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STUDY OF THE EFFECT OF NITROGEN-FIXING CYANOBACTERIA ON THE GROWTH RATE OF THE STRAWBERRY SUNRISE T-4 STRAWBERRY VARIETY

The article presents the results of the study of nitrogenase activity of five strains of cyanobacteria from the collection of phototrophic microorganisms of Al-Farabi Kazakh National University and their effect on the growth of strawberries. Nitrogen-fixing cyanobacteria were studied by strains Anabaena variabilis R-1-5, Anabaena sp. 7912, Anabaena sp. Z-1, Nostoc calisicola RI-3, Nostoc sp. S-2, and according to the results obtained high activity of nitrogenase in the strain Anabaena variabilis R-1-5. In the study of the effect of cells of different concentrations of the strain Anabaena variabilis R-1-5 on the growth of strawberry Strawberry Sunrise T-4, it was found that its suspension of 24x10⁶ cells/ml and 48x10⁶ cell/ml had a positive effect on strawberry growth. In addition, an increase in the number of strawberry roots was observed in the suspension of cyanobacterial biomass 24x10⁶ cell/ml. An increase in the number of strawberry leaves, root length, stem height and a significantly higher yield of dry biomass of strawberries were observed in the suspension of the study in the amount of 48x10⁶ cell/ml. The optimal cell count of the nitrogen-fixing cyanobacterium Anabaena variabilis R-1-5 suspension, which has a positive effect on the growth of strawberry plants, was 48x10⁶ cell/ml. The obtained results allow to use the nitrogen fixing strain Anabaena variabilis R-1-5 for obtaining biological products in agrobiotechnology.

Key words: Nitrogen-fixing cyanobacteria strains, grow of Strawberry Sunrise T-4 variety, molecular nitrogen absorption in air.
Изучение влияния азотфиксирующих цианобактерий на показатели роста сорта клубники Strawberry Sunrise T-4

В статье представлены результаты исследования КазНУ им. аль-Фараби по нитрогеназной активности пяти штаммов цианобактерий из коллекции фототрофных микроорганизмов и их влиянию на рост земляники. Азотфиксирующие цианобактерии исследовали на штаммах Anabaena variabilis R-I-5, Anabaena sp. 7912, Anabaena sp. Z-1, Nostoc calsicola RI-3, Nostoc sp. S-2 и по результатам получили высокую активность нитрогеназы в штамме Anabaena variabilis R-I-5. При изучении влияния клеток штамма Anabaena variabilis R-I-5 с различной концентрацией на рост клубники Strawberry Sunrise T-4 было обнаружено, что его суспензия, содержащая 24×10^6 клеток (кл)/мл и 48×10^6 кл/мл, оказывает положительное влияние на рост клубники. Кроме того, увеличение количества корней клубники наблюдалось в суспензии биомассы цианобактерий 24х10^6 кл/мл. Увеличение количества листьев клубники, длины корня, высоты стебля и значительно более высокий выход сухой биомассы клубники наблюдали в суспензии в количестве 48×10^6 кл/мл. Оптимальное количество клеток в суспензии азотфиксующей цианобактерии Anabaena variabilis R-I-5, которая положительно влияет на рост растений клубники, составляло 48×10^6 кл/мл. Полученные результаты позволяют использовать азотфиксирующий штамм Anabaena variabilis R-I-5 для получения биопрепаратов в агробиотехнологии.

Ключевые слова: азотфиксирующие штаммы цианобактерий, рост сорта Strawberry Sunrise T-4, поглощение молекулярного азота на воздухе.

