

Application of Pyrazole Derivatives As New Substitutes of Auxin IAA To Regulate Morphometric and Biochemical Parameters of Wheat (*Triticum Aestivum* L.) Seedlings.

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Abstract

The comparative analysis of plant growth regulating the activity of new synthetic low molecular weight heterocyclic compounds, pyrazole derivatives, and auxin IAA (1*H*-Indol-3-ylacetic acid) was conducted. It was shown that the synthetic compounds used at the concentration 10^{-8} M revealed stimulating auxin-like and cytokinin-like effects on growth and development of wheat (*Triticum aestivum* L.) seedlings cultivar Antonivka during the six weeks. The morphometric parameters of the 6th-week-old wheat seedlings grown in the water solution of the pyrazole derivatives used at the concentration 10^{-8} M were similar or higher to the morphometric parameters of the wheat seedlings grown in the distilled water (control) or water solution of auxin IAA used at the same concentration 10^{-8} M on average: to 31 - 54 % – for total number of roots, to 39 – 104 % – for total length of roots, to 53 - 66 % – for length of the longest root. The content of photosynthetic pigments in the leaves of the 6th-week-old wheat seedlings grown in the water solution of the pyrazole derivatives used at the concentration 10^{-8} M increased on average: to 15-34 % – for content of chlorophyll a, to 10 - 61 % – for content of chlorophyll b, to 16 – 34 % – for content of chlorophylls a+b, to 14 – 28 % – for content of carotenoids, as compared with content of photosynthetic pigments in the leaves of the wheat seedlings grown in the distilled water (control) or in the water solution of auxin IAA used at the same concentration 10^{-8} M. The obtained results confirmed the possibility of the practical application of pyrazole derivatives as new effective substitutes for plant hormones for regulating the growth and development of wheat (*Triticum aestivum* L.) plants during the vegetative stage.

Keywords: *Triticum Aestivum* L., IAA, Pyrazole Derivatives, Plant Growth Regulation.

Introduction

Today, a new promising strategy for plant growing is developing of environmentally friendly plant hormones and plant growth regulators [1 – 17], natural bio stimulants or biofertilizers [18 – 23] that are capable of accelerating the growth and development of plants, reducing the phytotoxicity of chemical agents, and enhancing the plant immune response to abiotic and biotic stresses [1, 24 – 27].

In this regard, the elaboration of new effective ecologically safe plant growth regulators on the base of synthetic low molecular weight heterocyclic compounds is a very strategic approach. As is known, the various classes of low molecular weight heterocyclic compounds, derivatives of pyrimidine, pyrazole, and oxazole are widely applied in the agriculture as plant growth regulators, herbicides, fungicides and antibacterial agents [28 - 35].

The advantage of application of synthetic low molecular weight heterocyclic compounds is their high efficiency at their application at very low concentrations and ecological safety due to lack of toxic effect on the human, animal and plant cells; moreover they are widely used in the medical practice as therapeutic agents for treatment of nervous, allergic, gastroesophageal, cancer, bacterial, viral, fungal, infectious, and inflammatory diseases [36 - 44].

Wheat (*Triticum aestivum* L.) is the major strategic cereal crop cultivated over the world [45 - 47]. Wheat provides by 30 % of the food calories consumed by world population [46, 47]. Wheat seeds contain bioactive compounds that are important to the pharmaceutical industry: alkaloids, saponins, glycosides, terpenoids, steroids, flavonoids, and tannins [48]. Wheat is used as a raw material for the production of malt and beer [47]. However, despite the rapidly increasing of wheat sowing, there are significant problems with the increasing of the productivity of this crop because the world population is expected to reach 9.7 billion people by 2050 [49]. Abiotic and biotic stress-factors hurt the vegetative growth of wheat, thereby reducing plant productivity [25]. The plant growth regulators and fertilizers are used to accelerate the growth of wheat plant during the vegetative stage and increase crop yields [52 - 54].

