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INFLUENCE OF *AZOTOBACTER VINELANDII* IMV B-7076 ON THE GROWTH OF WHEAT AND THE SYNTHESIS OF EXOMETABOLITES IN HYDROPONIC CONDITIONS

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Objective. *Determine the influence of Azotobacter vinelandii IMV B-7076 on the in vitro synthesis of exometabolites by wheat plants.* ***Methods.*** *Experiments were carried out with the winter wheat of Shestopalivka variety, which seeds first underwent surface sterilized and then grown for 14 days under the conditions of hydroponics with A. vinelandii IMV B-7076. Bacteria were grown in 700 mL E-flasks contained 100 mL of Ashby medium and incubated on the orbital shakers at 220 rpm at 28 °C during two days. The wheat plants were grown in 1.5 L cylindrical glass vessels of 115 mm in diameter and 160 mm height. Stainless steel meshes were placed in the vessels at a distance of 5 mm from the bottom. The vessels were sterilized, after which 75 mL of sterile Farreus medium was added. After seed germination, five sprouts were placed on stainless steel meshes in glasses. Azotobacter suspensions were added to the medium in three different dilutions (10^5, 10^6 and 10^7 cells/mL, respectively). The effect of bacteria on plant morphometric parameters, chlorophyll and carotenoid content in plant leaves, and the accumulation of carbohydrates, proteins and phenolic compounds in the medium with root exudates were determined.* ***Results.*** *It was found that growing wheat on a medium with A. vinelandii IMV B-7076 had a positive effect on plant growth, the content of photosynthetic pigments in leaves and the accumulation of proteins, phenols and carbohydrates in root exudates. The stimulating effect of Azotobacter increased with an increase in the content of cells in the medium. When growing plants on a medium with 10^7 cells/mL, the length of sprouts increased by 26.4 % compared to the control (without Azotobacter), and the weight of plants increased by 63.6 %. When growing wheat plants under such conditions, the content of proteins and carbohydrates in the medium with plant root exudates increased more than twice compared to the control, and phenolic compounds — by 79.8 %.* ***Conclusion.*** *A. vinelandii IMV B-7076 has the positive effect on the growth and development of the wheat plants and synthesis of exometabolites.*

Key words: *Azotobacter vinelandii, wheat, phenols, carbohydrates, chlorophylls, carotenoids.*

Introduction. The most critical factor for life on Earth is vegetation, which ensures the functioning of all the living organisms. In the process of plant growth, about 40 % of the photosynthesis products are released into the basal zone of the soil [1]. Due to the release of root exudates around plant roots at a distance of up to 4–6 mm from its surface, in the rhizosphere, a unique microbial community is formed for each plant species [2] the number of which significantly exceeds similar indicators of the soil layer, distant from the surface of the root [3].

Microorganisms of the rhizosphere play a significant role in the growth, development, and productivity of plants. They can improve the nitrogen and phosphorus nutrition of plants, the availability of a number of mineral elements for them, stimulate their growth and development through the synthesis of phytohormones and other biologically active compounds [4–11].

Microorganisms of the rhizosphere are able to protect plants from the influence of a number of negative environmental factors [12–14]. At the same time, the influence of the microbiota of the rhizosphere and its individual components on the synthesis of root exudates has hardly been investigated.

Analysis of recent studies and publications. An important direction of increasing the positive influence of microorganisms on the growth and productivity of plants is the use of effective microbial preparations. For this purpose, a significant number of such preparations have been developed and are widely used in crop production [6–8; 15].

Complex microbial preparations created on the basis of two or more types of microorganisms show the most noticeable positive effect on the growth and productivity of plants [9; 14; 15]. Based on the interaction of nitrogen-fixing bacteria *Azotobacter vinelandii* IMV B-7076 and phosphate-mobilizing bacteria *Bacillus subtilis* IMV B-7023 with natural mineral particles, we developed a complex bacterial preparation Azogran, which significantly improves the growth and productivity of a significant number of plants [9]. The effect of the components of this preparation on the synthesis of plant exometabolites has not been studied. Considering this, the **objective** of the work was to determine the effect of *Azotobacter vinelandii* IMV B-7076 on the synthesis of exometabolites by wheat plants.

Materials and methods. In the experiments, *Azotobacter vinelandii* IMB B-7076 was used, which was isolated in the Department of Microbiological Processes on Hard Surfaces of the D. K. Zabolotny Institute of Microbiology and Virology, the NAS of Ukraine [16]. The bacteria were grown in 700 mL Erlenmeyer flasks containing 100 mL of Ashby medium [17] and incubated on orbital shakers at 220 rpm at 28 °C for two days. The population of azotobacter in the suspension was determined by serial dilution and plating onto agarized Ashby medium, followed by cultivation and colony counting.