Introduction

Intensive cultivation of agricultural crops in the country has led to a decrease in soil fertility due to a lack of organic and mineral fertilizers, including nitrogen. According to soil scientists in Kazakhstan, 60% of the country’s soil cover is subject to soil erosion. Over the past 20-40 years, the loss of the humus layer amounted to 8-30%, which became the basis of soil fertility, including a decrease in the most valuable humic acids and hydrolyzed nitrogen 45% and 48% respectively. Nitrogen is known to be one of the most important nutrients for plants. Nitrogen makes up 1-5% of their dry biomass and is an important component of chemical processes. Crop yields in different agricultural regions are often closely linked to soil nitrogen reserves. The capacity of biological nitrogen is three times higher than that of chemical nitrogen introduced as a fertilizer. Therefore, the current issue is to carry out concrete work to restore the soil. On this basis, it is important to replace the traditionally used chemical fertilizers with biofertilizers that produced from biological processes. The amount of nitrogen fixed and consumed from the air is directly related to the activity of the enzyme nitrogenase in the species of cyanobacteria. The amount of nitrogen in the soil depends on the intensity of microorganisms that fix N₂ in the air. Among them, nitrogen-fixing bacteria (azotobacteria) and cyanobacteria play a leading role in the formation of nitrogen in the air. Some species of cyanobacteria are able to fix atmospheric nitrogen in the absence of nitrogen in the environment. They use special cells called heterocysts to produce nitrogen [2]. Some heterocystic species produce high levels of nitrogen in the air, while some species have relatively low nitrogen consumption. All of them are similar in terms of biochemical mechanisms of fixation of molecular nitrogen. It is based on the process of reduction of N₂ which follows the following equation:

\[ \text{N}_2 + 6e^- + 6 \text{H}^+ \rightarrow 2 \text{NH}_3 \]

In the cell, this reaction takes place in the presence of the enzyme nitrogenase, which is present in the inner membrane of the cell. The amount of nitrogen fixed and consumed from the air is directly related to the activity of the enzyme nitrogenase in the species of cyanobacteria. Among cyanobacteria, heterocystic forms absorb nitrogen from the air and distribute it in the soil. It is known that the soil is the habitat of underground nitrogen-fixing microorganisms. Strains of cyanobacteria in the soil fix atmospheric nitrogen, bind soil particles together and help retain moisture and prevent erosion. In addition, nitrogen-fixing cyanobacteria increase the content of micro- and macronutrients in the soil and increase the supply of important plant growth hormones – phytohormones [3, 4].
In addition, the biomass of cyanobacteria is rich in amino acids and vitamins, which in turn are important chemical compounds necessary for plants [5,6,7,8]. Active cyanobacteria for nitrogen fixation are potential microorganisms that are suitable for the replacement of traditionally used chemical fertilizers [9,10]. The effect of nitrogen-fixing cyanobacteria is beneficial for crops such as wheat, strawberries, soybeans, oats, radishes, cotton, sugar cane, corn, chili, peas, tomatoes [11, 12]. Righini et al. (2018) proved in his study that the biomass of microalgae cells had a positive effect on the growth of strawberries and contributed to an increase in productivity growth [13].

Strawberry is a widely used crop around the world. In addition, strawberries are known for their aroma, bright red color, juicy texture and sweetness [14]. It should be noted that its antioxidant properties are beneficial to the human body, and its normal consumption has a positive effect on the neutralization of radicals in the body. Currently, China and the United States produce about 3.8 Mt and 1.4 Mt of strawberries per year. Mexico, Turkey and Spain produced about 468 kt, 415 kt and 366 kt of strawberries in 2016. Currently, strawberries are grown commercially and their seeds are prepared in vitro and propagated in artificial plants [15].

Strawberry seedlings can be grown in soil, hydroponic and soilless production systems, in the open field or in a protected area. The choice of cultivation system depends on climatic conditions, market demand and exports. In China, open field and protected field systems are used [16], open field is the most widely used system in the United States, and Italian manufacturers use special greenhouses. In our study, we studied the effect of nitrogen-fixing cyanobacteria on strawberries, as this plant has a short reproductive period and a large number of roots and shoots. Therefore, the impact of environmental factors can be clearly seen.

The article covers the study of the activity of the enzyme nitrogenase of cyanobacterial strains. In addition, the effect of different concentrations of Anabaena variabilis R-I-5 strain that has high nitrogen fixation activity compared to other cyanobacterial species was studied on the growth rates of Strawberry Sunrise T-4 strawberry.

**Materials and methods**

The object of research is 6 different strains of cyanobacteria from the collection of phototrophic microorganisms of Al-Farabi Kazakh National University – Anabaena sp. 7912, Anabaena sp. Z-1, Anabaena variabilis R-I-5, Nostoc calsicola RI-3, Nostoc sp. S-2, Synechocystis sp. PCC 6803 strains and Strawberry Sunrise T-4 strawberry variety.

**Cultivation of strains of cyanobacteria**

Collection strains of cyanobacteria were actively grown in 250 ml flasks at a temperature of 25°C, 450 μmol photon m\(^{-2}\)/sec. The light was transmitted from one side of the flask and a BONY air pump (PRC) was used during cultivation.

