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Research Article

Synergistic Effect of Synthetic Plant Growth Regulators Ivin, Methyur, Kamethur and Biofertilizers Rostok Extra and Radix Tim Forte+ On Wheat Growth During the Vegetation Period

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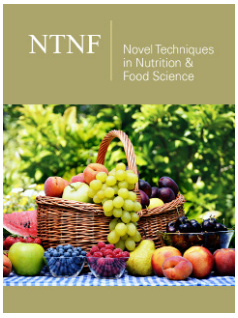

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Abstract

The effect of new environmentally friendly synthetic plant growth regulators Ivin, Methyur, Kamethur and biofertilizers Rostok Extra and Radix Tim forte+, used separately and in combination, on the growth of an important cereal crop - winter wheat (*Triticum aestivum* L.) variety Mudríst' Odes'ka during the vegetation period was studied. Their regulatory activity on wheat growth was compared with the regulatory activity of auxins NAA and 2,4-D. In wheat plants treated with the synthetic plant growth regulators Ivin, Methyur, Kamethur and biofertilizers Rostok Extra and Radix Tim forte+, used separately and in combination, an increase in such morphometric indicators as length of the shoots (mm), length of the roots (mm) and plant biomass (g), as well as biochemical indicators such as the content of chlorophylls and carotenoids (mg/g FW) and the content of total soluble protein (g/100 g FW) was observed compared to similar indicators in control wheat plants treated with distilled water. The regulatory activity of the synthetic plant growth regulators Ivin, Methyur, Kamethur and biofertilizers Rostok Extra and Radix Tim forte+, used separately and in combination, on wheat indicators was similar to or exceeded the regulatory activity of the synthetic auxins NAA and 2,4-D. The obtained results allow us to conclude that synthetic plant growth regulators and biofertilizers exhibit a synergistic auxin-and cytokinin-like effects in vital processes of wheat, namely: they enhance the division, elongation and differentiation of plant cells, improve the uptake of water and nutrients by the root system, increase the efficiency of photosynthesis and protein metabolism, which contributes to the enhanced growth and development of wheat. The findings from this study could be valuable for farmers looking to optimize wheat production and for researchers interested in plant growth regulation.

Keywords: *Triticum aestivum* L; Synergistic effect of plant growth regulators and biofertilizers; Plant growth; Uptake of water and nutrients; Photosynthesis; Protein metabolism

Introduction

A pressing issue in agriculture is the development of new environmentally friendly technologies for growing an important grain crop - wheat, which is cultivated throughout the world, in order to obtain organic agricultural products to provide food for the world's population and reduce environmental pollution [1-4]. Wheat is already crucial for food security; its yield is the agronomically most important aspect of the crop phenotype, resulting from a tripartite interaction between genotype, environment and management; since a significant increase in the area under cultivation is unlikely, higher yields per unit area and time must match the expected increase in demand for wheat [5]. Over the past decades, wheat yields have declined significantly due to global climate change, drought and heat stress, soil

salinization and depletion, shortening the duration of the growing season, as well as the spread of pathogens and pests that negatively impact plant growth and development [4]. In addition, various factors that have an important impact on wheat yield, such as sowing depth and dates, irrigation and weather conditions, as well as simplified crop rotations based on cereals, which are widely used for economic reasons, lead to the cultivation of wheat after wheat and the associated yield losses [6-8]. A study conducted in Northern Germany on a Stagnic Luvisol from 2006 to 2018 with winter wheat after the four most widely used preceding crops in the region (sugar beet, winter wheat, silage maize and winter oilseed rape) showed that crop rotations with winter oilseed rape and sugar beet were found to be favorable preceding crops for winter wheat and led to a significantly higher yield of about 1.00 and 0.43t/ha, respectively, compared to wheat after wheat [8].

As is well known, increasing crop yields and improving the quality of agricultural products depends primarily on a scientifically based fertilization system, as evidenced by FAO data indicating that Western European countries and the United States obtain a third of their crop yields through the use of fertilizers [9,10]. The introduction of fertilizer application technologies into agricultural practice makes it possible to obtain high yields with good product quality in virtually all climatic zones [9,10]. Practical use of breeding tools to improve economically important characteristics of varieties in combination with exogenous application of phytohormones, synthetic plant growth regulators, natural biostimulants, organic and mineral fertilizers contribute to improving wheat growth at the vegetative and reproductive stages, increasing yield and increasing resistance to abiotic and biotic stress factors [11-23]. It should also be noted that the combined use of synthetic plant growth regulators with biofertilizers or Plant Growth Promoting Rhizobacteria (PGPR) opens up broad prospects for managing plant growth and development processes and increasing their yield [24,25]. A promising strategy for organic farming is the development of new effective plant growth regulators based on synthetic analogues of auxins and cytokinins, which have a chemical structure similar to or different from that of natural auxins and cytokinins-essential phytohormones that play a key role in regulating plant growth and development at all stages of ontogenesis, as well as in controlling plant resistance to various abiotic and biotic stresses that significantly limit crop yields [26-35]. New opportunities for screening compounds with auxin-and cytokinin-like activity are opened by synthetic low-molecular-weight azaheterocyclic compounds, pyridine and pyrimidine derivatives, which have found practical application in agriculture as effective and environmentally friendly substitutes for traditional plant growth regulators, herbicides, fungicides and antimicrobial agents [36- 44].

Scientists of the V.P. Kukhar Institute of Bioorganic Chemistry and Petrochemistry of the National Academy of Sciences of Ukraine have developed methods for synthesis of new environmentally friendly plant growth regulators based on synthetic low-molecular-weight azaheterocyclic compounds, derivatives of N-oxide-2,6-dimethylpyridine (Ivin), sodium and potassium salts of 6-methyl-2-mercapto-4-hydroxypyrimidine (Methyur and Kamethur), as well as new synthetic azaheterocyclic compounds, pyridine and pyrimidine derivatives, which exhibit a regulatory effect related to the phytohormones auxins and cytokinins on cereals, legumes and industrial crops, including wheat, increase their yield and enhance adaptive properties to abiotic stress factors [45-57]. In our previous studies it was found that the use of synthetic low-molecular-weight azaheterocyclic compounds such as Ivin, Methyur and Kamethur separately or in combination with biofertilizers Rostock Extra produced by LLc "Ukrainian Agrarian Resource" company and Radix Tim forte+ produced by "Forcrop" Company (Spain), improves the growth and development of wheat, sunflower, oilseed flax and rapeseed during the vegetative phase [58-61]. Thanks to the use of new environmentally friendly plant growth regulators based on synthetic compounds such as Ivin, Methyur, Kamethur in non-toxic low molar concentrations separately and in combination with biofertilizers, it is possible to improve seed germination, enhance the organogenesis of roots, shoots, leaves and reproductive organs of plants, activate photosynthesis in plant leaves, increase crop yields and reduce the use of environmentally toxic agrochemicals for plant protection and improve the ecological state of the entire agricultural system.