The experiments were carried out on Shestopalivka wheat provided by the National Scientific Center "Institute of Agriculture" of the NAS of Ukraine. Wheat plants were grown in 1.5 liters cylindrical glass vessels with a diameter of 115 mm, and a height of 160 mm. Stainless steel meshes were placed in these vessels at a distance of 5 mm from the bottom. The vessels were sterilized at 160 °C, after which 75 mL of sterile Farreus medium of the following composition per liter was added: $CaCl_2$ — 0.1 g, $MgSO_4 \cdot 7H_2O$ — 0.12 g, KH_2PO_4 — 0.1 g, $Na_2HPO_4 \cdot 12H_2O$ — 0.15 g, Fe citrate — 0.005 g, and trace amounts of Cu, Zn, B, Mn, Mo, pH 6.5 [18].

Before application in the experiments, these wheat seeds were sterilized for 7 minutes in 96 % ethanol and 50 % hydrogen peroxide with the 1:1 ratio of these solutions. Then, the seeds were thoroughly washed five times with sterile distilled water and placed on the surface of potato agar in Petri dishes. After the germination, five sterile seeds were placed on stainless meshes in glasses. The study was conducted in four variants. Bacteria were not added to the medium of the 1st variant (control). In the following variants, the concentration of azotobacter in the medium was 10^5, 10^6, and 10^7 cells/mL, respectively.

Plants were grown in a phytotron for 14 days at a temperature of 20 °C with 16 hours of light. After that, the medium with the root exudate was sterilely drained for the subsequent determination of the metabolite content; the length of the plant shoots and roots and their weight were determined.

To determine the content of chlorophylls *a* and *b* and carotenoids in plants, 1 g fresh crushed leaves were placed in a mortar and ground with a small amount of magnesium carbonate; 10 mL of cooled 96 % ethanol was added under thorough subsequent grinding for 2–3 minutes. The resulting extract was carefully drained and filtered through a membrane filter. The extraction was carried out several times until the filtrate stopped decolorizing. The resulting filtrate was placed in a 25 mL volumetric flask, and the required volume was made up with 96 % ethanol. The amount of green and yellow pigments was determined spectrophotometrically on SF-46 LOMO [19]. The absorption maximum of chlorophyll *a* was determined 665 nm, that of chlorophyll *b* — at 649 nm, and that of carotenoids — at 441 nm.

The concentration of chlorophyll *a* (C*a*, mg/L) and *b* (C*b*, mg/L) was calculated using the following formulas:

$$C_a = 13.70 \cdot A_{665} - 5.76 \cdot A_{649},$$

$$C_b = 25.80 \cdot A_{649} - 7.60 \cdot A_{665},$$

where A_{665} is the optical density of the solution at 665 nm;

A_{649} is the optical density of the solution at 649 nm.

The concentration of carotenoids (C_c, mg/L) was calculated using the formula:

$$C_c = 4.695 \cdot A_{441} - 0.268 (C_a + C_b),$$

where A_{441} is the optical density of the solution at 441 nm;

($C_a + C_b$) is the total content of chlorophyll *a* and *b* in the solution, mg/L.

After determining the pigment concentrations in the extract, the quantitative content of the pigments (X, mg/mL) in the raw material was calculated using the formula:

$$X = V \cdot C / m \cdot 1000,$$

where: V is the volume of the alcoholic extract, mL;

C is the concentration of chlorophyll in the alcoholic solution, mg/L;

m is the weight of the raw material, g.

The protein content in cell-free supernatants was determined according to the Bradford method [20]. This method is based on the reaction of Coomassie with arginine and hydrophobic amino acid residues.

The quantitative determination of carbohydrates was carried out using the colorimetric method, which is based on the ability of both free sugars and those composed of monosaccharide residues of homo- and heteropolymers to create a yellow-brown color during interaction with phenol and sulfuric acid [21].

The total content of phenols was determined using the Folin-Ciocalteu reagent [22]. The reaction mixture contained the following components: the test sample — 1 mL, Folin-Ciocalteu reagent : H_2O = 1 : 9 — 2.5 mL. After mixing, the samples were placed in a dark place for three minutes, then 2 mL of 3.5 % sodium carbonate were added to each sample and placed in a dark place again for two hours. The total content of phenols was determined at 765 nm. The calibration curve was constructed using gallic acid. Statistical analysis of experimental data was performed using Microsoft Excel 2010.

