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Nano, Bio and Green – Technologies for a Sustainable Future

SGEM

VOLUME I

16th INTERNATIONAL MULTIDISCIPLINARY SCIENTIFIC GEOCONFERENCE SGEM 2016

Book 6

Nano, Bio and Green – Technologies for a Sustainable Future

CONFERENCE PROCEEDINGS

Volume I

MICRO & NANO TECHNOLOGIES

ADVANCES IN BIOTECHNOLOGY

16th INTERNATIONAL MULTIDISCIPLINARY SCIENTIFIC GEOCONFERENCE

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VOLUME I

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MICRO AND NANO TECHNOLOGIES,

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regarding the applicability on the results of patients alive who have pathology similar to that included in our study.

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

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ABSTRACT

Synthesis of new potential plant growth regulators (analogues of natural phytohormones) was carried out by stirring of equimolar mixture of aryloxypropyne with 1-methylpiperidine-4-one – in terms of Favorski reaction. New derivatives of acetylene amino alcohols series ZhoT have been produced, which structures were identified by using the methods of IR and ^{1}H NMR spectroscopy. The most efficient preparations ZhOT-4 and ZhOT-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 standards – known phytohormones such as heteroauxin (indolyl-3-acetic acid), 6-BAP (6-benzylaminopurine) and "agrostimulin" (Ukraine).

Keywords: regulators of plants growth, piperidols, analogues of phytohormones, agriculture

1 RELEVANCE AND CURRENT STATE OF THE PROBLEM

Kazakhstan historically is the agrarian country but despite this, there are still some unsolved problems connected with the increase of efficiency, productivity and quality of agricultural products, including grain crops, increase of stability to adverse environmental conditions, weeds, various illnesses and insects. As cultivation of grain crops is getting more intensified a need in application of better means of protection grows that leads to a considerable increase of the product price.

One of the perspective ways providing the increase of efficiency of agriculture, namely, crop production is application of regulators of plants growth (RPG) - 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 in which occur diverse biochemical processes, operates a complex system that coordinates the functioning of its individual parts and regulation at the level of the whole plant. 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 regulate 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 abiotic, biotic and anthropogenic factors. The problem of stability of plants is actively studied in many countries around the world and an extensive and various experimental material has been collected to date[1], [2].

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 synthetic analogues of phytohormones have been produced on the basis of natural chemical substances, which are more stable in organism because of the absence of corresponding enzymes which can cause their degradation.

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 Russia 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 [3]. 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 and has received limited permission to use (only on vegetable crops: potatoes, carrot and beets) [4]. Despite that, 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.

Therefore, development of economically effective, little step and high effective methods of synthesis of domestic preparations for crop production, including RGP, which increase productivity and improve the quality of plant become very urgent.

2. SYNTHESIS OF HIGHLY EFFICIENT PLANTS GROWTH REGULATORS

By 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) was synthesized derivatives of acetylene amino alcohol (Fig. 1). Synthesis 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 – quaternising aminoalcohols for production of water soluble salts.

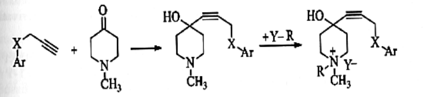

Fig. 1. Synthesis of aryloxypropargyl piperidols

The optimum conditions of all stages of synthesis have been selected 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°C and equimolar ratio of reagents. For propargylation of *n*-chloroaniline, the most suitable medium is methanol, and condensing agent is potassium acetate at 50^{0}C.

Condensation of aryloxi- and arylaminopropargyls with 1-methylpiperidine-4 proceeds effectively in diethyl ether medium with three-fold excess of potassium hydroxide at ambient temperature.

Quaternization of acetylenic alcohols with practically 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°C.

Crystalline substances of white, light-yellow color, well-soluble in water are formed under these conditions. Compound structures have been established using IR- and NMR ^{1}H and ^{13}C spectroscopy. They are coded ZhOT for further testing [5].

3. STUDY OF GROWTH-REGULATING ACTIVITY OF SYNTHESIZED PREPARATIONS

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

The influence of the growth regulating activity of the synthesized acetylene amino alcohols was studied on the cultures of the callus of wheat (Triticum aestivum L.),which produced from "Otan" grade. Isolated explants were cultivate in the agarized nutritional Murashige and Skoog medium (MS) at 22–24°C under dark conditions within one month. The results of experiments were evaluated by the increment of callus tissue, calculated by the following formula: K_r= final weight – initial weight/initial weight.

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. It is established that synthesized compounds have growth regulating activity and optimal growth stimulating concentration is 1 mg/l or 0.0001%. At this concentration the growth of callus of wheat is maximum. For ZhOT-1 it is equal to 0,84 ± 0.002 g, which is significantly higher than in the control in MS medium without hormones - 0,67 ± 0,003 g and slightly smaller than the growth of

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callus (1,03 ± 0.001 g), which grown in MS medium with plant hormone for stimulation of callusogenesis - 2,4 –dichlorophenoxyacetic acid (2,4-D), a concentration is 2 mg/l. In the application of greater and lesser concentrations ZhOT-1 the growth of the callus of wheat are: at 10 mg/l or 0.001 % - 0.75± 0.001 g and at 0.1 mg/l or 0,00001% - [illegible] ± 0.002 g, accordingly. A similar pattern was observed with other derivatives of acetylene amino alcohols.