**Determination of nitrogenesis activity by acetylene method**

Nitrogenase activity was determined by injecting a 10% acetylene/90% argon gas mixture into a vial for 30 min [17]. Cells were cultured for 24 hours at 250 μmol photon m\(^{-2}\)/sec. After incubation, 500 μl of gas samples were taken and the concentration of ethylene in the gas mixture was determined. The redox activity of acetylene was determined on a gas chromatograph GC-15A (Shumadzu, USA), and was shown in nmol ethylene/mg dry biomass (mg)/h. Nitrogen-fixing collection strains were cultured under light under anaerobic conditions (90% argon/10% acetylene) for 24 hours and the amount of ethylene released on a gas chromatograph was determined.

**Obtaining biomass of the Anabaena variabilis R-I-5 strain**

Nitrogen-fixing cyanobacteria strain Anabaena variabilis R-I-5 was intensively grown in a 5-liter photobioreactor PBR-5 (Bioreactors, Latvia) under a light of 450 μmol photon m\(^{-2}\)/sec at a temperature of 25°C and aerated with an Airpump 350 (China). BG\(_{\text{o}}\)-11 culture medium was used to increase the accumulation of cyanobacteria. When the optical density was 0.6 the cell biomass was collected the rotating with a Centrifuge 5810 (Eppendorf, USA) at a speed of 5,000 rpm for 15 minutes. The rate of cell growth was measured with an optical density spectrophotometer KFK-3-01 (Russian Federation). The optical densities of the suspension at 6 different concentrations were prepared and measured at a wavelength of 720 nm (Figure 1).

*Figure 1 – PBR-5 photobioreactor*
Growing strawberries

Strawberry Sunrise T-4 strawberry was obtained from the Biotechnology laboratory of Dankook University (South Korea). In the laboratory, 2-week-old strawberry seedlings grown in vitro are cultivated in a mirror camera “Plant Factory” (Allen, Republic of South Korea). The roots of the seedlings were cut to a length of 3 cm. The length of the plant was 3-4 cm and the number of leaves was the same in all samples. The temperature of the culture chamber was 25ºC. The light is on for 12 hours in the dark/12 hours in the light mode. Special red spectrum light bulbs were used for normal growth of strawberries.

Obtaining dry biomass of strawberries

At the end of the experiment, the strawberry seedlings were removed from the soil, and washed three times. Dried in a Petri dish for 3 days in a dark thermostat “SNOL 67/350” (AB Utenos Electrotechnika) at a temperature of 70 ºC. Then, the dry biomass was measured using a laboratory scale (Clever, China).

Preparation of suspension

Strawberry Sunrise T-4 strawberry seedlings were grown in 200 ml hydroponics in a 250 ml moisture storage container. The duration of the study was 30 days. 50 ml of liquid biomass of Anabaena variabilis R-I-5 was poured on 50 ml of Murashige and Skoog culture medium and 100 ml of liquid was prepared in 6 different cell concentrations of suspension:

- Control 1 - nitrogen in 100 ml of Murashige and Skoog medium (+ KNO₃);
- Control 2 - 100 ml of nitrogen-free Murashige and Skoog medium (- KNO₃);
- Option 1 (0.1 optical density) - 50 ml nitrogen-free Murashige and Skoog medium + 50 ml suspension of Anabaena variabilis R-I-5 strain - where the total number of cells in 100 ml was 6x10⁶ cell/ml;
- Option 2 (0.3 optical density) - 50 ml nitrogen-free Murashige and Skoog medium + 50 ml suspension of Anabaena variabilis R-I-5 strain - where the total number of cells in 100 ml was 12x10⁶ cell/ml;
- Option 3 (0.6 optical density) - 50 ml nitrogen-free Murashige and Skoog medium + 50 ml suspension of Anabaena variabilis R-I-5 strain - where the total number of cells in 100 ml was 24x10⁶ cell/ml;
- Option 4 (1.2 optical density) - 50 ml nitrogen-free Murashige and Skoog medium + 50 ml suspension of Anabaena variabilis R-I-5 strain - where the total number of cells in 100 ml was 48x10⁶ cell/ml;
- Option 5 (2.4 optical density) - 50 ml nitrogen-free Murashige and Skoog medium + 50 ml 2.4 optical density Anabaena variabilis RI-5 strain suspension - where the total number of cells in 100 ml was 96x10⁶ cell/ml;
- Option 6 (4.8 optical density) - 50 ml nitrogen-free Murashige and Skoog medium + 50 ml suspension of Anabaena variabilis R-I-5 strain - the total number of cells in 100 ml was 192 x10⁶ cell/ml.