As is known the main disadvantages of the practical application of traditional plant growth regulators are low growth regulating activity when used at high concentrations, their storage instability, and toxicity to humans, animals and environment [55 – 57]. In addition, excessive use of chemical agents of plant protection and mineral fertilizers leads to a number of negative consequences: the formation of resistant races of pathogens which cause plant diseases; depletion of the quantitative and qualitative composition of soil microbial coenoses, mainly due to the reduction in the number of beneficial members of soil microbiota; accumulation of toxic residues in the environment [58 – 61].

At present, various classes of synthetic low molecular weight heterocyclic compounds are synthesized at the Institute of Bioorganic Chemistry and Petrochemistry of National Academy of Sciences of Ukraine as new plant growth regulators. Our numerous studies have shown that chemical compounds belonging to derivatives of pyridine, pyrimidine, and pyrazole revealed a high stimulating effect on seed germination and growth of seedlings of various crops [62 – 69]. Since synthetic low molecular weight heterocyclic compounds are applied at very low non-toxic for human, animal and plant concentrations $10^{-8}\text{M} - 10^{-9}\text{M}$, it is possible to prevent the negative effects on environmental pollution in comparison with chemical agents and plant growth regulators currently used in high concentrations and to have a significant half-life [55 – 61]. Therefore, the great theoretical and practical interest is an elaboration of new effective and environmentally friendly plant growth regulators on the base of synthetic low molecular weight heterocyclic compounds to intensify the vegetative growth of wheat.

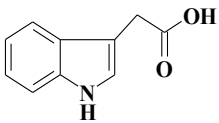
This work is devoted to studying of the stimulating effect of synthetic low molecular weight heterocyclic compounds, pyrazole derivatives on growth and development of wheat (*Triticum aestivum* L.) seedlings cultivar Antonivka during the vegetative stage.

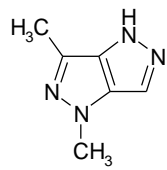
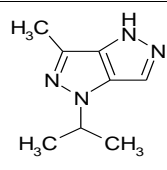
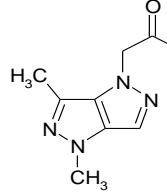
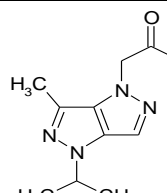
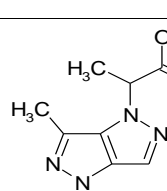
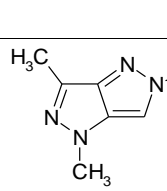
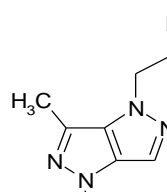
1. Materials and Methods

1.1. Chemical structure of pyrazole derivatives and plant hormone auxin used for bioassays

We conducted a comparative analysis of the plant growth regulating the activity of new synthetic low molecular weight heterocyclic compounds (LMWHC), pyrazole derivatives (compounds № 1-7) and plant hormone auxin IAA (1*H*-Indol-3-ylacetic acid) (Table 1).

Table 1. The chemical name, structure and relative molecular mass of plant hormone auxin IAA and synthetic LMWHC, pyrazole derivatives

Ccompounds	Chemical structure of compounds	Chemical name and relative molecular mass of compounds
IAA		(1 <i>H</i> -Indol-3-yl)-acetic acid;

		MM=175.19
1		1,3-Dimethyl-1,4-dihydro-pyrazolo[4,5-c] pyrazole; MM=136.16
2		1-Isopropyl-3-methyl-1,4-dihydro-pyrazolo[4,5-c]pyrazole; MM=164.21
3		(4,6-Dimethyl-4H-pyrazolo[4,5-c]pyrazol-1-yl)-acetic acid; MM=194.19
4		(4-Isopropyl-6-methyl-4H-pyrazolo[4,5-c]pyrazol-1-yl)-acetic acid; MM=222.25
5		2-(4-methyl-6-methyl-4H-pyrazolo[4,5-c]pyrazol-1-yl)-propionic acid; MW=208.22
6		(4,6-Dimethyl-1,4-dihydro-pyrazolo[4,5-c]pyrazol-2-yl)-acetic acid; MW=194.19
7		5-(4,6-Dimethyl-4H-pyrazolo[4,5-c]pyrazol-1-ylmethyl)-4-methyl-4H-[1,2,4]triazole-3-thiol; MW=263.33