The hormone-like action of ZhOT-1 is also confirmed by its inducing effect on the risogenesis process (root formation) in callus tissue of wheat. Addition of 0.1-1 mg/l of ZhOT-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 ZhOT-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 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 ZhOT; 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 $KMnO_4$ 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 and underground parts and were measured their length on day 3, 6, 9 and 12 (Fig. 2). 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 also adding known phytohormones (IAA and BAP) if appropriate concentrations

Advances in Biotechnology

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

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.

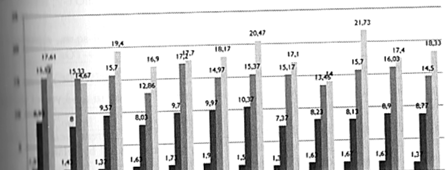

Fig. 2. Influence of preparations upon development of the above-ground part

The most efficient preparations ZhOT-4 and ZhOT-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) [6].

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

1. Synthesized derivatives of acetylene amino alcohols have growth-regulatory activity.
2. Optimum concentration of action of synthesized derivatives of piperidine is 0.0001%.
3. 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 and barley.
4. The most promising compounds ZhOT-4 and ZhOT-7 were identified in the series of synthesized derivatives of piperidine.
5. 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 wheat "Kazakhstan 10", barley "Baisheshek".

4. Soil: dark chestnut, moderate loamy, humus 3.0 – 3.5% , pH 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 ZhOT-4 and ZhOT-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 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°C 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°C higher than the average many-years temperature of 20.3°C.

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

In the course of conducting and analyzing experiments it was found that all synthesized compounds showed high growth stimulating effect in optimal concentration. Moreover, it should be noted that the combined use of synthesized RPG with protectants were more effective. Combination of ZhOT-4 (0.0001%) + chemical seed disinfectant Vial (0.4 l/t) showed the highest field emergence - 396 pcs./sq.m and option ZhOT-4 (0.0001%) – 385 pcs./sq.m , that exceeds the index of field emergence of seeds of the well- known standard - Agrostimulin (371 pcs./sq.m) and significantly higher than the control (349 PCs/m2) (Table I).

Table I – 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	ZhOT-4	96	105	98	97	396
2	ZhOT-7	99	100	95	96	388
3	Agrostimulin	94	93	98	86	371
4	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 II).

Table II – Influence of preparations upon field emergence of barley "Baisheshek" grade

Item №	Item	Option				
		I	II	III	IV	Thickness of emergence, pcs./sq.m
1	ZhOT-4	77	82	80	80	296
	ZhOT-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 ZhOT-4, ZhOT-7, and agrostimulin as reference, it was identified that ZhOT-4 and ZhOT-7 had a better effect upon tilling capacity of wheat (1.5-2.7 pcs), which is comparatively higher than that of the control; in addition the highest mass is registered in ZhOT-4 – 1000 grains (41.7 g).

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

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

Table III – 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	ZhOT-4	2.7	82.1	7.4	14.4	41.7	20.9	2.5
2	ZhOT-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	-

Processing of barley seeds by synthesized preparations did not affect the productivity of barley, the maximum increment of crop yield of 1.6 dt/ha comes on ZhOT-4. As to other options, these parameters did not exceed 0.2-1.5 dt/ha.

Conclusion: Detailed laboratory and demonstration comparative testing of ZhOT-4, ZhOT-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 ZhOT-4 and ZhOT-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 ZhOT-4 and ZhOT-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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DIFFERENT SOWING DATES – AN IMPORTANT STRATEGY FOR COEXISTENCE OF GENETICALLY MODIFIED CORN AND CONVENTIONAL CORN

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ABSTRACT

The adventitious presence of GM material in conventional corn crops is due to several factors including the cross-pollination from GM to non-GM crop. An important strategy for reducing the rate of cross-pollination is the reduction in flowering synchronization by sowing at different dates. Therefore, our study has included experiments with narrow plots (14 rows) were the transgenic corn MON 810 was sown in the same time (simultaneous) and before the conventional corn (staggered), with different isolation distances and cardinal points. The cross - pollination rates have been monitored by xenia effect. A delay of flowering of 10-12 days between MON 810 and the conventional corn, combined with the isolation distance over the source pollen donor has reduced the gene flow by 65.7%, as compared with the same time flowering making it possible to coexist even at a distance isolation of 10 m. This demonstrates the possibility of improving corn coexistence through temporal isolation based of different sowing dates.

Keywords: cross-pollination rate, flowering synchronous, MON 810, xenia effect

INTRODUCTION

Transgenesis represents one of the techniques of agricultural biotechnology by which the obtaining of genetically modified plants becomes possible [1]. Genetically modified plants have experienced a spectacular spreading. Thus, since 1996 until the end of 2014, the global area cultivated by GMOs has increased, currently reaching 181.5 million hectares. At European Union (EU) level, in 2014, the Bt-corn has been cultivated only by 5 countries from EU, occupying 143.016 hectares [2]. In Romania, in 2014, the GM MON 810 has been cultivated on 770.7 hectares [3].The introduction of GM crops and foods derived from them in the European market led to implementation of a legislative framework including in Romania as EU member [4].

Corn is a species of allogamous pollination, mostly windy and that fact makes it an ideal plant for study the spreading of pollen [5]. This species expressed the xenia effect, making possible the rate of cross-pollination between two corn types with different color of seeds [6].