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## Pyrimidine Derivatives As Analogues Of Plant Hormones For Intensification Of Wheat Growth During The Vegetation Period

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## Abstract.

Wheat is one of the economically important cereals that are widely used in the agricultural sector of many countries. A very important issue is the development of new effective growth regulators to improve the growth and development of this crop in order to increase its productivity. In our investigations the regulating activity of the synthetic low molecular weight heterocyclic compounds, pyrimidine derivatives on growth and development of wheat (*Triticum aestivum* L.) variety Tyra was studied. It was found that the use of pyrimidine derivatives in a concentration of 10<sup>-7</sup>M in water solution revealed a positive effect on the growth and development of both shoot and root systems of wheat plants during the vegetation period. Comparative analysis of the growth regulating activity of synthetic compounds showed that the activity of pyrimidine derivatives as new effective regulators of growth and development of wheat (*Triticum aestivum* L.) variety Tyra was proposed.

Key words: wheat (Triticum aestivum L.), auxins, pyrimidine derivatives, plant growth regulators.

## 1. Introduction

Wheat is one of the economically important cereals that are widely used in the agricultural sector of many countries [1, 2]. A very important issue is to increase the productivity and the resistance of wheat plants to stress factors of abiotic and biotic origin, which negatively affect the growth of this crop [3-7], reducing the use of toxic to the environment and human health pesticides used in high concentrations and with a long half-life [8, 9].

Today, new effective and environmentally friendly regulators of growth of wheat (*Triticum aestivum* L.) based on synthetic low molecular weight heterocyclic compounds derivatives of different classes are being developed [10, 11]. The prospects for the practical application of these compounds are due to the fact that synthetic compounds of these classes are able to reveal related to natural plant hormones auxins and cytokinins biological activity in the range of low concentrations, non-toxic to humans, animals and plants.

It is known that the major plant hormones auxins and cytokinins play an important role in regulating plant growth and development at all stages of ontogenesis and in protecting plants from biotic and abiotic stresses [12-23]. Auxins and cytokinins include natural substances, such as IAA (1*H*-Indol-3-ylacetic acid), Zeatin ((*E*)-2-methyl-4-(7*H*-purin-6-ylamino)but-2-en-1-ol)) and their synthetic analogues, for example, indole-3-butyric acid (IAA), indole-butric acid (IBA), NAA (1-Naphthylacetic acid), BAP (*N*-(Phenylmethyl)-7*H*-purin-6-amine), Kinetin (*N*-(2-Furylmethyl)-7*H*-purin-6-amine), which exhibit biological activity that may exceed the activity of the corresponding natural growth substances, and many other classes of auxin- and cytokinin-related compounds [16, 17].

Plant hormones auxins are involved in control of plant embryogenesis, germination of seeds, cell elongation and cell division in plant hypocotyls and coleoptiles, apical dominance, cambium cell division, plant tropisms, formation of root and shoot meristems of plants, increasing the biomass of seedlings, promotion of fruit setting and prevention of leaf abscission [12, 13, 18, 19].

Plant hormones cytokinins take an important part in control of embryo patterning, germination of seeds, deetiolation, cell cycle control and protein synthesis, chloroplast differentiation and photosynthesis, formation of root and shoot meristems of plants, increasing the biomass of seedlings, overcoming of apical dominance,



releasing of lateral buds from dormancy, stomata opening, delaying of leaf senescence, elevation of the sink strength, flower and fruit development, and nutritional signaling [12, 13, 20-23].

Considerable attention is currently being given to the study of the plant growth regulatory activity of synthetic low molecular weight heterocyclic compounds, derivatives of pyrimidine, which have already found practical application in the agriculture as new effective plant growth regulators, herbicides and fungicides [24 - 29].