2.2. Plant Treatment and Growing Conditions

Seeds of wheat (*Triticum aestivum* L.) cultivar Antonivka were surface sterilized by 1 % KMnO₄ solution for 5-10 min followed by treatment with 96 % ethanol solution for 1 min, and then washed three times with sterile distilled water. After this procedure seeds were placed in the cuvettes (each containing 25-30 seeds) in perlite moistened with distilled water (control), or with the solutions of synthetic LMWHC, pyrazole derivatives used at the concentration 10⁻⁸M, or plant hormone auxin IAA (1*H*-Indol-3-ylacetic acid) used at the same concentration 10⁻⁸M. 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 seedlings (length of shoots (mm), total number of roots (pcs), total length of roots (mm), and length of the longest root (mm) was carried out at the end of the 6th week after seed germination according to the guideline [70].

2.2. Determination of Content of Photosynthetic Pigments in The Leaves of The Wheat Seedlings

The content of chlorophyll a, chlorophyll b and carotenoids was determined in the leaves of the 6th-week-old wheat (*Triticum aestivum* L.) seedlings cultivar Antonivka grown either in the distilled water (control), or in the water solution of synthetic LMWHC, pyrazole derivatives used at the concentration 10⁻⁸M, or in the water solution of phytohormone auxin IAA used at the same concentration 10⁻⁸M. To perform extraction of pigments the sample (500 mg) of leaves isolated from 6th-week-old wheat (*Triticum aestivum* L.) seedlings was homogenized 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 [71]:

$$C_{chl\ a} = 13.36 \times A_{664.2} - 5.19 \times A_{648.6},$$

$$C_{chl\ b} = 27.43 \times A_{648.6} - 8.12 \times A_{664.2},$$

$$C_{chl\ (a + b)} = 5.24 \times A_{664.2} + 22.24 \times A_{648.6},$$

$$C_{car} = (1000 \times A_{470} - 2.13 \times C_{chl\ a} - 97.64 \times C_{chl\ b}) / 209,$$

Where, C_{chl} – concentration of chlorophylls (mg/ml), C_{chl a} – concentration of chlorophyll a (mg/ml), C_{chl b} – concentration of chlorophyll b (mg/ml), C_{car} – concentration of carotenoids (mg/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 wheat leaves was calculated by the following formula (separately for chlorophyll a, chlorophyll b and carotenoids):

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

Where, A₁ – content of chlorophyll a, chlorophyll b, or carotenoids (mg/g FW), C - concentration of pigments (mg/ml), V - volume of extract (ml), a₁ - sample of plant tissue (g).

The content of chlorophyll a, chlorophyll b, and carotenoids determined in the leaves of the 6th-week-old wheat seedlings grown in the water solution of synthetic LMWHC, pyrazole derivatives used at the concentration 10⁻⁸M was calculated in % according to similar indices determined in the leaves of control 6th-week-old wheat

seedlings grown in the distilled water, or in the water solution of phytohormone auxin IAA used at the same concentration 10^{-8} M.

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 \pm SD [72].

3. Results and Discussion

3.1. Impact of pyrazole derivatives on morphometric parameters of wheat seedlings

The conducted researchers showed that synthetic LMWHC, pyrazole derivatives used at the concentration 10^{-8} M revealed auxin-like stimulating effect on growth and development of the roots and shoots of wheat (*Triticum aestivum* L.) seedlings cultivar Antonivka during the six weeks (Fig. 1).