Thus, based on the literature data and the results of our previous studies, the use of breeding tools to improve economically important traits of varieties in combination with exogenous application of phytohormones, natural biostimulants, synthetic plant growth regulators, biofertilizers, especially in the tillering stage, ensuring regular irrigation, especially during dry periods, to support the crop's water needs and accelerate growth, the selection of favorable predecessors for wheat in crop rotation and appropriate sowing dates, post-harvest technologies, as well as integrated pest and weed control, especially in warm summer conditions, to protect against high levels of disease are extremely important strategies for managing wheat yield and crop quality. This work is devoted to a very promising and relevant topic: the development of new environmentally friendly agrobiotechnological approaches to improving the growth of an important cereal crop - wheat using new plant growth regulators based on synthetic low-molecular-weight azaheterocyclic compounds, such as Ivin, Methyur, Kamethur, as well as the biofertilizers Rostock Extra and Radix Tim forte+.

Materials and Methods

Phytohormones, plant growth regulators and biofertilizers used in research

A comparative study of the regulatory effect of synthetic plant hormones auxins NAA (1-Naphthylacetic acid) and 2,4-D (2,4-dichlorophenoxyacetic acid) and new plant growth regulators based on synthetic low-molecular-weight azaheterocyclic compounds, derivatives of N-oxide-2,6-dimethylpyridine (Ivin), sodium and potassium salts of 6-methyl-2-mercapto-4-hydroxypyrimidine (Methyur and Kamethur), used separately or in combination with biofertilizers Rostock Extra and Radix Tim forte+, on the growth of an important cereal crop - winter

wheat (*Triticum aestivum* L.) variety Mudríst' Odes'ka during the vegetation period was conducted. Synthetic plant hormones auxins NAA (1-Naphthylacetic acid) and 2,4-D (2,4-dichlorophenoxyacetic acid) were manufactured by Sigma-Aldrich, USA (Figure 1).

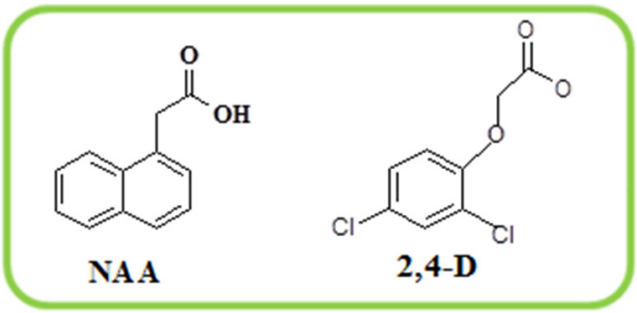

Figure 1: Chemical structures and relative molecular weight (MW, g/mol) of synthetic plant hormones auxins NAA (1-Naphthylacetic acid, MW=186.21) and 2,4-D (2,4-dichlorophenoxyacetic acid, MW=221.04).

New plant growth regulators based on synthetic low-molecular-weight azaheterocyclic compounds, derivatives of N-oxide-2,6-dimethylpyridine (Ivin), sodium and potassium salts of 6-methyl-2-mercapto-4-hydroxypyrimidine (Methyur and Kamethur) have been developed at the Department for Chemistry of Bioactive Nitrogen-Containing Heterocyclic Compounds, V.P. Kukhar Institute of Bioorganic Chemistry and Petrochemistry of the National Academy of Sciences of Ukraine (Figure 2).

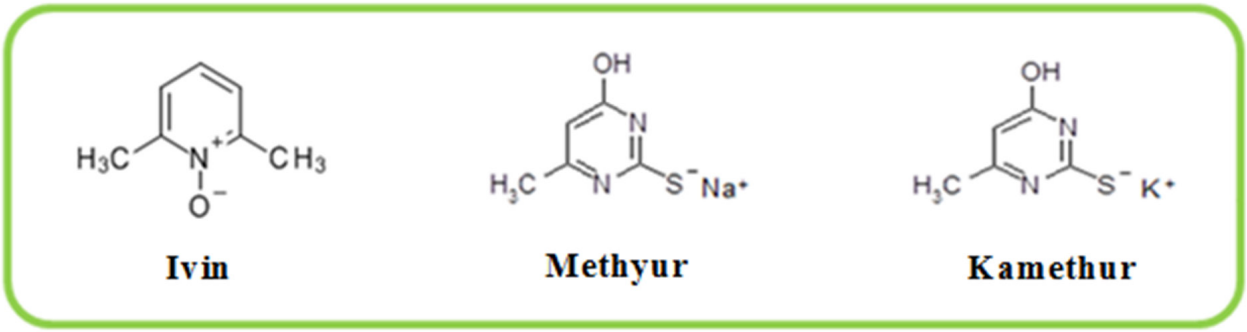

Figure 2: Chemical structures and relative molecular weight (MW, g/mol) of new synthetic plant growth regulators: Ivin (N-oxide-2,6-dimethylpyridine, MW=125.17), Methyur (sodium salt of 6-methyl-2-mercapto-4-hydroxypyrimidine, MW=165.17) and Kamethur (potassium salt of 6-methyl-2-mercapto-4-hydroxypyrimidine, MW=181.28).

Biofertilizer Rostok Extra produced by the LLc "Ukrainian Agrarian Resource" company is complex, liquid, chelated fertilizer intended for pre-sowing seed treatment, foliar fertilization and drip irrigation. It is a universal fertilizer that improves seed germination, plant resistance to stress factors, increases yield and improves the quality of products of most agricultural, vegetable, fruit and berry crops. Its chemical composition is as follows (g/l): Nitrogen (N)-100, Phosphorus (P_2O_5)-300, Potassium (K_2O)-100, Magnesium (MgO)-22, Sulfur (SO_3)-55, trace elements (Mn-10, B-2, Zn-25, Cu-5), amino acids, humic substances [58]. Biofertilizer Radix Tim forte plus, produced by the "Forcrop" Company (Spain), is a root growth stimulator for fruit, berry, decorative and vegetable crops grown in open and closed soil. It provides the root system with activators and minerals for its proper development and rejuvenation and also protects the root system from stress and fungal attacks that can harm its maximum development. It is used both for better rooting after transplantation and throughout the growing season of the plant to maintain the root system in a healthy and functional state. Its chemical composition is as follows (W/V): Nitrogen (N) - 3.7%, Potassium (K_2O)-4.1% Phosphorus (P_2O_5) - 11.3%, free amino acids - 5.7%, trace elements (Zn, Mn, B, Mo and Fe) [58].

Seed treatment and plant growing conditions

Seeds of winter wheat (*Triticum aestivum* L.) variety Mudríst' Odes'ka were soaked with auxins NAA and 2,4-D at a concentration of 10^{-6}M, or with synthetic plant growth regulators: Methyur, Kamethur, Ivin at a similar concentration of 10^{-6}M or with biofertilizer Rostok Extra at a concentration of 100 ml per 1liter of distilled water, or with biofertilizer Radix Tim forte plus at a concentration of 50 ml per 1liter of distilled water, or with a combination of each of the synthetic plant growth regulator Methyur, Kamethur, Ivin with fertilizers Rostok Extra or Radix Tim

forte plus, used in the above mentioned concentrations. After this procedure, the seeds were placed in a thermostat for germination in the dark at a temperature of 22-23 °C for 48h. The germinated seedlings were then placed in a climatic chamber and grown for four weeks under a light/dark regime of 16/8h, at a temperature of 22-23 °C, a light intensity of 3000lux and an air humidity of 60-80 %. Plant morphometric indicators, such as length of the shoots (mm), length of the roots (mm) and biomass (g) of 10 plants, were measured according to the methodological manual [62]. Morphometric indicators determined on experimental plants, compared with similar indicators of control plants, were expressed in %.