The number of cells was measured at different optical densities (0.05, 0.5, 1, 1.5, 2, 3, 4, 5) and the 6 different suspension concentrations was measured using a linear measurement using the Excel trend line calculated according to the formula – \( y = 4E+07x \). The mean deviation of the measured optical densities was \( R² = 0.9883 \) (Figure 2).

![Figure 2](image)

Figure 2 – Trend line of the ratio of the optical density of the strain Anabaena variabilis R-I-5 to the number of cells

Statistical analysis

The study was conducted 3 times. The results were processed according to the statistical system “ANOVA”. The figures show the arithmetic results of research and deviations from their standards. For the discussion of the results, the standard deviation did not exceed 10% [18].

Results

Determination of nitrogenase activity based on acetylene oxidation is the most important method that demonstrates the ability of microorganisms to fix nitrogen. Microorganisms (Azotobacter, Cyanobacteria) capable of fixing nitrogen in the air and convert acetylene \( (\text{C}_2\text{H}_2) \) to ethylene \( (\text{C}_2\text{H}_4) \) [19]. Because the reduction processes of molecular nitrogen and acetylene are similar, they are widely used to conduct and study nitrogen fixation. If \( \text{N}_2 \) and \( \text{C}_2\text{H}_2 \) gases are present simultaneously in the gas chromatograph vial, then acetylene is reduced.
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primarily due to the presence of electrons in the carbon atom. Therefore, it is important to create anaerobic conditions (without nitrogen, oxygen, carbon) during the experiment [20,21].

The purpose of this work is to determine the nitrogenase activity of nitrogen-fixing cyanobacteria. Research was conducted to determine the high nitrogenase activity of five different nitrogen-fixing strains from the collection. *Synechocystis* sp. PCC 6803 heterocyst-free strain was obtained as a control.

The results of the obtained gas chromatograph showed that all strains of nitrogen-fixing cyanobacteria showed nitrogenase activity in acetylene atmosphere. However, their ability to reduce acetylene to ethylene has been shown to vary. According to the results, among the studied species, the strain of *Anabaena variabilis* R-I-5 accumulated relatively low ethylene, and the strain of *Anabaena variabilis* R-I-5 accumulated the highest amount of ethylene (3.57 ±0.26 mmol ethylene/mg dry weight (DW)/h). In addition, an experiment with *Nostoc calsicola* R-I-3 strain showed a value of 1.82 ±0.06 mmol ethylene/mg DW/h. As expected, nitrogenase activity was not observed with *Synechocystis* sp. PCC 6803 strain (Figure 3).

![Figure 3](image)

During the experiment, *Nostoc* sp. S-2 (1.69±0.05 mmol ethylene/mg DW/h), *Anabaena* sp. 7912 (1.2±0.03 mmol ethylene/mg DW/h) *Anabaena* sp. Z-1 strains (1.13 ±0.014 mmol ethylene/mg DW/h) showed close results.

The *Anabaena variabilis* R-I-5 strain showed high levels of ethylene and was selected for further research.

The *Anabaena variabilis* R-I-5 strain was characterized by high activity of nitrogen fixation properties. In this context, it is important to study the dynamics of growth. The next step was to study the growth of cells of the *Anabaena variabilis* R-I-5 strain (Figure 4). Active cell growth was recorded from day one and had an optical density of 0.03 units. In the following days (2-11) there was a linear increase and an increase in the number of cells. On the 11th day, the log phase of the culture was completed, the biomass of the cells was obtained in the stationary phase and the high optical density was 2.6 units. On the 14th day, the death phase began.

The next step was to determine the effect of nitrogen-fixing cyanobacteria strain on the growth rates of strawberries. In this context, the effect of cyanobacteria *Anabaena variabilis* R-I-5 with high nitrogenase activity on the growth rates of *Strawberry Sunrise* T-4 was studied. In some published articles [22,23,24], *Anabaena* and *Nostoc* strains have shown the ability to replace chemical nitrogen in the nutrient medium with biological nitrogen. This is because they can fix the free nitrogen in the air with the help of the nitrogenase enzyme in heterocysts and distribute it in the soil [25].
In the study of the biomass effect of different suspension concentrations of the *Anabaena variabilis* R-I-5 strain on the variety *Strawberry Sunrise* T-4, the number of cells per 1 ml was taken into account.