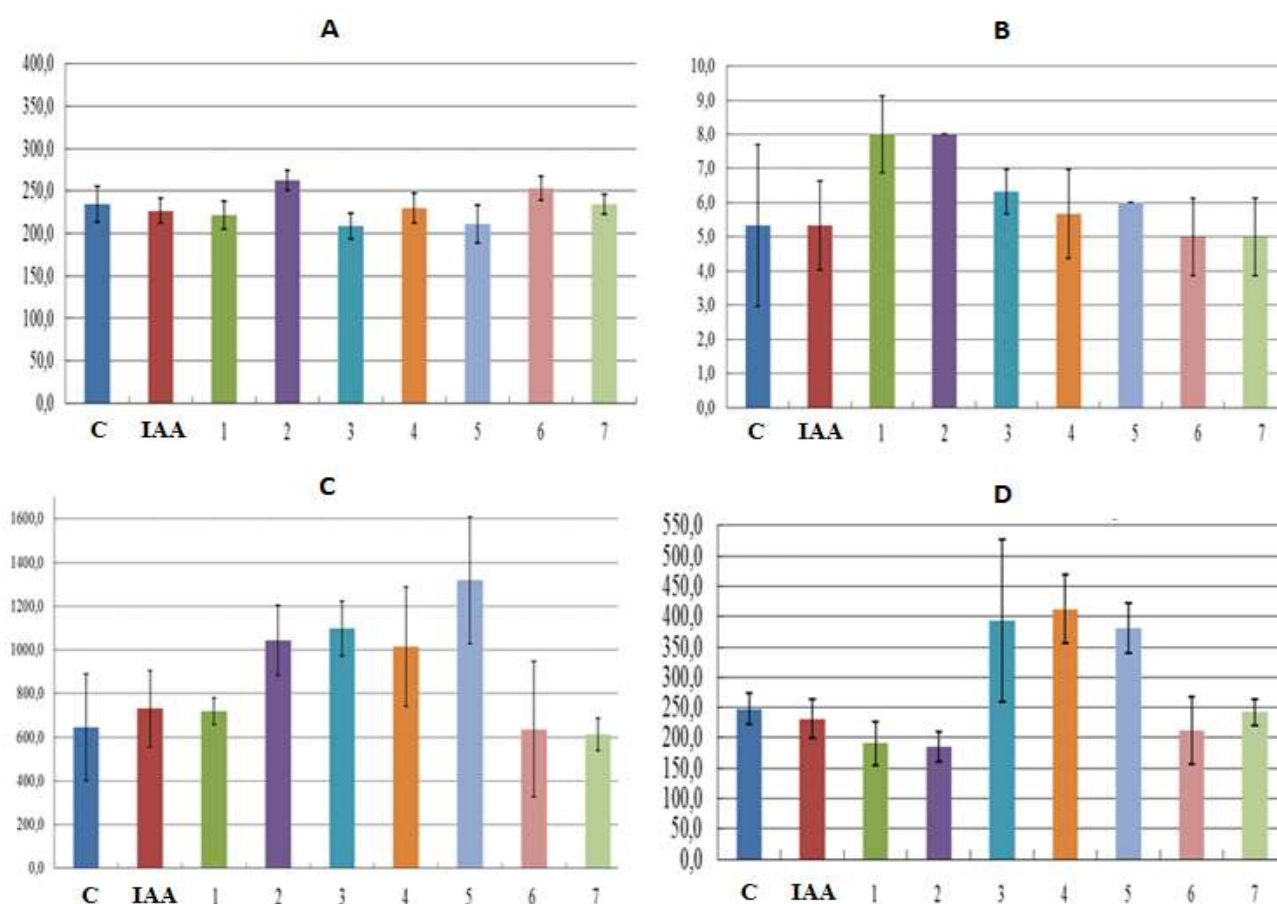


Fig. 1. Effect of synthetic LMWHC, pyrazole derivatives (compounds № 1-7) and plant hormone auxin IAA (1*H*-Indol-3-ylacetic acid) used at the concentration 10^{-8} M on morphometric parameters of the roots and shoots of wheat (*Triticum aestivum* L.) seedlings cultivar Antonivka during the 6 weeks as compared with morphometric parameters of the roots and shoots of control wheat seedlings grown in the distilled water (C). **A** - length of shoots (mm); **B** - total number of roots (pcs); **C** - total length of roots (mm); **D** - length of the longest root (mm)

