**CREATION AND INTRODUCTION OF HIGHLY EFFECTIVE ECOLOGICALLY SAFE REGULATORS OF PLANTS GROWTH FOR INCREASE OF AGRICULTURAL CROPS**

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**Abstract**

*Synthetic analogues of natural phytohormones series ZhOT have been produced. Detailed laboratory and demonstration comparative tests have shown a high efficiency of new preparations ZhOT-4 and ZhOT-7 application, which exceeded the indicators of the reference preparation Agrostimulin permitted in Kazakhstan*

**Key words:** *regulators of plants growth, piperidols, analogues phytohormones, agriculture*

1. **BACKGROUND**

Kazakhstan is the agrarian country but in spite of the fact that agriculture has been actively used for a long time, there are still some unresolved problems connected with the increase of efficiency, increase of stability to environmental conditions, weeds, illnesses and insects. As cultivation of grain crops is getting more intensified a need in application of better means of protection arises that leads to a considerable increase of the product price.

One of the perspective ways providing the increase of efficiency is application of regulators of plants growth (RPG). Regulators of plants growth are natural and synthetic compounds, which when used in small concentrations are capable to initiate changes in plant life processes.

The vegetative organism is made of a number of various cells, tissues and organs and consequently a system, capable to ensure coordination of functioning of its separate parts and regulation at level of the whole plant, is necessary. In view of the modern science such regulation is carried out by a system of hormonal regulation with the help endogenous the chemical compounds named phytohormones or phytoregulators.

Phytohormones take active part in regulation of many biochemical and physiological processes in plants carrying out the functions both under ordinary (normal) conditions and at various adverse effects. It is especially important as naturally plants constantly or periodically are affected by certain adverse environmental factors, in particular, abiotic ones. During the last years global climate changes have led to strengthening of its instability shown, among other things, in sharp temperature drops. On the other hand, the constantly increasing anthropogenous load on the environment leads to the increase of salted territories and environmental contamination by heavy metals. Therefore, the problem of stability of plants to adverse environment factors of abiotic nature has become even more urgent. No wonder that it is actively studied in many countries around the world and an extensive and various experimental material has been collected to date.

Despite of considerable achievements of the science, natural phytohormones have not been widely used in practice due to complexity and costs in connection with their extraction from producer organisms, different actions of easy metabolic deactivation by vegetative enzymes. Mass application of phytohormones (regulators of plants growth (RPG)) has become possible only after analogues of phytohormones have been produced on the basis of natural and chemical substances, which are more stable in organism because of the absence of corresponding enzymes for destruction of new products.

Therefore, the creation of new highly effective and low-cost synthetic analogues of natural phytohormones (RPG) with complex properties (regulating, antistress, immunostimulant, etc.) becomes very urgent since the need in highly effective phytoregulators grows day in and day out.

Kazakhstan being in a great need in preparations of different designation for plant growing. They are not produced but imported today. RPG are currently imported to Kazakhstan from 14 countries of the world. The CIS countries importing RPG are the Russian Federation and Ukraine. The most stable suppliers are the Russian Federation, Ukraine, Germany, Switzerland. Some 1272 tons of RPG, (on the average 212 tons per year) were imported to Kazakhstan for six years.

Only 14 regulators of plants growth are on the list of pesticides (weed and pest-killer chemicals) permitted for agricultural purposes in Kazakhstan. Three of them only are domestic, but they have passed only the registration stage and are not allowed for application (because of not adaptability to manufacture, i.e. complexity of production). In addition such preparations have been allowed for application only with vegetable crops (potato, carrot and beet).

The main sector of crop production in Kazakhstan has traditionally been grain farming where the wheat farming dominates. This sector provides food to the people and fodder to animals. Though, Kazakhstan is the important exporter of grain in the international market and sown area of grain crops occupies over 80% (16.5 million hectares of 21.5 million hectare) of the area under agriculture crops, no domestic regulator of plants growth for grain crops has been permitted in Kazakhstan.



Fig 1. In 2014 the yield of grain crops is 17 million tons in bunker weight.