Determination of chlorophyll and carotenoid content in plant leaves

The content of photosynthetic pigments such as chlorophylls a, b and carotenoids (mg/g FW) in plant leaves was analyzed according to methodological recommendations [63,64]. To perform the extraction of photosynthetic pigments, we homogenized a sample (500 mg) of plant leaves in the porcelain mortar in a cooled at the temperature 10 °C 96 % ethanol at the ratio of 1:10 (weight: volume) with addition of 0,1-0,2 g $CaCO_3$ (to neutralize the plant acids). The 1 ml of obtained homogenate was centrifuged at 8000g in a refrigerated centrifuge K24D (MLW, Engelsdorf, Germany) during 5 min at the temperature 4 °C. The obtained precipitate was washed three times, with 1 ml 96% ethanol and centrifuged at above mentioned conditions. After this procedure, the optical density of chlorophyll a, chlorophyll b and carotenoid in the obtained extract was measured using spectrophotometer Specord M-40 (Carl Zeiss, Germany).

The content of chlorophyll a, chlorophyll b and carotenoids in plant leaves was calculated in accordance with formula [63,64]:

Cchl a=13.36×A664.2-5.19×A648.6,

Cchl b=27.43×A648.6-8.12A×664.2,

Cchl (a+b)=5.24×A664.2+22.24×A648.6,

Ccar = (1000×A470-2.13×Cchl a-97.64×Cchlb)/209,

Where, Cchl - concentration of chlorophylls (μg/ml), Cchl a - concentration of chlorophyll a (μg/ml), Cchl b - concentration of chlorophyll b (μg/ml), Ccar - concentration of carotenoids (μg/ml), A - absorbance value at a proper wavelength in nm.

The chlorophyll and carotenoids content per 1g of Fresh Weight (FW) of plant leaves was calculated by the following formula (separately for chlorophyll a, chlorophyll b and carotenoids):

$A_1=(C\times V)/(1000\times a_1)$,

Were, A_1 - content of chlorophyll a, chlorophyll b, or carotenoids (mg/g FW), C-concentration of pigments (μg/ml), V-volume of extract (ml), a_1-sample of plant leaves (g).

The content of photosynthetic pigments determined in experimental plants, compared with similar parameters of control plants, was expressed in %.

Determination of total soluble protein content

The content of total soluble protein (g/100 g FW) in plant leaves was determined by the Bradford technique [65]. To make the plant extracts, a sample (100 mg) of plant leaves was homogenized in a porcelain mortar in a 0.1 M sodium phosphate buffer (pH 6.0-8.0) at a weight-to-volume ratio of 1:5 at 4 °C for 1h. The resulting homogenates were centrifuged at 8000 g in a refrigerated centrifuge K24D (manufactured by MLW, Engelsdorf, Germany) at 4 °C for 15 min. A volume of 1.5 ml of distilled water and 1.5 ml of Coomassie Brilliant Blue G-250 reagent (manufactured by Bio-Rad, 500-0006) were added to 50ml of the obtained supernatant. The resulting mixture was stirred for 10 min. The Optical Density (OD) of total soluble protein was then determined using spectrophotometer Specord M-40 (Carl Zeiss, Germany) at a wavelength of 595 nm. The total soluble protein content (g of protein per 100 g of Fresh Weight (FW) of plant leaves) in the sample was quantified using a calibration curve based on the Optical Density (OD) measurements of standard samples containing 1.5 ml of Bovine Serum Albumin (BSA) solution and 1.5ml of Coomassie Brilliant Blue G-250 reagent (manufactured by Bio-Rad, 500-0006). The total soluble protein content determined in experimental plants, compared with similar parameters of control plants, was expressed in %.

Statistical data analysis

Statistical processing of the data of the experiments performed in three replications was carried out according to the student's-t variance test with a significance level of $P\leq0.05$; the values are average ± SD.

Results and Discussion

Analysis of morphometric indicators of wheat

A comparative study of the regulatory effect of synthetic plant hormones auxins NAA (1-Naphthylacetic acid) and 2,4-D (2,4-dichlorophenoxyacetic acid) and new plant growth regulators based on synthetic low-molecular-weight azaheterocyclic compounds, derivatives of sodium and potassium salts of 6-methyl-2-mercapto-4-hydroxypyrimidine (Methyur and Kamethur) and N-oxide-2,6-dimethylpyridine (Ivin), used separately or in combination with biofertilizers Rostock Extra and Radix Tim forte+, on such morphometric indicators of wheat as length of the shoots (mm), length of the roots (mm) and biomass (g) of 10 plants was carried out. It has been shown that treatment of wheat seeds with synthetic plant growth regulators, such as Methyur, Kamethur, Ivin, used at a concentration of 10^{-6}M separately or in combination with biofertilizers Rostock Extra and Radix Tim forte+, considerably improved the growth and development of roots and shoots of wheat plants during the vegetative phase (Figure 3).

