей активности симбиоза (в 1,7 раза) и функциональной способности каждой морфоструктурной симбиотической единицы (в 1,4 раза). Это обеспечило более высокую (от 14 до 29%) семенную продуктивность данной системы по сравнению с симбиозом, образованным немодифицированными углеводом ризобиями. Активизация азотобактером, модифицированным глюкозамином, основных физиологических процессов пшеницы — азотфиксации (активность ризосферной микробиоты возросла в 1,1–1,4 раза) и фотосинтеза (содержание хлорофиллов в листьях — в 1,1–1,2 раза) обеспечила более высокий уровень реализации продуктивного потенциала этой системы по сравнению как с небактеризованными растениями (на 15%), так и с вариантом инокуляции семян монокультурой азотобактера (на 7%). При положительном влиянии инокулянтов бактерии + глюкозамин на семенную продуктивность симбиотической и ассоциативной систем не отмечено достоверное изменение биологической продуктивности растений сои и пшеницы. Таким образом, применение N-ацетил-D-глюкозамина как дополнительного агента углеводной природы в инокулянтах с клубеньковыми бактериями сои и почвенными диазотрофами рода *Azotobacter* способствовало более полной реализации симбиотического и продуктивного потенциала фитобактериального симбиоза и ассоциации по сравнению с использованием одних диазотрофов, что указывает на возможность практического применения ацетилюванного глюкозосодержащего аминосахара при создании комплексных инокулянтов на основе азотфиксирующих бактерий.

**Ключевые слова:** соєво-ризобіальний симбіоз, асоціація пшениця–азотобактер, N-ацетил-D-глюкозамін, нодуляція, азотфіксація, ризосферна мікробіота, хлорофіл, урожай.