Therefore, creation of unique domestic preparations for protection of plants that increase the yield of crops and quality of crop products, and development and implementation of low-cost and highly effective new technologies for creation thereof become very urgent.

**2. SYNTHESIS OF HIGHLY EFFICIENT PLANTS GROWTH REGULATORS**

The goal of this work is to develop methods of synthesis of highly efficient plants growth regulators (analogues of natural phytohormones) on the basis of aromatic propargyl piperidols, production of water soluble forms thereof and running tests to increase the crop yield, growth and development of cereal (wheat and barley), vegetable (potato, carrot, cabbage, onion, etc.) and other crops.

The methods of fine organic and combinatorial synthesis, varying aromatic radical (naphthyl-, phenyl-, n-chlorinephenyl-) and quaternising the tertiary atom of nitrogen of piperidine ring of agents (hydrochloric acid, methyl iodide, amber acid) have resulted in synthesis of derivative acetylene aminoalcohol by the following reaction scheme:

Combinatorial synthesis of compounds was carried out to study the dependence of phenyl substitution with naphthyl, phenyl substitution with chlorinephenyl and phenyloxy substitution with phenylamino, as well as salt-forming radicals (hydrochloride – methyl iodide – amber acid).

Synthesis and production were carried out in three stages: 1 – synthesis of propargyl ethers using Williamson reaction, 2 – synthesis of tertiary acetylenic alcohols using Favorsky reaction and 3 – production of water soluble salts by quaternising aminoalcohols.



Fig 2. Synthesis of aryloxypropargyl piperidols

All stages of synthesis have been worked out and it was found that it is better to carry out propargylation of phenols and naphthols with bromic propargyl in acetone medium with soda ash at 60°С and equimolar ratio of reagents. For propargylation of *п*-chloroaniline, the most suitable medium is methanol, and condensing agent is potassium acetate at 500С.

Condensation of aryloxi- and arylaminopropargyls with 1-methylpiperidine-4 can show best results in diethyl ether medium with three-fold excess of potassium hydroxide at ambient temperature.

Quaternization of acetylenic alcohols practically with quantitative yield is in the medium of dehydrate alcohols with addition of ether hydrochloric acid or equimolar quantity of methyl iodide or amber acid at 50°С.

Crystalline substances of white to light-yellow color well-soluble in water are formed under these conditions. Compound structures have been established using IR- and NMR 1Н and 13С spectroscopy. They are coded ZhОТ for further testing.

**3. STUDY OF GROWTH-REGULATING ACTIVITY OF SYNTHESIZED PREPARATIONS**

*3.1.* *Bioscreening of synthesized compounds to determine growth and development of wheat seeds*

Test systems of wheat callus crops (*Triticum aestivum* L.) produced from Otan grade were created to evaluate the hormone-like activity of ZhОТ-1 in *in vitro* system. Isolated explants were cultivate in the agarized nutritional Murashige and Skoog medium (MS) at 22–24оС under dark conditions within one month. The results of experiments were evaluated by the increment of callus tissue, calculated by the following formula: Кr= final weight – initial weight/initial weight.

Test scheme: control 1 – hormone-free MS medium; control 2 – MS medium with phytohormones to stimulate callusogenesis for wheat calluses 2 mg/l of 2,4 –dichlorophenoxyacetic acid (2,4-D); pilot options: final concentrations of ZhOT-1 in nutritional medium is 0.001% (10 mg/l), 0.0001% (1 mg/l), 0.00001 (0.1 mg/l).

Table 1 – Influence of ZhOT-1 upon increment of biomass of wheat callus tissue

|  |  |  |
| --- | --- | --- |
| # | Test option, concentration, % | Increment of wheat callus |
| 1 | control 1 (hormone-free) | 0.67 |
| 2 | ZhOT-1 10 mg/l (0.001%) | 0.75 |
| 3 | ZhОТ-1 1 mg/l (0.0001%) | 0.84 |
| 4 | ZhОТ-1 0.1 mg/l (0.00001%) | 0.62 |
| 5 | control 2 (with hormones) | 1.03 |

Evaluation of accumulation of wheat callus biomass in the reference and pilot options was carried out during the exponential growth phase. Each option was repeated ten times in five calluses per each repetition. The results are summarized in Table 1.