As shown in Figure 5, the growth rates of strawberry seedlings were different. First of all, the number of seedlings was registered. At the end of the experiment, different suspension concentrations of cyanobacterial strains were recorded, respectively, different numbers of leaves. In control 1, 35±6.3 leaves were observed. In option 1, the number of growing leaves was 26±5.4, and in control 2, a slightly lower value (23 ± 4.3 leaves) observed. In the 3rd and 4th options there was an increase in the number of leaves. Although the positive growth rates were higher at 48x10^6 cell/ml, the increase in cyanobacteria to 96x10^6 cell/ml was lower and the number of leaves in this option of the experiment was 34 pieces. In addition, it should be noted that the concentration of cyanobacterial suspension 192x10^6 cell/ml had a negative effect on the growth of strawberries. Figure 5 shows a microphoto of strawberries in the control and experimental option on the 30th day of the experiment.

Figure 6 shows the results of different suspension concentrations effects of cyanobacterial strains on strawberry seedlings. Next, the number of roots of *Strawberry Sunrise* T-4 seedlings was recorded. It is known that the increase in the number of roots ensures the normal growth of plants and the normal transfer of macro and micronutrients in the soil throughout the plant [26,27]. Therefore, it is important to study the effect of a cyanobacterial suspension on the root system of plants.

In control 1, 15±2.2 lateral and umbilical roots were found. In control 1, 10±2.1 roots were registered. In the 1st and 2nd options, the number of growing roots did not differ significantly from control 2 – 10 ±3.2 and 11 ±2.2. However, in option 3, the highest roots value was recorded, which was 1 unit higher than in the nitrogen version of the control (16±1.5). In option 4, there was a decrease in the number of roots, and this control showed an equal result. The results of the study showed that the suspension concentration of cyanobacteria in option 3 had a positive effect on the growth of *Strawberry Sunrise* T-4. As shown in Figure 7, many side effects have been found to have a positive effect on root growth. In option 5 and 6 in this case, 12±3 roots were recorded. It shows that the suspension concentration of excess cyanobacterial cells had a toxic effect on plant growth. When too much suspension was introduced, plant growth slowed down, air circulation was disrupted due to the proliferation of cells on the soil surface, and harmful microorganisms accumulated on the soil surface. This, in turn, leads to rapid disease of plants [28].
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**Figure 5** – Effect of different suspension concentrations of cyanobacterial strain *Anabaena variabilis* R-I-5 on the growth of *Strawberry Sunrise* T-4.

Note: 1 – control 1; 2 – control 2; 3 – 6x10^6 cell/ml; 4 – 12x10^6 cell/ml; 5 – 24x10^6 cell/ml; 6 – 48x10^6 cell/ml; 7 – 96x10^6 cell/ml; 8 – 192x10^6 cell/ml

**Figure 6** – Effect of different suspension concentrations of nitrogen-fixing *Anabaena variabilis* R-I-5 on the growth of strawberry leaves
Next, we studied the length of the root. This is because root length plays an important role in plant growth and molecular nitrogen has a high effect on root growth. Since all macro- and micronutrients come to the plant through the roots, the large number and length of roots affect the normal growth of the plant. At different suspension concentrations of cyanobacteria species, respectively, grew different root lengths. Control 1 seedlings showed higher values than seedlings with control 2 – 7 ± 0.5 cm and 5 ± 1.2 cm.
In control 1, an average root length of 7±0.5 cm was recorded. In the 1st and 2nd options, the growth rates are slightly higher – 7±0.8 cm and 5.9±1.3 cm. In addition, the average length of the growing roots in option 4 with a cell concentration of 48x10^6 cell/ml was 8.2±1.12 cm, which was the highest. In option 5, a decrease in root length was observed. The lowest root length was observed in control 2 and option 6 – 5±1.12 cm and 5.6±0.67 cm. The results showed that the optimal suspension concentration of cells stimulated the growth of strawberries, and the excess reduced the growth of plant roots. Version 4 of *Anabaena variabilis* R-I-5 in 48x10^6 cell/ml was found to have a positive effect on the growth of seedlings of *Strawberry Sunrise* T-4 (Fig. 8).