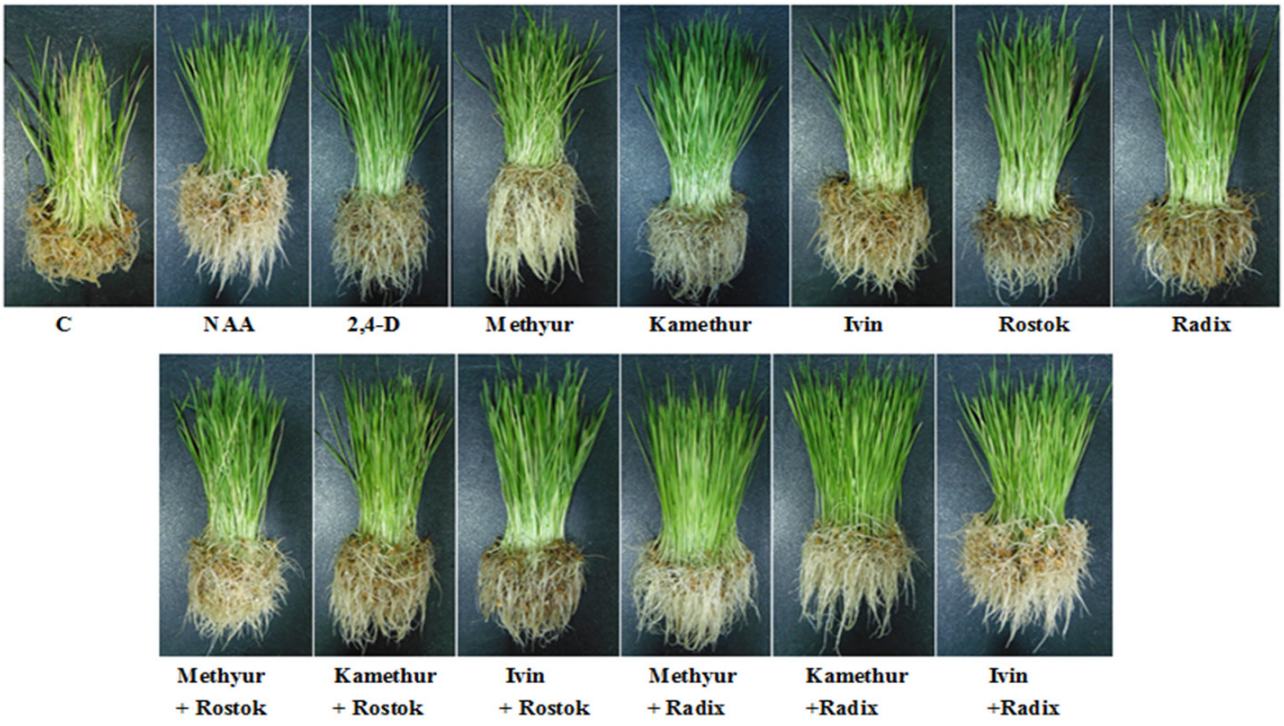

Figure 3: Shoots and roots of 4-week-old wheat (*T. aestivum* L.) variety Mudrist' Odes'ka, grown under the regulatory effect of auxins NAA and 2,4-D and synthetic plant growth regulators: Methyur, Kamethur, Ivin, used at a concentration of 10^{-6}M separately or in combination with biofertilizers Rostock Extra and Radix Tim forte+, in comparison with control plants.

Morphometric indicators of plants, such as length of the shoots (mm), length of the roots (mm) and biomass (g) of 10 plants, treated with synthetic plant growth regulators Methyur, Kamethur, Ivin, used separately or in combination with biofertilizers Rostock Extra and Radix Tim forte+, were similar to or higher than the indicators of plants treated with auxins NAA and 2,4-D and considerably higher than the indicators of the control plants (Figures 4-6). The indicators of the length of the shoots (mm) increased: by 40,39% - under treatment with NAA, by 38,46% - under treatment with 2,4-D, by 48,1% - under treatment with Methyur, by 46,15% - under treatment with Kamethur, by 16,35% - under treatment with Ivin, by 31,25% - under treatment with Rostock Extra, by 36,54% - under treatment with Methyur+Rostock Extra, by 42,79% - under treatment with Kamethur+Rostock Extra, by 36,54% - under treatment with Ivin+Rostock Extra, by 29,52% - under treatment with Radix Tim forte+, by 38,46% - under treatment with Methyur+Radix Tim forte+, by 32,69% - under treatment with Kamethur+Radix Tim forte+, by 19,23% - under treatment with Ivin+Radix Tim forte+, compared to the indicators of the control plants (Figure 4).

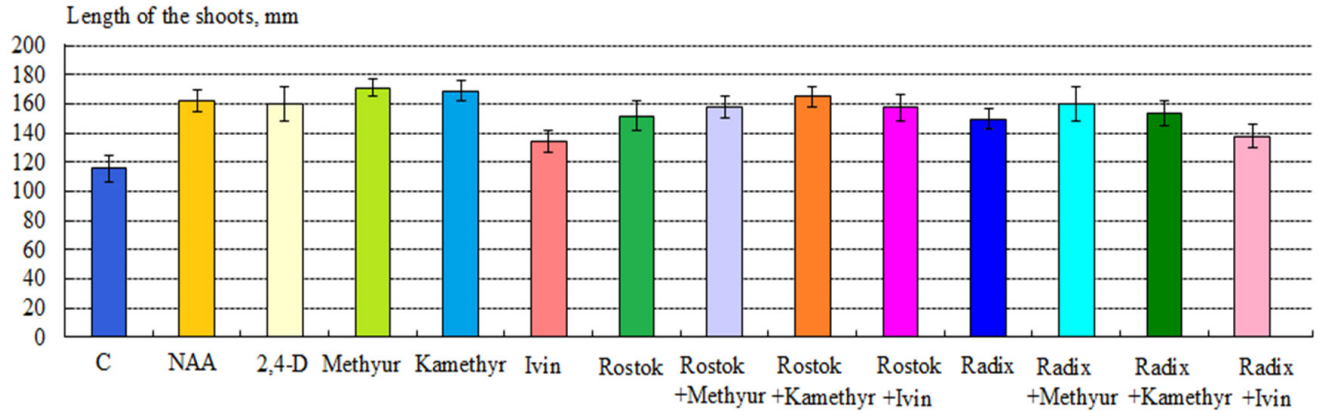

Figure 4: Length of the shoots (mm) of 4-week-old wheat (*T. aestivum L.*) variety Mudrist' Odes'ka, grown under the regulatory effect of auxins NAA and 2,4-D and synthetic plant growth regulators: Methyur, Kamethur, Ivin, used at a concentration of 10^{-6}M separately or in combination with biofertilizers Rostock Extra and Radix Tim forte+, in comparison with control plants.

The indicators of the length of the roots (mm) increased: by 91,18%-under treatment with NAA, by 176,47% -under treatment with 2,4-D, by 276,47% -under treatment with Methyur, by 288,24%-under treatment with Kamethur, by 79,42% -under treatment with Ivin, by 102,94% -under treatment with Rostock Extra, by 164,71% -under treatment with Methyur+Rostock Extra, by 182,35% -under treatment with Kamethur+Rostock Extra, by 100% -under treatment with Ivin+Rostock Extra, by 76,47% -under treatment with Radix Tim forte+, by 194,12% -under treatment with Methyur+Radix Tim forte+, by 123,53% - under treatment with Kamethur+Radix Tim forte+, by 100% -under treatment with Ivin+Radix Tim forte+, compared to the indicators of the control plants (Figure 5). The indicators of the biomass (g) of 10 plants increased: by 54,53% - under treatment with NAA, by 58,55% -under treatment with 2,4-D, by 59,55% -under treatment with Methyur, by 64,63% - under treatment with Kamethur, by 39,44% - under treatment with Ivin, by 57,75% -under treatment with Rostock Extra, by 87,45% - under treatment with Methyur+Rostock Extra, by 90,53% -under treatment with Kamethur+Rostock Extra, by 85,29% -under treatment with Ivin+Rostock Extra, by 59,85% -under treatment with Radix Tim forte+, by 73,46% -under treatment with Methyur+Radix Tim forte+, by 80,28% -under treatment with Kamethur+Radix Tim forte+, by 63,95% - under treatment with Ivin+Radix Tim forte+, compared to the indicators of the control plants (Figure 6).