Analysis of data contained in Table 1 demonstrates that addition of ZhOT-1 into the nutritional MS medium stimulates callusogenesis in *in vitro* wheat test system. Thus 1 mg/l concentration is the most efficient for accumulation of callus tissue of wheat.

It should be mentioned that hormone-like action of ZhOT-1 is also confirmed by its inducing effect on the risogenesis process (root formation) in callus tissue of wheat (Figure 2).

Reference 10 mg/l 1 mg/l 0.1 mg/l

Fig 3. Influence of ZhОТ-1 on risogenesis of *in vitro* in callus tissue of wheat

As one can see from the figure above, addition of 0.1-1 mg/l of ZhОТ-1 in the nutritional medium stimulates root formation in the callus tissue up to 80%, whereas in the reference (hormone-free MS environment) the risogenesis does not exceed 60%.

Therefore, the conducted researches have proved stimulating effect of ZhОТ-1 under *in vitro* conditions in the form of accumulation of callus biomass of wheat, and induction of risogenesis in callus tissue of wheat.

Screening of growth-regulating activity of other synthesized compounds coded ZhOT on wheat seeds was carried out thereafter. The work was carried out in accordance with the generally-accepthed methods.

*Subjects of research:* synthesized water-soluble aromatic propargyl piperidols coded ZhОТ; autumn wheat of grade Kazakhstan 10, Almaty super-elita and spring wheat of grade Glassy, and barley of grade Baisheshek.

The following was used as a reference: potable water, phytohormones – indolyl-3-acetic acid (IAA) Sigma, 6-benzylaminopurine (BAP) Sigma.

*Preparation of wheat seeds:* wheat seeds were washed in a soap solution, thoroughly washed with running water and treated with 1% solution of KMnO4 during 10-15 minutes for disinfection, whereupon it was washed in the running water and dried on the filter paper.

*Experiments were carried out as follows:* a) the first batch of seeds was grown in solutions of synthesized derivatives of piperidine in concentration of 0.01%, 0.001%, 0.0001% and 0.00001%, preliminarily watered in potable water during 6 hours; b) the second batch was grown after being watered during 6 hour in solutions of synthesized derivatives of piperidine in concentration of 0.01%, 0.001%, 0.0001% and 0.00001%. Plants grown using potable water were used as reference. In the both cases the seeds were growing during three days in Petri dishes using filter paper, and then under hydroponic conditions. Biometric parameters were measured by common methods. The plants were divided into above-ground part and roots, and measured the length of the root and above-ground part on day 3, 6, 9 and 12. These experiments were repeated three times using 15–20 seeds per Petri dish.

Screening of preparations with regulating activity was carried out on the basis of biometric parameters. Selection of the most efficient synthesized derivatives of piperidine characterized by regulating activity was carried out by growing wheat plants of different grades of four concentrations of the plant in question (0.01%, 0.001%, 0.0001% and 0.00001%) comparing with plants grown using potable water (control) and adding known phytohormones (IAA and BAP) if appropriate concentrations.

There are different ways to treat seeds with agricultural chemicals of different designation: plant spaying at different stages of plant growth and development, preliminary preplanting cultivation of seeds, addition of agricultural chemicals while planting seeds, etc.

In our experiments we used two methods of seeds treatment with agricultural chemicals: preliminary 6-hour long watering of seeds in synthesized derivatives of piperidine of different concentrations and directly grew seeds in solutions of compounds in question in different concentration and comparing their biometric parameters on day 3, 6, 9 and 12 dividing the plants into above-ground part and roots. Then measured the length of roots and plant stems and the number of roots. Seeds preliminary watered and grown using potable water were used as reference.

Comparison of biometric parameters of the above-ground part of wheat of different grades has shown that in case of preliminary watering the plants are characterized by better parameters in comparison with those grown directly in solutions of the same concentration, thus in case of 0.0001% concentration of synthesized derivatives of piperidine the values are maximum. Therefore, 0.0001% concentration is optimum.