Today the new plant growth regulating substances are elaborated on the base of synthetic low molecular weight heterocyclic compounds, derivatives of pyrimidine, synthesized in the V.P. Kukhar Institute of Bioorganic Chemistry and Petrochemistry of National Academy of Sciences of Ukraine. Our numerous studies have shown that synthetic low molecular weight heterocyclic compounds, derivatives of pyrimidine have a high stimulating auxin-like and cytokinin-like effect on vegetative growth of various crops and increase their yield [30-36]. The advantages of using synthetic low molecular weight heterocyclic compounds are their high efficiency when used in very low concentrations and environmental safety due to lack of toxic effects on the human, animal and plant cells.

The aim of this work was to study new biologically active compounds among new synthetic low molecular weight heterocyclic compounds, derivatives of pyrimidine, which may have auxin-like and cytokinin-like stimulating activity on the growth and development of wheat (*Triticum aestivum* L.) variety Tyra during the vegetation period.

## 2. Materials and methods

## 2.1. Chemical compounds studied in the experiment

Synthetic low molecular weight heterocyclic compounds, derivatives of pyrimidine (compounds № 1 - 10) were synthesized in the Department for Chemistry of Bioactive Nitrogen-Containing Heterocyclic Compounds, V.P. Kukchar Institute of Bioorganic Chemistry and Petrochemistry of the National Academy of Sciences of Ukraine (Table 1).

The plant growth regulatory activity of compounds, derivatives of pyrimidine was compared with the activity of plant hormone auxin IAA (1*H*-Indol-3-ylacetic acid) (Table 1).

Chemical compound №	Chemical structure	Chemical name and relative molecular weight
ΙΑΑ	OH N H	1 <i>H</i> -Indol-3-ylacetic acid MW=175,19
1.		1-(2,3-dihydroxypropyl)-3-phenyl-5-(phenylsulfonyl)pyrimidine- 2,4(1 <i>H</i> ,3 <i>H</i> )-dione MW=402
2.		1-(2,3-dibromopropyl)-3-phenyl-5-(phenylsulfonyl)pyrimidine- 2,4(1 <i>H</i> ,3 <i>H</i> )-dione MW=528

Table 1. Chemical name, structure and relative molecular weight of chemical compounds and auxin IAA



	~	1 (2 hydrogeneropyl) 2 phased 5 (phasedeulfsee the size of the
3.	S S N S	1-(3-hydroxypropyl)-3-phenyl-5-(phenylsulfonyl)pyrimidine- 2,4(1 <i>H</i> ,3 <i>H</i> )-dione MW=386
4.		N-(1-(5-fluoro-2,4-dioxo-3-phenyl-3,4-dihydropyrimidin-1(2H)-yl)-2- oxo-2-phenylethyl)furan-2-carboxamide MW=381
5.		3-(4-chlorophenyl)-1-(2-hydroxyethyl)-5-(phenylsulfonyl)pyrimidine- 2,4(1 <i>H</i> ,3 <i>H</i> )-dione MW=406
6.		2,4-dioxo-N-(2-(trifluoromethyl)phenyl)-1,2,3,4- tetrahydropyrimidine-5-sulfonamide MW=349
7.		N-(1,1-dioxidotetrahydrothiophen-3-yl)-6-methyl-2,4-dioxo-1,2,3,4- tetrahydropyrimidine-5-sulfonamide MW=323
8.		(4-(benzylthio)-6-oxo-2-phenyl-1,6-dihydropyrimidin-5- yl)triphenylphosphonium chloride MW=591
9.		(2-methyl-6-oxo-4-((2-oxo-2-phenylethyl)thio)-1,6- dihydropyrimidin-5-yl)triphenylphosphonium bromide MW=602
10.		(4-(butylthio)-6-oxo-2-(p-tolyl)-1,6-dihydropyrimidin-5- yl)triphenylphosphonium iodide MW=663