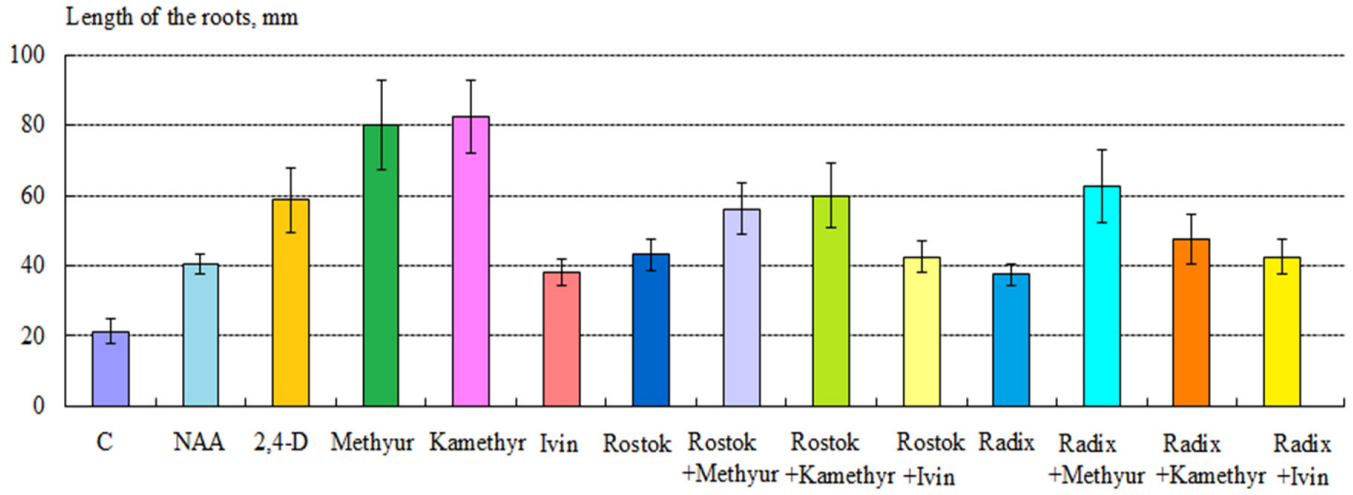

Figure 5: Length of the roots (mm) of 4-week-old wheat (*T. aestivum* L.) variety Mudrist' Odes'ka, grown under the regulatory effect of auxins NAA and 2,4-D and synthetic plant growth regulators: Methyur, Kamethur, Ivin, used at a concentration of 10^{-6}M separately or in combination with biofertilizers Rostock Extra and Radix Tim forte+, in comparison with control plants.

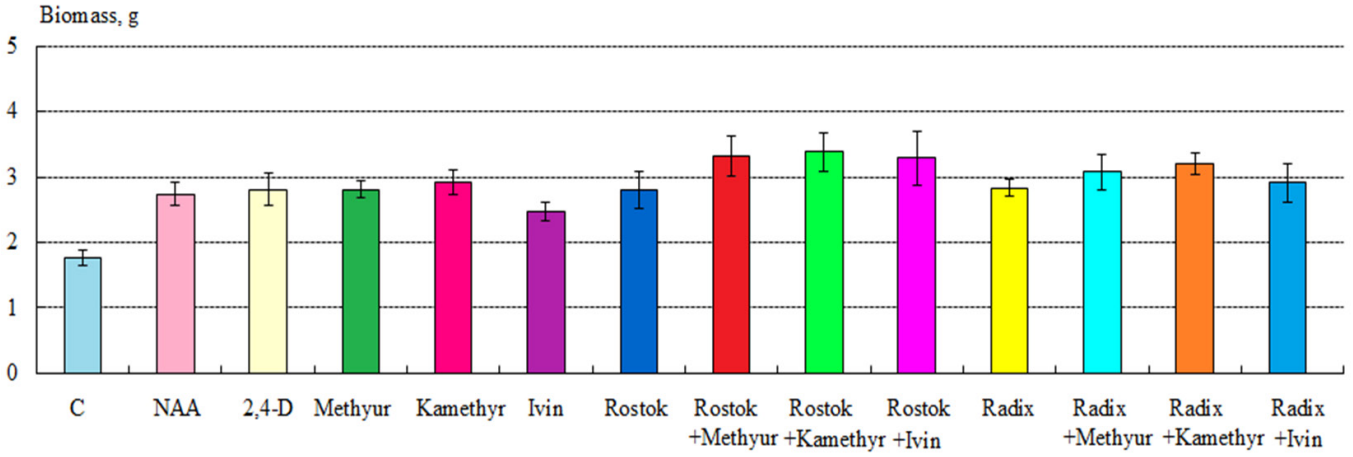

Figure 6: Biomass (g) of 10 plants of 4-week-old wheat (*T. aestivum* L.) variety Mudrist' Odes'ka, grown under the regulatory effect of auxins NAA and 2,4-D and synthetic plant growth regulators: Methyur, Kamethur, Ivin, used at a concentration of 10^{-6}M separately or in combination with biofertilizers Rostock Extra and Radix Tim forte+, in comparison with control plants.

Analysis of Biochemical Indicators of Wheat

Determination of chlorophyll and carotenoid content

A comparative study of the regulatory effect of synthetic plant hormones auxins NAA (1-Naphthylacetic acid) and 2,4-D (2,4-dichlorophenoxyacetic acid) and new plant growth regulators based on synthetic low-molecular-weight azaheterocycle compounds, derivatives of sodium and potassium salts of 6-methyl-2-mercapto-4-hydroxypyrimidine (Methyur and Kamethur), N-oxide-2,6-dimethylpyridine (Ivin), used separately or in combination with biofertilizers Rostock Extra and Radix Tim forte+, on such biochemical indicators as the content of chlorophylls and carotenoids (mg/g FW) in wheat leaves was conducted. The results obtained shows that the treatment of wheat seeds with new plant growth regulators, such as Methyur, Kamethur, Ivin, used at a concentration of 10^{-6}M separately or in combination with biofertilizers Rostock Extra and Radix Tim forte+, contributed to the improvement of the photosynthesis process in wheat leaves grown during the vegetation period. Biochemical indicators of plants, such as the content of chlorophylls and carotenoids (mg/g FW), treated with synthetic plant growth regulators Methyur, Kamethur, Ivin, used separately or in combination with biofertilizers Rostock Extra and Radix Tim forte+, were similar to the indicators of plants treated with auxins NAA and 2,4-D and higher than the indicators of the control plants (Figure 7). The content of chlorophyll a increased: by 23,13% - under treatment with NAA, by 6,51%-under treatment with 2,4-D, by 15,81%-under treatment with Methyur, by 7,33%-under treatment with Kamethur, by 22,73%-under treatment with Ivin, by 8,1%-under treatment with Methyur+Rostock Extra, by 10,65%-under treatment with Kamethur+Rostock Extra, by 7,4%-under treatment with Ivin+Rostock Extra, by 5,98%-under treatment with Radix Tim forte+, by 6,05%-under treatment with Methyur+Radix Tim forte+, by 11,27%-under treatment with Kamethur+Radix Tim forte+, by 4,62%-under treatment with Ivin+Radix Tim forte+, compared to the indicators of the control plants (Figure 7). At the same time, no statistically significant changes in content of chlorophyll a in-wheat leaves treated with Rostock Extra were detected compared to the indicators of the control plants (Figure 7).