Experimental data have shown that the use of higher concentrations of synthesized derivatives of piperidine (0.01% and 0.001% solutions) leads to a slight inhibition of growth of wheat as compared to plants grown at 0.0001% concentration. The maximum growth of wheat of different grades occurs at 0.0001% concentration. Further reduction of concentration leads to reduction of plant’s stem length.



Fig4. Influence of preparations upon development of the above-ground part

The most efficient preparations ZhОТ-4 and ZhОТ-7 have been selected by screening their water-soluble forms on seeds of wheat and barley, whose biometric parameters exceed those of the control and reference – known phytohormones such as heteroauxin (indolyl-3-acetic acid), 6-BAP (6-benzylaminopurine) by 30% to run further tests in order to increase crop yield, growth and development of wheat and barley.

Based on the results of the researching the biological activity of synthesized substances the following conclusions have been made:

* Optimum concentration of action of synthesized derivatives of piperidine is 0.0001.
* Biometric parameters of plant regulators preliminary watered in solutions is higher than those of grown directly in solutions of synthesized derivatives of piperidine of wheat.
* The screening has demonstrated that a number of compounds has only a stimulating effect and identified the most promising compounds in the series of synthesized derivatives of piperidine, namely ZhОТ-4 and ZhОТ-7.
* In terms of the salt-forming agent hydrochloric acid – hydrochloride is the most efficient; and use of phenyl radical the most preferable among aryl radicals.

*3.2. Wheat and barley growth demonstration testing of preparations.*

1. *Test venue*: Experimental base of the Kazakh Research Institute of Agriculture and Plant Growing, Almaty region.

3. *Crop, grade*: spring Kazakhstan 10, barley Baisheshek.

4. *Soil*: dark chestnut, moderate loamy, humus 3.0 – 3.5% , рН 7.0.

5. *Agrotechnics*: Forecrop – spring wheat, 20-22 cm deep ploughing, preplant cultivation, compaction after sowing. Spring wheat seeds were sown on April 17 (manual sowing), rate of seeding 3.5 million seeds per hectare, depth 5-6 cm.

6. *Test options*: preparations – 0.0001% solutions of ZhОТ-4 and ZhОТ-7; reference – Agrostimulin; control – untreated seeds.

7. *Test type, area of test plot, repetition*: field plot, plot size – 2.0 sq.m., repetition – four-fold.

8. *Preparations application terms and methods*: pre sowing seed treatment.

9. *Type of devices, working fluid consumption rate*: seed shaking in a 5 l vessel during 3-5 minutes, water consumption rate 10 l/t.

10. *Specific characteristics of weather conditions of the current year*: Weather conditions during the vegetation period in Almaty region show that year 2014 is characterized by relatively warm weather.

In April, precipitations almost twice exceeded the average monthly rate, the temperature was at the level of average many-years temperatures. The temperature in May was 2.2°С higher as compared to the average many-years temperature.

In June only 6.6 mm of precipitations fell down while the average many-years level is 24.4 mm, air temperature was 3.1°С higher than the average many-years temperature of 20.3°С (Table 1).

Treated seeds of spring wheat (Kazakhstan 10 grade) and barley (Baisheshek grade) were sown on April 17, 2014 on the test fields of the Kazakh Research Institute of Agriculture and Plant Growing, Almaty region.

Field emergence of treated seeds was determined that showed a high field emergence of all options, while the highest field emergence comes on ZhОТ-4+ Vial (0.0004%+0.4 l/t) 396 pcs./sq.m, and option ZhОТ-4 (0.0001%) – 385 pcs./sq.m, this parameter of the reference option of agrostimulin made 371 pcs./sq.m, in control - 349 pcs./sq.m. According to our observations all tested preparations caused a positive effect upon field emergence of wheat seeds. Furthermore, combination of ZhOT-4 (0.0001%) + chemical seed disinfectant Vial (0.4 l/t) showed the highest field emergence (Table 2).