Among tested synthetic LMWHC, pyrazole derivatives, the highest stimulating activity on growth and development of the wheat seedlings revealed the compounds № 2, 3, 4 and 5.

The morphometric parameters of the 6th-week-old wheat seedlings grown in the water solution of the compounds № 2, 3, 4 and 5 used at the concentration 10^{-8} M were similar or higher to the morphometric parameters of the wheat seedlings grown in the distilled water (control) or water solution of auxin IAA used at the same concentration 10^{-8} M on average: to 31 - 54 % – for total number of roots, to 39 – 104 % – for total length of roots, to 53 - 66 % – for length of the longest root (Fig. 1).

3.2. Impact of pyrazole derivatives on the content of photosynthetic pigments in wheat seedlings

We conducted the comparative analysis of the effect of synthetic LMWHC, pyrazole derivatives and phytohormone auxin IAA on content in the leaves of wheat (*Triticum aestivum* L.) seedlings cultivar Antonivka of the photosynthetic pigments (chlorophyll a, chlorophyll b, and carotenoids) ensuring wheat productivity [73 - 76]. Plant hormones cytokinins are known to play an important role in the biosynthesis of photosynthetic pigments such as chlorophylls and carotenoids and delayed degradation of chlorophylls in plant cells [77 - 79].

The obtained results showed the cytokinin-like stimulating effect of synthetic LMWHC, pyrazole derivatives used at the concentration 10^{-8} M on increasing of content (mg/ml) of photosynthetic pigments (chlorophyll a, chlorophyll b, and carotenoids) in the leaves of wheat (*Triticum aestivum* L.) seedlings cultivar Antonivka grown during the 6 weeks (Fig. 2).