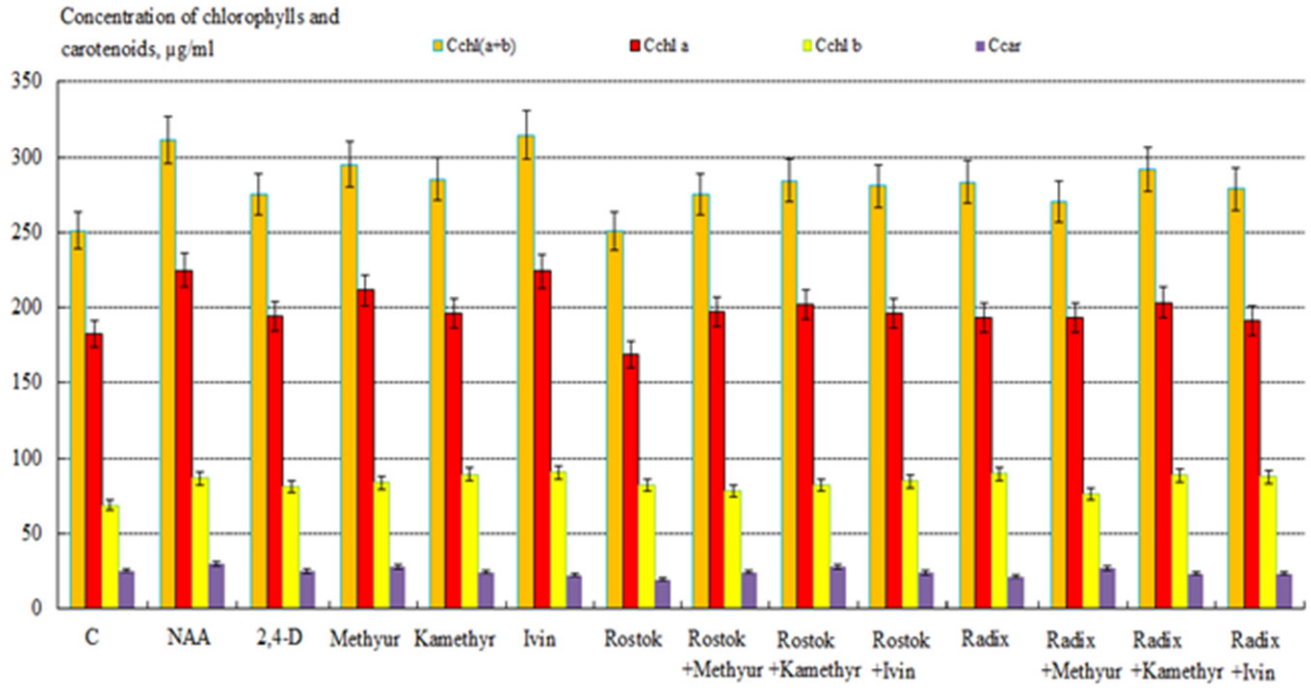

Figure 7: The content of chlorophylls and carotenoids (mg/g FW) in the leaves of 4-week-old wheat (*T. aestivum* L.) variety Mudrist' Odes'ka, grown under the regulatory effect of auxins NAA and 2,4-D and synthetic plant growth regulators: Methyur, Kamethur, Ivin, used at a concentration of 10^{-6}M separately or in combination with biofertilizers Rostock Extra and Radix Tim forte+, in comparison with control plants.

The content of chlorophyll b increased: by 26,25%-under treatment with NAA, by 17,76%-under treatment with 2,4-D, by 21,83% - under treatment with Methyur, by 29,87%-under treatment with Kamethur, by 31,85%-under treatment with Ivin, by 19,1%-under treatment with Rostock Extra, by 13,8%-under treatment with Methyur+Rostock Extra, by 19,55% - under treatment with Kamethur+Rostock Extra, by 23,37%-under treatment with Ivin+Rostock Extra, by 30,64%-under treatment with Radix Tim forte+, by 11,43%-under treatment with Methyur+Radix Tim forte+, by 29,16%-under treatment with Kamethur+Radix Tim forte+, by 27,88%-under treatment with Ivin+Radix Tim forte+, compared to the indicators of the control plants (Figure 7). The content of chlorophylls a+b increased: by 23,99%-under treatment with NAA, by 9,58% -under treatment with 2,4-D, by 17,45%-under treatment with Methyur, by 13,49%-under treatment with Kamethur, by 25,22%-under treatment with Ivin, by 9,65%-under treatment

with Methyur+Rostock Extra, by 13,09%-under treatment with Kamethur+Rostock Extra, by 11,77%- under treatment with Ivin+Rostock Extra, by 12,72% -under treatment with Radix Tim forte+, by 7,53%- under treatment with Methyur+Radix Tim forte+, by 16,16% - under treatment with Kamethur+Radix Tim forte+, by 10,99% -under treatment with Ivin+Radix Tim forte+, compared to the indicators of the control plants (Figure 7). At the same time, no statistically significant changes in content of chlorophylls a+b in wheat leaves treated with Rostock Extra were detected compared to the indicators of the control plants (Figure 7). The content of carotenoids increased: by 16,45% -under treatment with NAA, by 9,36% -under treatment with Methyur, by 11,5%-under treatment with Kamethur+Rostock Extra, by 5,11%-under treatment with Methyur+Radix Tim forte+, compared to the indicators of the control plants (Figure 7). At the same time, statistically significant changes in content of chlorophyll a in wheat leaves treated with 2,4-D, Kamethur, Ivin, Rostock Extra, Methyur+Rostock Extra, Ivin+Rostock Extra, Radix Tim forte+, Kamethur+Radix Tim forte+, Ivin+Radix Tim forte+, compared to the indicators of the control plants were not detected (Figure 7).