## 2.2. Plant growth conditions

Study of the stimulating activity of new synthetic low molecular weight heterocyclic compounds, derivatives of pyrimidine on the growth and development of wheat (*Triticum aestivum* L.) variety Tyra for six weeks was carried out. For this purpose, the wheat seeds were superficially sterilized by 1 % KMnO<sub>4</sub> solution for 3 min



followed by treatment with 96 % ethanol solution for 1 min, and then washed three times with sterile distilled water. After this procedure wheat seeds were placed in the cuvettes (each containing 25-30 seeds) in perlite moistened with distilled water (control), or with the water solutions of synthetic compounds, derivatives of pyrimidine, or plant hormone auxin IAA used in a concentration of 10<sup>-7</sup>M. Afterward, wheat seeds were placed in the thermostat for germination in the darkness at the temperature 23 °C during 48 hours. Sprouted wheat seedlings were placed in the growth chamber where seedlings were grown for six weeks at the 16/8 h light/dark conditions, at the temperature 24-25 °C, light intensity 3000 lux and air humidity 60-80 %. The comparative analysis of the morphometric parameters of wheat plants (length of the shoots and roots (mm), number of the roots (pcs)) was carried out at the end of the six week after seed germination according to the guideline [37].

## 2.3. Determination of the content of photosynthetic pigments in the leaves of wheat plants

In order to evaluate the content of photosynthetic pigments (chlorophylls a and b, and carotenoids) we used leaves isolated from wheat (*Triticum aestivum* L.) variety Tyra grown for 6 weeks in the laboratory conditions. To perform the extraction of 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 CaCO3 (to neutralize the plant acids). The 1 ml of obtained homogenate was centrifuged at 8000 g 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 was calculated in accordance with formula [38, 39]:

Cchl a = 13.36×A664.2 - 5.19×A648.6,

 $Cchl b = 27.43 \times A648.6 - 8.12A \times 664.2,$ 

 $Cchl (a + b) = 5.24 \times A664.2 + 22.24 \times A648.6,$ 

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

Where,

Cchl – concentration of chlorophylls ( $\mu$ g/ml), Cchl a– concentration of chlorophyll a ( $\mu$ g/ml), Cchl b – concentration of chlorophyll b ( $\mu$ g/ml), Ccar – concentration of carotenoids ( $\mu$ g/ml), A – absorbance value at a proper wavelength in nm.

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

 $A1=(C \times V)/(1000 \times a1),$ 

Where, A1 - 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 tissue (g).

The content of chlorophyll a, chlorophyll b, and carotenoids (in %), determined in the leaves of the experimental wheat plants grown in water solutions of derivatives of pyrimidine, or plant hormone auxin IAA, used in a concentration of  $10^{-7}$ M, was calculated in accordance with similar indices determined in the leaves of control wheat plants grown in distilled water.

**2.4. Statistical Analysis.** All experiments were performed in three replicates. Statistical analysis of the data was performed using dispersive Student's-t test with the level of significance at P $\leq$ 0.05, the values are mean ± SD [40].

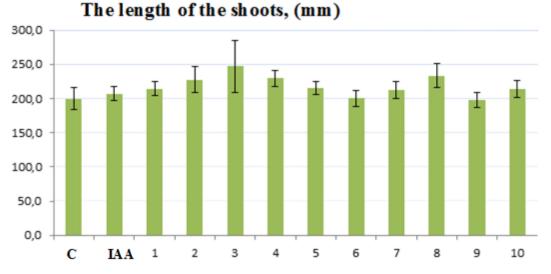


## 3. Results and discussion

## 3.1. Effect of pyrimidine derivatives on the morphometric parameters of wheat plants

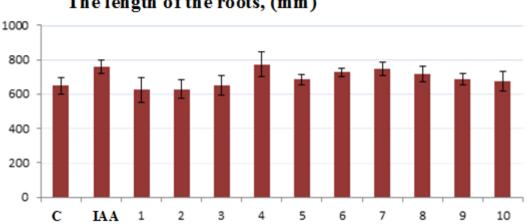
A study of the morphometric parameters of wheat (Triticum aestivum L.) variety Tyra have found that synthetic compounds, derivatives of pyrimidine used in a concentration of  $10^{-7}$  M show auxin-like activity, stimulating the growth and development of shoots and roots of wheat plants for 6 weeks. The highest stimulating activity on plant morphometric parameters (length of the shoots and roots (mm), number of the roots (pcs)) was found for compounds № 2-5, 7, 8 and 10 (Figure 1 – Figure 3).