Results. It was established that during the cultivation of wheat for 14 days in the Farreus medium, which contained 10^5 cells/mL of *A. vinelandii* IMV B-7076, the stimulating effect of these bacteria on the growth and mass of plants was insignificant. The length of the root grew by only 1.8 %, the length of the stem — by 8.8 %. At the same time, the average weight of plants increased by 27.3 % compared to the control (azotobacter was not used) (Table 1).

When wheat was grown in the medium with 10^6 cells/mL of these bacteria, the morphometric indicators of the plants increased more noticeably. The indices for the length of the root were 12.3 % higher, for the stem — 8.2 % higher, and for the average plant weight — 27.3 % higher than those in the control.

The most noticeable stimulatory effect was observed for growing wheat in the medium with the addition of 10^7 cells/mL. Under these conditions, compared to the control, the root length increased by 45.6 %, the stem length — by

Table 1. ***The influence of different contents of Azotobacter vinelandii IMV B-7076 on the morphometric indicators of wheat plants when grown in the Farreus medium***

Variants of research	Average root length, cm / %	Average stem length, cm / %	Average mass of plants, g / %
Medium without *A. vinelandii*	5.7 ± 1.4 100.0	18.2 ± 1.8 100.0	0.11 ± 0.01 100.0
A. vinelandii, (3.00 ± 0.06) · 10^5 cells/mL	5.8 ± 1.4 101.8	19.8 ± 1.9 108.8	0.14 ± 0.02 127.3
A. vinelandii, (2.32 ± 0.02) · 10^6 cells/mL	6.4 ± 0.8 112.3	19.7 ± 0.9 108.2	0.14 ± 0.01 127.3
A. vinelandii, (2.23 ± 0.03) · 10^7 cells/mL	8.3 ± 1.0 145.6	23.0 ± 1.8 126.4	0.18 ± 0.02 163.6

26.4 %, and the average plant weight — by 63.6 % (Table 1).

It was established that after 14 days of growing plants in a medium containing *A. vinelandii* IMV B-7076 in the amount of 10^5 cells/mL, the content of chlorophyll *a* in the leaves was 4 % higher than the value in the control (Table 2), and those for chlorophyll *b* and carotenoids were lower than the indicators in the control.

When plants were grown in the medium with *A. vinelandii* IMV B-7076 in the amount of 10^6 cells/mL, the content of chlorophyll *a* increased by 9 % compared to the control, and that for chlorophyll *b* increased slightly. Growing wheat in a medium containing 10^7 cells/mL increased the content of chlorophyll *a* and chlorophyll *b* by 13 and 11 %, respectively. At the same time, the concentration of carotenoids was slightly lower than the values in the control.

The cultivation of wheat plants in Farreus medium containing *A. vinelandii* IMV B-7076 cells increased the protein content in root exudates (Fig. 1).

At the level of 10^5 and 10^6 cells/mL of these bacteria in this medium, the protein content in the exudates increased by 68 % and 55 %, respectively, compared to the control. When wheat was grown in the medium with the addition of 10^7 cells/mL, the protein content in root exudates increased more than twice compared to the control.

The cultivation of wheat plants in the Farreus medium, which contained the *A. vinelandii* IMV B-7076 bacteria, influenced the accumulation of phenolic compounds in it (Fig. 2). When these plants were grown in the control variant (without bacteria), the content of these compounds in the medium was 0.66 µg/mL.

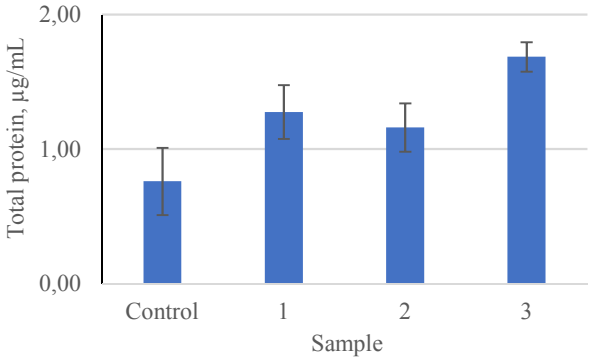

Fig. 1. Protein content in the Farreus medium in case of wheat cultivation for 14 days and introduction of different amounts of A. vinelandii bacteria: 1 — 10^5 cells/mL; 2 — 10^6 cells/mL; 3 — 10^7 cells/mL.