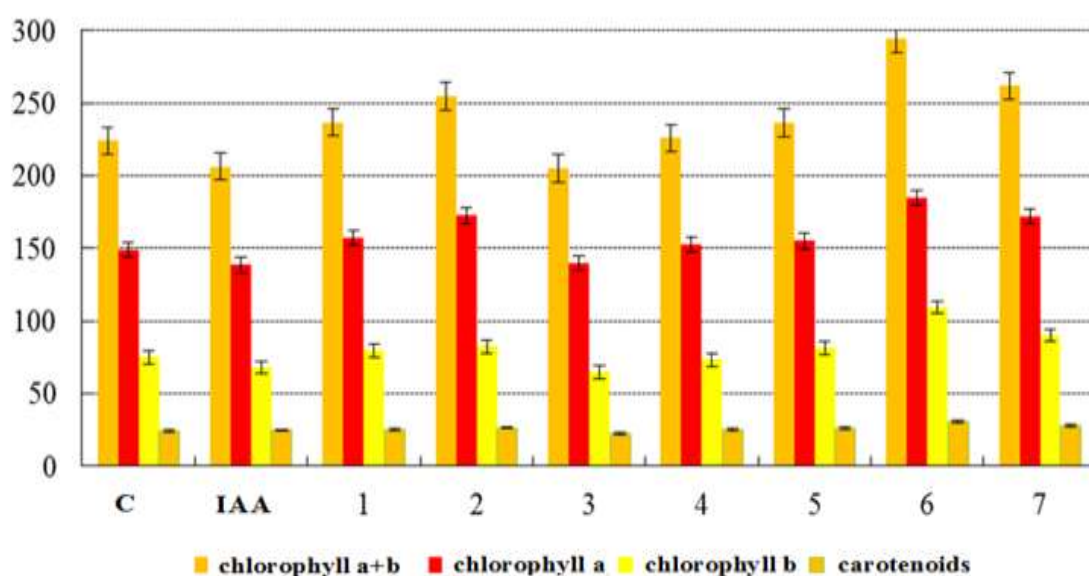


Fig. 2. Effect of synthetic LMWHC, pyrazole derivatives (compounds № 1-7) and plant hormone auxin IAA (1*H*-Indol-3-ylacetic acid) used at the concentration 10^{-8} M on content (mg/ml) of chlorophyll a, chlorophyll b and carotenoids in the leaves of wheat (*Triticum aestivum* L.) seedlings cultivar Antonivka grown during the six weeks

Among tested synthetic LMWHC, pyrazole derivatives, the highest stimulating activity on the content of photosynthetic pigments in the leaves of wheat seedlings revealed the compounds № 2, 6 and 7. The content of chlorophyll a in the leaves of wheat seedlings grown in the water solution of the compounds № 2, 6 and 7 used at the concentration 10^{-8} M increased on average at the 15 - 34 %, respectively, as compared with content of photosynthetic pigments in the leaves of wheat seedlings grown on the distilled water (control), or on the water solution of auxin IAA used at similar concentration 10^{-8} M (Fig. 2).

The content of chlorophyll b in the leaves of wheat seedlings grown in the water solution of the compounds № 2, 6 and 7 used at the concentration 10^{-8} M increased on average at the 10 - 61 %, respectively, as compared with content of photosynthetic pigments in the leaves of wheat seedlings grown on the distilled water (control), or on the water solution of auxin IAA used at similar concentration 10^{-8} M (Fig. 2).

The content of chlorophylls a+b in the leaves of wheat seedlings grown in the water solution of the compounds № 2, 6 and 7 used at the concentration 10^{-8} M increased on average at the 16 – 34 %, respectively, as compared with content of photosynthetic pigments in the leaves of wheat seedlings grown on the distilled water (control), or on the water solution of auxin IAA used at similar concentration 10^{-8} M (Fig. 2).

The content of carotenoids in the leaves of wheat seedlings grown in the water solution of the compounds № 2, 6 and 7 used at the concentration 10^{-8} M increased on average at the 14 – 28 %, respectively, as compared with content of photosynthetic pigments in the leaves of wheat seedlings grown on the distilled water (control), or on the water solution of auxin IAA used at similar concentration 10^{-8} M (Fig. 2).

Obviously, the cytokinin-like effect of pyrazole derivatives is associated with an increase in the synthesis of photosynthetic pigments such as chlorophylls and carotenoids and delayed degradation of chlorophylls in plant cells [77 - 79].

Thus, the conducted studies have shown that synthetic LMWHC, pyrazole derivatives revealed auxin-like and cytokinin-like stimulating effect on growth and development of wheat (*Triticum aestivum* L.) seedlings cultivar Antonivka during the 6 weeks.

It is possible to assume that the molecular mechanisms of action of these synthetic LMWHC, pyrazole derivatives might be associated with their regulatory action (by analogy with plant hormone auxin) on the network of key auxin-binding proteins (ABPs) that may be the auxin receptors involved in auxin signalling and transport, network of auxin response transcription factors (ARFs) that are DNA-binding proteins, which recognize and bind to auxin responsive *cis*-acting promoter elements (AuxREs) in early/primary auxin response genes, and network of transcription factors that bind to promoter elements in genes encoding protein-enzymes responsible for plant cell division and extension [80 – 103].