The obtained morphometric parameters - the length of the shoots of 6-week-old wheat plants grown in water  $10^{-7}$ M solution of compounds Nº 2-4 and 8, exceeded the length of the shoots of plants grown on distilled water (control) and plants grown in water  $10^{-7}$ M solution of auxin IAA on average by 13-24% and 11-20%, respectively (Figure 1).



**Figure 1.** The effect of pyrimidine derivatives and auxin IAA used in a concentration of  $10^{-7}$ M on the length of the shoots of 6-week-old wheat (Triticum aestivum L.) variety Tyra, compared with control plants (C)

The obtained morphometric parameters - the length of the roots of 6-week-old wheat plants grown in water 10<sup>-7</sup>M solution of compounds № 4, 7, 8, exceeded on average by 10-19% the length of the roots of plants grown on distilled water (control) and were similar to the length of the roots of plants grown in water  $10^{-7}$ M solution of auxin IAA (Figure 2).

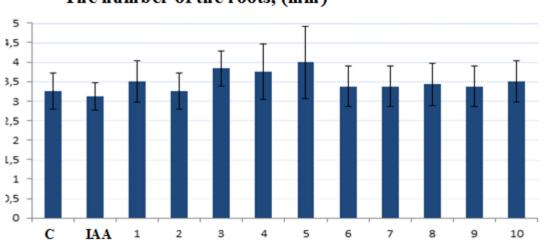


The length of the roots, (mm)

**Figure 2.** The effect of pyrimidine derivatives and auxin IAA used in a concentration of  $10^{-7}$ M on the length of the roots of 6-week-old wheat (Triticum aestivum L.) variety Tyra, compared with control plants (C)



The obtained morphometric parameters - the number of the roots of 6-week-old wheat plants grown in water  $10^{-7}$ M solution of compounds Nº 3-5, 8 and 10 exceeded the number of the roots of plants grown on distilled water (control) and plants grown in water  $10^{-7}$ M solution of auxin IAA on average by 15-28 %, respectively (Figure 3).



The number of the roots, (mm)

**Figure 3.** The effect of pyrimidine derivatives and auxin IAA used in a concentration of 10<sup>-7</sup>M on the number of the roots of 6-week-old wheat (*Triticum aestivum* L.) variety Tyra, compared with control plants (C)

Comparative analysis of the growth regulating activity of synthetic compounds showed that the activity of pyrimidine derivatives was differentiated and depended on substituents in their chemical structure. The highest stimulating activity on plant morphometric parameters (length of the shoots and roots (mm), number of the roots (pcs)) was found for compounds N<sup>o</sup> 2-5, 7, 8 and 10 (Figure 1 – Figure 3).

Compound Nº 2 has a phenylsulfonyl group residue in position 5, and a 2,3-dibromopropyl group residue in position 1. Compound Nº 3 has a phenylsulfonyl group residue in position 5, and a 3-hydroxypropyl group residue in position 1. Compound Nº 4 has a furan residue 2-carboxamide group in position 5 and fluorine in position 1. Compound Nº 5 has a phenylsulfonyl group residue in position 5, and 2-hydroxyethyl group in position 1. Compound Nº 7 contains in position 5 a sulfonamide group with a sulfolane residue and a methyl group in position 1. Compound Nº 8 contains in position 4 a benzylthio group and in position 5 a triphenylphosphonium group. Compound Nº 10 contains a butylthio group at position 4 and a triphenylphosphonium group at position 5.