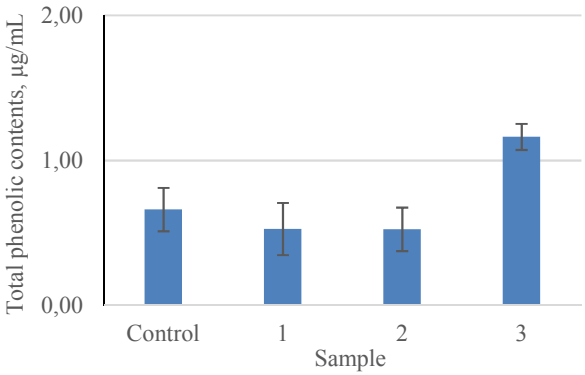

Fig. 2. The soil of phenolic compounds in the Farreus medium for the cultivation of wheat for 14 days and the introduction of different amounts of A. vinelandii bacteria: 1 — 10^5 cells/mL; 2 — 10^6 cells/mL; 3 — 10^7 cells/mL.

When 10^5 or 10^6 cells/mL of these bacteria were added to the medium, the content of these compounds decreased by almost 20 % compared to the control. At the same time, during

Table 2. ***The content of chlorophyll a and b and carotenoids in wheat plants when grown in the Farreus medium with different contents of Azotobacter vinelandii IMV B-7076***

Variants of research	Content of photosynthetic pigments, mg/g		
	chlorophyll *a*	chlorophyll *b*	carotenoids
Control (plants without azotobacter)	1.28 ± 0.18	0.80 ± 0.09	0.35 ± 0.04
Wheat plants + *A. vinelandii*, $(3.00 \pm 0.06) \cdot 10^5$ cells/mL	1.33 ± 0.07	0.66 ± 0.08	0.34 ± 0.04
Wheat plants + *A. vinelandii*, $(2.32 \pm 0.02) \cdot 10^6$ cells/mL	1.40 ± 0.02	0.82 ± 0.09	0.32 ± 0.04
Wheat plants + *A. vinelandii*, $(2.23 \pm 0.03) \cdot 10^7$ cells/mL	1.45 ± 0.14	0.88 ± 0.09	0.34 ± 0.05

the cultivation of these plants in the medium containing 10^7 cells/mL of azotobacter, the content of phenolic substances increased by 79.8 % compared to the control (Fig. 2).

The cultivation of wheat plants in an environment with the introduced *A. vinelandii* IMV B-7076 had a noticeable effect on the accumulation of carbohydrates in root exudates (Table 3). While the carbohydrate content of dry exudate in the control was 26.8 μg/mg, during the cultivation of plants in a medium containing 10^5 cells/mL the amount of carbohydrates increased to 41.8 μg/mg of dry exudate mass, and with azotobacter in the amount of 10^6 and 10^7 cells/mL, the concentration of carbohydrates increased to 58.7 and 65.7 μg/mg.

Table 3. ***Total carbohydrate content in dried preparations of exudates of wheat grown with Azotobacter vinelandii IMV B-7076***

Variants of research	Carbohydrate content in exudates, μg/mg
Control (plants without azotobacter)	26.80 ± 1.30
Wheat plants + *A. vinelandii,* $(3.00 \pm 0.06) \cdot 10^5$ cells/mL	41.80 ± 2.10
Wheat plants + *A. vinelandii,* $(2.32 \pm 0.02) \cdot 10^6$ cells/mL	58.73 ± 2.55
Wheat plants + *A. vinelandii,* $(2.23 \pm 0.03) \cdot 10^7$ cells/mL	65.77 ± 3.29

Thus, the cultivation of Shestopalivka wheat plants in hydroponic conditions with *A. vinelandii* IMV B-7076 in the medium caused a noticeable stimulating effect on the morphometric indicators of the plants, the content of photosynthetic pigments in them, the accumulation of proteins, phenolic substances, and carbohydrates in root exudates.

Discussion. The intensive use of chemical fertilizers, pesticides, and other chemical compounds in crop production harms the environment, reduces the quality of the obtained plant products, and can have a negative impact on people's health. With this in mind, in the last decade, considerable attention has been paid to the biologization of crop production [23]. An essential component of this approach is the use of microbial preparations in crop production, which can improve the growth, development, and productivity of plants without harming the environment. One of them is the complex bacterial preparation Azogran. This drug improves the growth and productivity of plants significantly [9; 10]. Its component is *Azotobacter vinelandii* IMV B-7076.

The results of our research show that under hydroponic cultivation of Shestopalivka wheat plants, the effect of this strain on the growth activity of plants depends on the concentration of these bacteria in the cultivation medium. After 14 days of growing wheat with 10^7 cells/mL of *A. vinelandii* IMV B-7076 in the medium, the length of the plant stems was 26.4 % longer compared to the control. The obtained data confirm the results of our previous research on the stimulating effect of the Azogran preparation on the morphometric indicators of wheat plants of other varieties [24].