Otherwise, there could be an alternative mode of action related to the inhibitory effect of synthetic LMWHC, pyrazole derivatives on the activity of a key enzyme IAA-oxidase, which is involved in the enzymatic destruction (degradation) of auxin [104]. As a result, the level of endogenously synthesized auxin IAA is increased in the plant cells, and auxin transport, perception, and signaling are restored leading to improved plant cell division and extension that are the main processes of plant growth and development [80 – 103]. Obviously also, that the cytokinin-like effect of synthetic LMWHC, pyrazole derivatives can be related to their direct influence on the metabolism of endogenous plant hormones or their inhibitory effect on the activity of an enzyme cytokinin oxidase, which is involved in the enzymatic destruction (degradation) of endogenous plant cytokinins [105]. As a result, the level of endogenously synthesized cytokinins is increased in the plant cells, and cytokinin transport, perception and signal transduction are restored [106, 107] leading to improved plant cell division and increased synthesis of photosynthetic pigments such as chlorophylls and carotenoids, and delayed leaf senescence [77 - 79].

In support of the bottom concept indicate published works [108, 109] which showed the effect of exogenously applied synthetic analogs of auxin on the decrease in the activity of IAA-oxidase and vice versa on the increase in the level of synthesis of endogenous auxin IAA in plant cells.

The authors of the work [110] also suggested that synthetic auxins might affect the level of synthesis of endogenous auxin modifying directly synthesis of enzyme IAA-oxidase and indirectly through effectors of IAA-oxidase.

Similar studies were conducted in work [111] that showed that synthetic 2-R substituted benzothiazole derivatives demonstrated dominant auxin-like plant growth promoting activity. Based on obtained results, showing that the plant growth promoting activity of synthetic benzothiazole compounds can be correlated with the activity of IAA synthetase, the authors have proposed that the mode of action of synthetic 2-R substituted benzothiazole derivatives as auxin-like substances is due to their possible regulation of synthesis or degradation of endogenous auxin indole-3-acetic acid (IAA) in plants. The assumptions discussed in the works [108 - 111]

are consonant with our early published work [112], which testified in favor of the indirect, mediated through endogenous phytohormones action of synthetic LMWHC, derivatives of pyridine – lutidine N-oxide (Ivin) and pyrimidine – 6-methylthiouracil (Methyur) on plant cell extension, and published works of other authors [113 - 118] that showed the effect of exogenously applied synthetic multi-dimensional plant growth regulator Thidiazuron (TDZ; N-phenyl-1,2,3-thidiazole-5ylurea) on increase in concentrations of endogenous cytokinins, auxin, ethylene and ABA in plant cells. Authors of work [117] suggested that the powerful cytokinin-like regulatory effect of TDZ on plant growth is associated with its influence on the metabolism of endogenous plant hormones, either directly or indirectly through prevention the breakdown of endogenous purines by inhibiting cytokinin oxidase, due to which plant cell division and regeneration occur.

4. Conclusion

The conducted comparative analysis of the plant growth regulatory activity of synthetic LMWHC; pyrazole derivatives showed that tested synthetic compounds used at the concentration used at the concentration 10^{-8} M revealed auxin-like and cytokinin-like stimulating effect on the growth of wheat (*Triticum aestivum* L.) seedlings cultivar Antonivka during the six weeks. It was found that plant growth regulatory activity of tested synthetic LMWHC; pyrazole derivatives was similar or higher of the activity of plant hormone auxin IAA used at the same concentration 10^{-8} M. It was found that the plant growth regulatory activity of synthetic LMWHC, pyrimidine derivatives was varied depending on their chemical structure. Among tested chemical compounds, the highest activity by the plant morphometric parameters revealed the compounds № 2, 3, 4 and 5, while the highest activity by the content of chlorophylls and carotenoids revealed the compounds № 2, 6 and 7. The obtained results confirmed the possibility of the practical application of pyrazole derivatives as new effective substitutes for plant hormones for regulating the growth and development of wheat (*Triticum aestivum* L.) plants during the vegetative stage.

5. Conflict of interest

Authors stated that there is no conflict of interest.

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