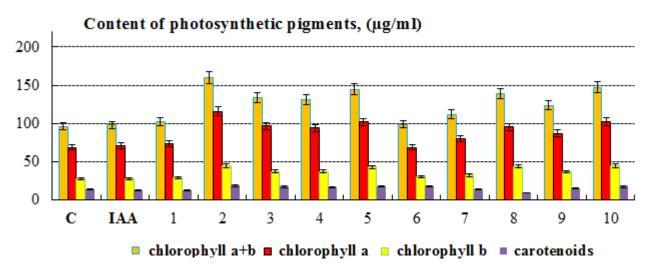
At the same time, compounds  $\mathbb{N}^{\mathbb{Q}}$  1, 6 and 9 did not show a significant effect on the morphometric parameters of wheat plants, which were not statistically significantly different from those of control wheat plants. Compound  $\mathbb{N}^{\mathbb{Q}}$  1 has a phenylsulfonyl group residue at position 5 and a 2,3-dihydroxypropyl group at position 1. Compound  $\mathbb{N}^{\mathbb{Q}}$  6 has a sulfonamide group residue at position 5 with a 2-(trifluoromethyl)phenyl substituent and a methyl group at position 1. Compound  $\mathbb{N}^{\mathbb{Q}}$  9 contains in position 4 a benzoylmethylthio group and in position 5 a triphenylphosphonium group.

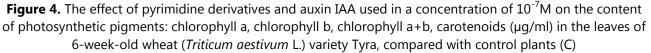
# 3.2. Effect of pyrimidine derivatives on the content of photosynthetic pigments in the leaves of wheat plants

It is known that plant hormones cytokinins take an important part in control of chloroplast differentiation and biosynthesis of photosynthetic pigments, which play an important role in ensuring plant productivity [20-23]. The cytokinin-like effect of pyrimidine derivatives used in a concentration of 10<sup>-7</sup>M on the biosynthesis of photosynthetic pigments: chlorophyll a, chlorophyll b, chlorophyll a+b, carotenoids in the leaves of 6-week-old wheat (*Triticum aestivum* L.) variety Tyra was studied.

Conducted studies have shown that the pyrimidine derivatives used in a concentration of 10<sup>-7</sup>M in water solution show cytokinin-like activity to increase the content of photosynthetic pigments in the leaves of 6-week-old wheat plants (Figure 4).







It was shown that the highest stimulating activity on the content of photosynthetic pigments (chlorophyll a, chlorophyll b, chlorophyll a+b, carotenoids) in the leaves of 6-week-old wheat (*Triticum aestivum* L) variety Tyra was found for compounds N<sup>o</sup> 2-5 and 8-10 (Figure 4).

The results showed that the treatment of plants with a  $10^{-7}$ M water solution of pyrimidine derivatives Nº 2-5 and 8-10 leads to an increase in the content of chlorophyll a on average by 23-68%, chlorophyll b on average by 34-65%, chlorophyll a + b on average by 26-68%, carotenoids on average by 13-48%, respectively, in the leaves of 6-week-old wheat plants, compared with similar parameters of control wheat plants grown in distilled water and plants grown in water 10-7M solution of auxin IAA (Figure 4).

## 4. Conclusions

Thus, summarizing the results obtained, we concluded that the derivatives of pyrimidine showed a regulatory effect similar to plant hormones auxins and cytokinins on growth and development of shoots and roots on wheat plants, and the increase of the content of photosynthetic pigments in the leaves of wheat plants. Obviously, these results are explained by auxin- and cytokinin-like regulatory effects of derivatives of pyrimidine on the processes of cell elongation, cell division, cell differentiation and chlorophyll biosynthesis in the plant cells [12, 13, 18-23].

The obtained data indicate the possibility of using selected synthetic low molecular weight heterocyclic compounds, pyrimidine derivatives N<sup>o</sup> 2-5, 7, 8 and 10 as new effective regulators of the growth and development of the wheat (*Triticum aestivum* L.) variety Tyra. These compounds showed the highest stimulating activity in relation to the morphometric parameters of wheat plant, increased the content of photosynthetic pigments in plant leaves.

## 5. Conflicts of Interest

The authors declare no conflict of interest.

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