Determination of total soluble protein content

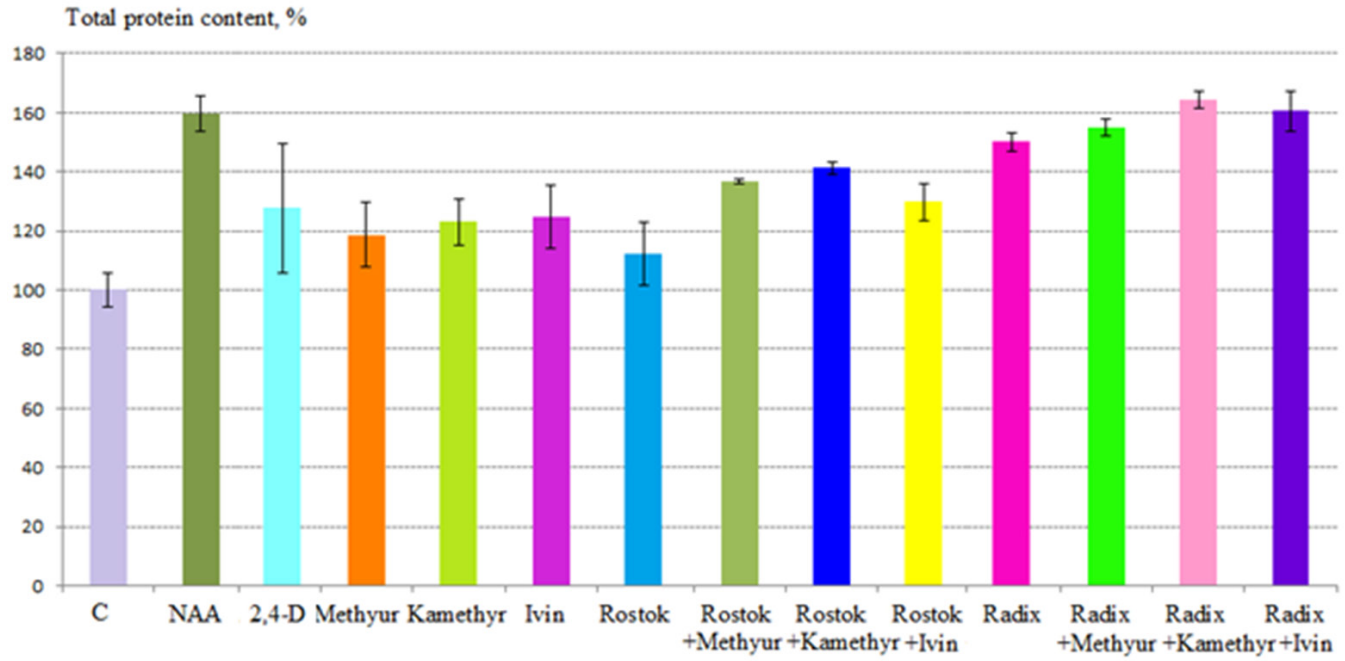

Figure 8: Total protein content (%) in the leaves of 4-week-old wheat (*T. aestivum* L.) variety Mudrist' Odes'ka, grown under the regulatory effect of auxins NAA and 2,4-D and synthetic plant growth regulators: Methyur, Kamethur, Ivin, used at a concentration of 10^{-6}M separately or in combination with biofertilizers Rostock Extra and Radix Tim forte+, in comparison with control plants.

A comparative study of the regulatory effect of synthetic plant hormones auxins NAA (1-Naphthylacetic acid) and 2,4-D (2,4-dichlorophenoxyacetic acid) and new plant growth regulators based on synthetic low-molecular-weight azaheterocyclic compounds, derivatives of sodium and potassium salts of 6-methyl-2-mercapto-4-hydroxypyrimidine (Methyur and Kamethur) and N-oxide-2,6-dimethylpyridine (Ivin), used separately or in combination with biofertilizers Rostock Extra and Radix Tim forte+, on such biochemical indicators as the content of total soluble protein (g/100 g FW) in wheat leaves was conducted. The results obtained shows that the treatment of wheat seeds with synthetic plant growth regulators, such as Methyur, Kamethur, Ivin, used at a concentration of 10^{-6}M separately or in combination with biofertilizers Rostock Extra and Radix Tim forte+, contributed to the increased of the protein biosynthesis in wheat leaves grown during the vegetation period. Biochemical indicators of plants, such as the total soluble protein content (g/100 g FW), treated with synthetic plant growth regulators Methyur, Kamethur, Ivin, used separately or in combination with biofertilizers Rostock Extra and Radix Tim forte+, were similar to the indicators of plants treated with auxins NAA and 2,4-D and higher than the indicators of the control plants (Figure 8). The total soluble protein content (g/100 g FW) increased: by 59,63% -under treatment with NAA, by 27,72% - under treatment with 2,4-D, by 18,58% -under treatment with Methyur, by 22,93% -under treatment with Kamethur, by 24,72% -under treatment with Ivin, by 12,14% -under treatment with Rostock Extra, by 36,56% -under treatment with Methyur+Rostock Extra, by 41,35% -under treatment with Kamethur+Rostock Extra, by 29,67% -under treatment with Ivin+Rostock Extra, by 50,05% -under treatment with Radix Tim forte+, by 54,84% -under

treatment with Methyur+Radix Tim forte+, by 64,28% - under treatment with Kamethur+Radix Tim forte+, by 60,38% - under treatment with Ivin+Radix Tim forte+, compared to the indicators of the control plants (Figure 8). Analyzing the results obtained in this work, we can come to the conclusion about the specificity of the regulatory activity of auxins NAA and 2,4-D, synthetic plant growth regulators such as Methyur, Kamethur, Ivin and biofertilizers Rostock Extra and Radix Tim forte+, on the morphometric and biochemical parameters of wheat. The highest regulatory effect on the morphometric indicators of wheat plants, such as length of the shoots (mm), length of the roots (mm) and biomass (g) of 10 plants, was exerted by auxin 2,4-D and synthetic plant growth regulators: Methyur and Kamethur and their combinations with biofertilizers: Methyur+Rostock Extra, Kamethur+Rostock Extra, Methyur+Radix Tim forte+, Kamethur+Radix Tim forte+; whereas a slightly lower regulatory effect on the morphometric indicators of wheat plants was exerted by auxin NAA, biofertilizers Rostock Extra and Radix Tim forte+ and synthetic plant growth regulator Ivin and its combinations with biofertilizers: Ivin+Rostock Extra and Ivin+Radix Tim forte+, in comparison with control plants. The highest regulatory effect on the biochemical indicators of wheat plants, such as the content of chlorophylls and carotenoids (mg/g FW), was exerted by auxin NAA, biofertilizer Radix Tim forte+ and synthetic plant growth regulators: Methyur, Kamethur, Ivin and some of their combinations with biofertilizers: Kamethur+Rostock Extra, Ivin+Rostock Extra, Kamethur+Radix Tim forte+, Ivin+Radix Tim forte+; whereas a slightly lower regulatory effect on these indicators of wheat plants was exerted by auxin 2,4-D, biofertilizer Rostock Extra and combinations of Methyur+Rostock Extra, Methyur+Radix Tim forte+, in comparison with control plants.

The highest regulatory effect on the biochemical indicators of wheat plants, such as the total soluble protein content (g/100 g FW), was exerted by auxin NAA, biofertilizer Radix Tim forte+ and combinations of synthetic plant growth regulators: Methyur, Kamethur, Ivin with biofertilizers: Methyur+Rostock Extra, Kamethur+Rostock Extra, Ivin+Rostock Extra, Methyur+Radix Tim forte+, Kamethur+Radix Tim forte+, Ivin+Radix Tim forte+; whereas a slightly lower regulatory effect on these indicators of wheat plants was exerted by auxin 2,4-D, synthetic plant growth regulators, such as Methyur, Kamethur, Ivin and biofertilizer Rostock Extra, in comparison with control plants. The obtained results indicate a synergistic effect of synthetic plant growth regulators: Methyur, Kamethur, Ivin and biofertilizers Rostock Extra and Radix Tim forte+ on the processes of shoot and root growth, an increase in plant biomass and an increase in the content of chlorophylls, carotenoids and proteins in leaves - key indicators of wheat yield. It is obvious that the synergistic effect of synthetic plant growth regulators: Methyur, Kamethur, Ivin and biofertilizers Rostock Extra and Radix Tim forte+ is associated with their auxin- and cytokinin-like effects [26-28] on the processes of division, elongation and differentiation of plant cells, better uptake of water and nutrients by the root system, increased efficiency of photosynthesis and improved protein metabolism in wheat cells.