Next, the indicators of plant length were recorded (Table 1). When studying the effect of cyanobacteria, which are able to fix nitrogen at different suspension concentrations, on the growth rate of strawberries, it was found that their length varies. The difference between control 1 and control 2 was 5 cm (Fig. 5). This shows that the strawberry plant is directly dependent on the concentration of nitrogen in the soil. The plant length of 16±3.5 cm was recorded in option 4, and in option 5 of 96x10^6 cell/ml cell fluid and in option 2 of 12x10^6 cell/ml the same low value was recorded – 13±2 cm.

### Table 1 - The effect of different concentrations of cyanobacteria of the nitrogen-fixing strain *Anabaena variabilis* R-I-5 on the growth of strawberries

| № | Options of experiment | Growth of strawberries, cm |
|---|----------------------|----------------------------|
| 1 | Control 1 – Murashige and Skoog medium with N₂ | 17±2,5 |
| 2 | Control 2 – Murashige and Skoog medium without N₂ | 12±1 |
| 3 | 1-option – 6x10⁶ cell/ml (OD–0,1) | 13±2 |
| 4 | 2-option – 12x10⁶ cell/ml (OD–0,3) | 13±2 |
| 5 | 3-option – 24x10⁶ cell/ml (OD–0,6) | 14±2,5 |
| 6 | 4-option – 48x10⁶ cell/ml (OD–1,2) | 16±3,5 |
| 7 | 5-option – 96x10⁶ cell/ml (OD–2,4) | 13±2 |
| 8 | 6-option – 192x10⁶ cell/ml (OD–4,8) | 14±1,5 |

In addition, it should be noted that the increase in the number of cells above 96x10^6 cell/ml had a negative impact on the growth of seedlings. In option 6 with the highest density, a strawberry length of 14±1.5 cm was recorded. It should be noted that option 4 has a positive effect on the normal growth of plants.

After measuring all the indicators of strawberry growth, the yield of biomass was determined. It should be noted that the dry biomass of roots, leaves and stems of plants was calculated during the registration of this indicator. At the end of the experiment, the seedlings were washed and placed at 60 °C for 72 hours. As expected, 2.9±0.6 g of dry biomass was recorded in 1 control of the nitrogen in the Murashige and Skoog medium, which was close to option 5 (Table 2).

The highest value was recorded in option 4, and this was higher than control 1 to 0.2 g (3.1±0.71 g). However, this indicator was 2.6±0.8 g of dry biomass of strawberry option 3 in 24x10^6 cell/ml suspension.

The biomass of strawberries affected by different suspension concentrations of cyanobacterial strains was different. The study took into account the length of the stems, the size of the cracks and the thickness of the roots. However, it was not presented in the form of a graph due to the lack of specific common indicators in the study.

In conclusion, it was observed that the strain of cyanobacteria *Anabaena variabilis* R-I-5 affects the growth of strawberries. It was found that this strain absorbs nitrogen from the air with the help of the enzyme nitrogenase and transfers it to the soil. It was found that different suspension concentrations of cells of the studied cyanobacterial strain have different effects on the growth rates of strawberries (number of leaves, number of roots, root length, length of growth, dry weight of the plant).
Table 2 – Effect of different suspension concentrations of cyanobacterium strain of nitrogen-fixing *Anabaena variabilis* R-I-5 on the weight of strawberries

| №  | Options of experiment                                                                 | DW of plant, g |
|----|----------------------------------------------------------------------------------------|----------------|
| 1  | Control 1 – Murashige and Skoog medium with N₂                                           | 2,9±0,6        |
| 2  | Control 2 – Murashige and Skoog medium without N₂                                        | 1,5±0,5        |
| 3  | 1-option – 6x10⁶ cell/ml (OD–0,1)                                                        | 2,1±0,65       |
| 4  | 2- option – 12x10⁶ cell/ml (OD–0,3)                                                       | 2,5±0,5        |
| 5  | 3- option – 24x10⁶ cell/ml (OD–0,6)                                                       | 2,6±0,8        |
| 6  | 4- option – 48x10⁶ cell/ml (OD–1,2)                                                       | 3,1±0,71       |
| 7  | 5- option – 96x10⁶ cell/ml (OD–2,4)                                                       | 2,8±0,53       |
| 8  | 6- option – 192x10⁶ cell/ml (OD–4,8)                                                      | 1,3±0,5        |