Table 2 – Influence of preparations upon field emergence of wheat of Kazakhstan 10 grade

|  |  |  |
| --- | --- | --- |
| Item # | Option | Option field emergence, pcs./sq.m |
| I | II | III | IV | Thickness of emergence, pcs./sq.m |
| 1 | ZhОТ-4 | 96 | 105 | 98 | 97 | 396 |
|  | ZhОТ-7 | 99 | 100 | 95 | 96 | 388 |
| 2 | Agrostimulin | 94 | 93 | 98 | 86 | 371 |
| 3 | Control | 88 | 84 | 81 | 96 | 349 |

Field emergence of barley per 1 sq.m was relatively lower. It is no doubt that the preparations stimulated emergence of barley seeds. Field emergence was 294–296 pcs./sq.m which is much higher as compared to the control option (Table 3).

Table 3 – Influence of preparations upon field emergence of barley of Baisheshek grade

|  |  |  |
| --- | --- | --- |
| Item # | Item # | Option |
| І | ІІ | ІІІ | ІV | Thickness of emergence,pcs./sq.m |
| 1 | ZhОТ-4 | 77 | 82 | 80 | 80 | 296 |
|  | ZhОТ-7  | 69 | 75 | 81 | 63 | 294 |
| 2 | Agrostimulin | 65 | 71 | 78 | 62 | 276 |
| 3 | Control | 63 | 58 | 66 | 75 | 262 |

As the result of determination of the efficiency of preparations for preplanting cultivation of wheat seeds with ZhОТ-4, ZhОТ-4, and agrostimulin as reference, it was identified that ZhОТ-4 and ZhОТ-7 had a better effect upon tilling capacity of wheat (2.5-2.7 pcs), which is comparatively higher than that of the control; in addition the highest mass is registered in ZhОТ-4 – 1000 grains (41.7 g).

As to crop yield, the highest increase of crop yield is attributed with ZhОТ-4 (2.5 dt/ha), in ZhОТ-4 it is 2.4 dt/ha, in the reference option – agrostimulin it is 2.0 dt/ha.

Therefore, all tested preparations resulted in increase of crop yield of wheat at the level of or slightly above the reference.

Table 4 – Influence of preparations on biometric parameters and crop yield of wheat

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Item # | Option | Tilling capacity, pcs. | Length, cm | No. of ears, pcs. | Grain mass, g per 1000 pcs. | Crop yield, dt/ha | Yield increase, dt/ha |
| stem | ear |
| 1 | ZhОТ-4 | 2.7 | 82.1 | 7.4 | 14.4 | 41.7 | 20.9 | 2.5 |
| 2 | ZhОТ-7  | 2.5 | 83.0 | 7.5 | 14.2 | 40.8 | 19.8 | 2.4 |
| 3 | Agrostimulin | 1.5 | 78.5 | 7.0 | 14.0 | 38.4 | 20.5 | 2.0 |
| 4 | Control | 1.5 | 72.9 | 6.0 | 11.0 | 37.4 | 18.5 | - |

For barley, increase of crop yield by options was clearly lower; the maximum increment of crop yield of 1.6 dt/ha comes on ZhОТ-4. As to other options, these parameters did not exceed 0.2-1.5 dt/ha.

Thus, detailed laboratory and demonstration comparative testing of ZhОТ-4, ZhОТ-7 and Agrostimulin (Ukraine) – the preparation permitted for application in Kazakhstan – demonstrated a high efficiency of application of new synthesized preparations on wheat and barley that increased parameters of Agrostimulin.

The use of ZhОТ-4 and ZhОТ-7 increases the germinating energy and capacity, number of ears, mass of grains, tilling capacity and crop yield of wheat and barley, it leads to accumulation of dry mass both in the above-ground part and under-ground parts of plants.

Advantages of ZhОТ-4 and ZhОТ-7 are as follows: high efficiency, wide range of crops, low dosage of application – 0.0001% on the acting substance (1 g per 1 t of water) or 13-50 mg per hectare that are comparative with natural phytohormones, good solubility in water, longer storage life and safety.

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