The results obtained in the present work, which showed that the treatment of wheat seeds with synthetic plant growth regulators, such as Methyur, Kamethur, Ivin, used at a concentration of 10^{-6}M separately or in combination with biofertilizers Rostock Extra and Radix Tim forte+, considerably improved the growth and development of roots and shoots of wheat plants during the vegetative phase, enhanced the processes of photosynthesis and protein biosynthesis in wheat leaves, correlate with the results of our previously published works [45-48, 58-61]. As our previous field studies have shown, pre-sowing treatment of seeds of agricultural crops such as wheat, sunflower, sorghum and cowpea with synthetic plant growth regulators, such as Methyur, Kamethur, Ivin, used at a concentrations of 10^{-4}M-10^{-7}M, enhances the growth and development of plant shoots and roots, activates photosynthesis in plant leaves and increases seed productivity and their nutritional value, especially protein content, as well as the biological productivity of plants, thereby increasing the yields of agricultural crops [45-48]. Our previous laboratory studies also showed that synthetic plant growth regulators such as Methyur, Kamethur, Ivin, when used at a concentration of 10^{-7}M in combination with biofertilizers Rostock Extra and Radix Tim forte+, exhibit a synergistic effect on improving the growth and development of wheat, sunflower, rapeseed and oilseed flax throughout the vegetation period [58-61]. Based on the data from the conducted studies, it was concluded that the synergistic effect of synthetic plant growth regulators and biofertilizers containing micro- and macroelements and other biologically active compounds is due to their auxin and cytokinin-like effect on the processes of division, elongation and differentiation of plant cells, which are the basis for the growth and development of root and shoot meristems in the vegetative stage [26-28]. The plant growth regulator Ivin contains the macroelement nitrogen; plant growth regulator Methyur contains the macroelements nitrogen, sulfur and sodium, which promote plant growth and their adaptation to salt and osmotic stress; plant growth regulator Kamethur contains the macroelements nitrogen, potassium and sulfur, which are necessary for growth and metabolism in plant cells. The biofertilizers Rostock Extra and Radix Tim forte+ contain such macro- and microelements as nitrogen, phosphorus, potassium, magnesium, sulfur, manganese, boron, zinc, iron, copper, free amino acids, humic substances, which improve growth and metabolism in plant cells [58].

The results obtained in the present work also correlate also with the results of published works by other authors [24,25] on the combined use of synthetic Plant Growth Regulators (PGRs) with biofertilizers or Plant Growth Promoting Rhizobacteria (PGPR) to regulate plant growth and development and increase their yield. The study [24] investigated the use of fertilizers (100% recommended dose of fertilizer, 150% recommended dose of fertilizer and 150% recommended dose of fertilizer+15 t farmyard manure (FYM)/ha) and plant growth regulator treatments (two sprays each of chlormequat chloride 0.2%, tebuconazole 0.1% and chlormequat chloride 0.2% + tebuconazole 0.1%) on wheat grown under different fertility conditions to achieve maximum yield. The study showed that application of the 150% recommended

dose of fertilizer together with FYM at a dose of 15 t/ha resulted in significantly higher values of all yield parameters, such as stem diameter, internode length and 1000-grain weight, while in the control variants where no fertilizer or manure was applied, significantly lower values of all these parameters were recorded. The highest absorption of nitrogen, phosphorus and potassium by wheat was also recorded with the combined application of 150% of the recommended fertilizer dose + 15 t FYM/ha. The use of the growth regulator tebuconazole alone resulted in a significant increase of wheat yield, while the effect of chlormequat chloride application was not significant. Thus, the authors recommend using an increased dose of fertilizer -150% of the recommended dose, together with the introduction of FYM at a dose of 15 t/ha, as well as tebuconazole at a concentration of 0.1%, achieving higher wheat yields.

The study [25] showed that wheat varieties (Galaxy-13 and Pak-2013) differing in drought sensitivity soaked in fresh cultures of isolates of PGPR strains such as Planomicrobium chinense (P1), Bacillus cereus (P2) and Pseudomonas fluorescens (P3) and sprayed at 150 mg/L at the three leaf stage of seedlings using PGRs such as salicylic acid and putrescine, significantly enhanced the yield traits (i.e. plant height, spike length, grain yield and weight), increased the biochemical indicators (i.e. chlorophyll, protein and sugar) and enhanced the production of proline, antioxidant enzymes and lipid peroxidation under drought stress conditions. It was also shown that accumulation of macronutrients, total NO_3-N and P concentrations and soil moisture in the wheat rhizosphere were also increased by PGPR inoculation. It was concluded that the combined effects of PGPR and PGRs have profound effects on the biochemical responses and drought tolerance of wheat grown on sandy soils. Thus, based on the data obtained in this work and the results of our previously published works [45-48, 58-61], as well as the published works of other authors [24,25] it can be concluded that the application in agricultural practice of biofertilizers Rostock Extra and Radix Tim forte+, synthetic plant growth regulators: Methyur, Kamethur, Ivin, used at a concentration of 10^{-6}M separately or in combination with biofertilizers Rostock Extra and Radix Tim forte+, to improve the growth and increase the yield of wheat plants is very promising approach.

Conclusion

In present research, new environmentally friendly agrobiotechnological approaches to improving the growth of an important cereal crop - wheat using new plant growth regulators based on synthetic low-molecular-weight azaheterocyclic compounds, such as Ivin, Methyur, Kamethur, as well as the biofertilizers Rostock Extra and Radix Tim forte+ were elaborated. When using synthetic plant growth regulators such as Ivin, Methyur, Kamethur, at a concentration of 10^{-6}M separately or in combination with biofertilizers Rostock Extra and Radix Tim forte+, their regulatory effects were revealed, similar to or higher to the effects of auxins NAA and 2,4-D on the growth and development of roots and shoots, an increase in the content of chlorophylls and carotenoids, as well as the content of total soluble protein in the leaves of wheat plants during the vegetative phase. The discovered regulatory effects are explained by the synergistic auxin- and cytokinin-like effects of the synthetic plant growth regulators such as Ivin, Methyur, Kamethur and biofertilizers Rostock Extra and Radix Tim forte+ on the enhancing the division, elongation and differentiation of plant cells, activating the processes of photosynthesis and protein biosynthesis, which leads to intensification of growth and development, improved metabolism and increased productivity of wheat (*T. aestivum* L.) variety Mudríst' Odes'ka.

Statement of Conflict of Interest

The authors are declared that they have no conflict with this research article.

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