A significant role in the growth and productivity of plants belongs to photosynthetic pigments, chlorophyll *a* and *b*, and carotenoids, which, in the process of functioning in the plant leaves, transform the energy of solar radiation into the energy of chemical bonds, which is the basis for the functioning of living organisms. We have shown that when growing wheat plants in the medium with *A. vinelandii* IMV B-7076, the content of chlorophyll *a* increases and is higher than the index of chlorophyll *b* and carotenoids.

It is known that photosynthetic pigments are extremely sensitive to the physiological state of plants. Even a short-term stress leads to a change in the total content of these pigments [25]. In plants, affected by phytopathogens, the content of chlorophyll *b* increases significantly [26], while the content of carotenoids can decrease considerably [27].

The results of our research demonstrate a positive effect of the preparation Azogran on the functioning of the photosynthetic system of wheat plants, on their development and accumulation of phenols, proteins, and carbohydrates in the root exudates of these plants.

Conclusions. The results of our study demonstrated a positive effect of *A. vinelandii* IMV B-7076 bacteria on the growth and development of wheat under conditions of hydroponic cultivation and the accumulation of biologically active substances in the root exudates of these plants. It was established that the highest stimulatory effect of these bacteria was

observed when growing plants in the medium with the addition of 10^7 cells/mL of *A. vinelandii* IMV B-7076. Under these conditions, the length of the stem was 26.4 % greater than the indices in the control, and the weight of the plants increased by 63.6 %. The content of chlorophyll *a* and *b* increased by 13 % and 11 %, respectively. When growing wheat in the medium containing such amounts of azotobacter, the content of proteins, phenolic compounds, and carbohydrates in plant root exudates increased significantly. The obtained results demonstrated a significant stimulating effect of *A. vinelandii* IMV B-7076 on the growth and development of wheat plants and the synthesis of exometabolites by these plants.

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ВПЛИВ *AZOTOBACTER VINELANDII* ІМВ В-7076 НА РІСТ ПШЕНИЦІ ТА СИНТЕЗ ЕКЗОМЕТАБОЛІТІВ В УМОВАХ ГІДРОПОНІКИ

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Мета. *Визначити вплив бактерії Azotobacter vinelandii ІМВ В-7076 на синтез екзометаболітів рослинами пшениці в умовах in vitro.* ***Методи.*** *Досліди проводили з пшеницею озимою сорту Шестопалівка, насіння якої після поверхневої стерилізації вирощували впродовж 14 діб в умовах гідропоніки з бактерією A. vinelandii ІМВ В-7076. Бактерії вирощували в колбах Ерленмейєра на 700 мл, що містили 100 мл середовища Ешбі, та інкубували на орбітальних шейкерах при 220 об./хв. за 28 °С протягом двох діб. Рослини пшениці вирощували в циліндричних скляних посудинах об'ємом 1,5 л, діаметром 115 мм, висотою 160 мм. Сітки*

з нержавіючої сталі поміщали в посудини на відстані 5 мм від дна. Посудини стерилізували, після чого додавали 75 мл стерильного середовища Фарреуса. Після пророщування насіння п'ять паростків поміщали на нержавіючі сітки в склянки. До середовища додавали суспензії азотобактера в трьох різних розведеннях (10^5, 10^6 і 10^7 клітин/мл відповідно). Визначали вплив бактерій на морфометричні показники рослин, вміст хлорофілів і каротиноїдів у листках рослин, накопичення вуглеводів, білків і фенольних сполук у середовищі з кореневими ексудатами. ***Результати.*** *Встановлено, що вирощування пшениці на середовищі з бактерією A. vinelandii IMB B-7076 позитивно впливало на ріст рослин, вміст фотосинтетичних пігментів у листках та накопичення білків, фенолів і вуглеводів у кореневих ексудатах. Стимулювальна дія азотобактера зростала зі збільшенням вмісту клітин у середовищі. При вирощуванні рослин на середовищі з 10^7 кл./мл довжина паростків збільшилася на 26,4 % проти контролю (без азотобактера), а маса рослин підвищилася на 63,6 %. При вирощуванні рослин пшениці за таких умов уміст білків і вуглеводів у середовищі з кореневими ексудатами рослин зріс більш ніж удвічі проти контролю, а фенольних сполук — на 79,8 %.* ***Висновки.*** *A. vinelandii IMB B-7076 позитивно впливає на ріст і розвиток рослин пшениці та синтез ними екзометаболітів.*

Ключові слова: *Azotobacter vinelandii, пшениця, феноли, вуглеводи, хлорофіли, каротиноїди.*

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