**Discussion**

The results of this experiment showed the effect of nitrogen-fixing cyanobacteria on the growth of strawberries. In the study of nitrogenase activity, it should be noted that the strain *Anabaena variabilis* R-I-5 showed the ability to fix high nitrogen. The results obtained in these experiments showed slight differences compared to controls. In the experimental versions, some growth rates were higher than in the standard Murashige and Skoog medium. The strain *Anabaena variabilis* R-I-5 was found to have a positive effect on root length, leaf growth, dry weight of the plant and increase in the number of roots. Studies based on this have shown that cyanobacteria can have a positive effect on root growth and improve soil water and nitrogen metabolism [29,30].

It should be noted that the growth of plants treated with cyanobacterial cells can be influenced by several factors, in particular, the high concentration of chemicals released by cyanobacteria and macro- and micronutrients in the nutrient medium [31]. These heterocystic cyanobacteria increase the amount of nitrogen and ammonium in the soil due to their ability to fix nitrogen. Cyanobacteria use a special enzyme nitrogenase to increase the amount of chemical nitrogen in the soil, and we have identified the activity of nitrogenesis in 5 different nitrogen fixing strains. Estimation of nitrogen formation rate of these 5 species shows that these heterocystic cyanobacteria can naturally form atmospheric N₂ (Figure 1). Among them, high nitrogenase activity was observed in the strain *Anabaena variabilis* R-I-5. Our results also showed that the presence of growth-promoting substances responds to the beneficial effects of these cyanobacterial cells on plant growth. In addition, Shariatmadari (2013) reported that the cells of some cyanobacteria releasing phytohormones IAA (indole 3-acetic acid) and IBA (indole 3-butyric acid) in addition to fixing atmospheric nitrogen [31]. Auxins (IBA) are plant hormones used to stimulate root growth [32]. In addition, some studies suggest that nitrogen-fixing cyanobacteria have a positive effect on plant growth and nutrient uptake [33]. *Nostoc* species increase the amount of organic matter in the soil - (C, N, etc.), increase plant growth and metabolic and energy activity [31]. In addition, polysaccharides released from cyanobacteria affect the structural stability of the soil, improve soil quality and normal plant growth [31].

In our study, slightly lower values were observed when strawberries were irrigated with a high-density cyanobacterial suspension (options 5 and 6). According to the cited literature, an excess of cells can disrupt the metabolism in the soil and prevent the respiration of beneficial microorganisms in the soil. In addition, cell biomass accumulated on the soil surface is likely to inhibit plant growth due to the release of excessive amounts of various chemicals (phytohormones, biologically active substances, etc.). According to Jacoby (2017), excess nitrogen in the soil is likely to adversely affect plant growth [28]. This is because the maximum accumulation of single-stranded heterocysts in a nitrogen-free BG-11 culture medium can reach 8-12 cells. Heterocysts in a humid environment absorb excess nitrogen from the air on the basis of the enzyme nitrogenesis and break it down into the soil [34]. In addition, high cell concentrations lead to soil contamination. In this case, the growth of lethal cyanobacterial cells can also cause bacteriological contamination.
Conclusion

The activity of the enzyme nitrogenosis of cyanobacterial strains was studied by the acetylene method. The effect of suspension of six different concentrations of strain *Anabaena variabilis* R-I-5 with relatively high nitrogen fixation intensity on strawberry growth rates (number of leaves, height, number of roots, root length, dry weight) was studied. In the study of the effect of nitrogen-fixing cyanobacteria strain on the growth of seedlings of *Strawberry Sunrise* T-4, it was found that the culture suspension 24x10⁶ cell/ml had a positive effect on plant root growth. The suspension of cyanobacteria 48x10⁶ cell/ml had a positive effect on the number of leaves, root and height of strawberries and the accumulation of dry biomass. Studies have shown that the strain *Anabaena variabilis* R-I-5 can improve the growth of strawberries and provide it with a normal source of nitrogen. In conclusion, 48x10⁶ cell/ml *Anabaena variabilis* R-I-5 cyanobacterial suspension was found to have a positive effect on the growth of *Strawberry Sunrise* T-4.

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