The reference to article publication: Tsygankova V.A., Stefanovska T.R., Andrusevich Ya.V., Ponomarenko S.P., Galkin A.P., Blume Ya.B. Induction of small regulatory si/miRNA biosynthesis in plant cells by growth regulators with antipathogenic and antiparasitic properties // Biotechnology (ukr.). – 2012. – V. 5, № 3. – P. 62 – 74.

INDUCTION of SMALL REGULATORY si/miRNA BIOSYNTHESIS IN PLANT CELLS BY GROWTH REGULATORS WITH ANTIPATHOGENIC AND ANTIPARASITIC PROPERTIES

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Development of economically feasible and environmentally friendly agrarian technologies to ensure stability of agricultural ecosystems, to promote wide use of biocontrol, and to guarantee high quality improvement is one of the challenges of modern agriculture. Pests (insects, mites, and nematodes), diseases (bacteria, viruses, fungi, nematodes) and weeds cause significant yield reduction in agricultural production over the world. According to the Food Agricultural organization of the UN (FAO) the global annual crop losses due to pests, diseases and weeds reach 20-25 %. European corn borer, cutworms, wireworms, grabs cereal flies, aphids, root-knot and leaf weevils, soybean pod borer, spider mites, trips, rape beetles, flea beetles, stink, bugs, white butterflies belong to the most widespread and dangerous pests that cause significant yield reduction of important agricultural crops such as corn, wheat, barley, soybean, rape. A problem of plant protection against widespread fungi (*Fusarium spp., Cercospora spp., Ascohyta spp., Perronospora spp., Blumeria spp., Pucinia spp., Sclerotinia spp., Verticillim spp.*); bacterial (*Pseudomonas spp*.) and viral diseases (Potyvirus spp.) is also

economically important [1].The total losses of agricultural harvest due to diseases caused by different pathogens in Ukraine is up 50 %.

Nematodes are one of widespread and harmful plant pathogen. Yield losses due to parasitic nematodes in different countries are from 25 % to 70 %, and in outbreaks' years can be 90-100 % [1]. The total annual global crop losses caused by nematodes in money equivalent are 100 billion USD [2]. Life cycle of all the soil nematodes (phytoparasitic and free living) are closely connected, on the one side, with plants, and on the other side, nematodes can be vectors for transmitting of phytopathogenic and saprophyte bacteria, fungi, viruses and other organisms.

Today the representatives of over 20 genera of plant nematodes are known as obligate parasites of higher plants. Most of them belong to order *Tylenchida*, family *Heteroderidae*. In the Central and Eastern countries, including Russia and Ukraine the cyst nematodes *Globodera rostochiensis*, *Globodera pallida* and *Globodera tabaccum* are most dangerous for crops. First two species cause Globodera desiase on potato. The crop loss could reach in some year up to 60%. Both species are included to the list of quarantine list of the European Plant Protection Organization (EPPO). Besides the above mentioned nematodes there are several nematodes of economical importance: the tuber nematode (*Ditylenchus destructor*), nematodes – vectors for transmitting virus, such as *Paratrichodorus teres, Xiphinema diversicaudatum, Longisporus elongates* and root nematodes (*Paratylenchus spp., Helycotylenchus spp.*). Association with viruses' disease above listed parasitic nematodes contributes to increasing of potato yield of potato in Central Europe. In some years it can cause 88 % yield reduction [3]. In greenhouses three types of root-knot nematodes – *Meloidogyne incognita Meloidogyne javanica* and *Meloidogyne arenaria* are most destructive plant parasite. They cause a dangerous Meloidogyne desease. These parasitic organisms affect the several vegetables (tomato, egg-plants, potato, sugar and red beet, carrot, water-melons etc) in open field also [4].

Sugar Beet cyst nematode *Heterodera schachtii Schmidt* is extremely harmful. It is dangerous pest which caused Heterodera disease of sugar beet in

almost 40 countries [5]. This nematode contributes 95 % of crop losses caused by all harmful organisms. Sugar beet nematode in Ukraine is widespread in Vinnitsa, Sumy, Chernihiv, Cherkassy, Kharkov, Kyiv, Zhitomir regions where it might cause 30 % crop losses [6]. An important feature of this parasite is ability to reproduce on plants of 25 families, in particular, on *Brassicaceae* cultivated crops (oilseed rape, cabbage, Chinese radish, mustard) and weeds. Also nematode can develop at cultivated crops and weeds of *Chenopodiaceae* family [6]. The crop rotation is most reliable preventive tool to control sugar beet cyst nematode. The most significant losses of nematode are observed during growing of sugar beet as a monoculture and in case large concentration (to 60-80 %) of its host crops in crop rotation. The most effective method of sugar beet cyst nematode control is the rotation of sugar beet with non host to *H. scachtii* crops: cereals, grain legumes, corn, potato, perennial grasses. Over the last years the risk of increase of *H. shcachtii* damage in sugar beet crop rotations has been increased significantly. It occurs as a result of increase sowing of oilseed rape and extension of growing area of this culture (which is suitable host for *H. schachtii*), which use for obtaining of bio-fuel. Consequently the control of this dangerous pest quantity is high priority [7].

The pest control options are limited. Sugar beet cyst nematode resistant varieties are not available on the market. Carbonate compounds and organophosphate pesticides have been banned due to the high toxicity therefore the application of compounds of chemical or biological origins which are less toxic and safer for an environment is extremely important for the nematode control. Preparations developed on the basis of avermectines, aversectines and abamectines – complex antiparasitic antibiotics (producer – soil streptomycete *Streptomyces avermitilis*) – belong to high-efficiency antiparasitic preparations. Results of researches conducted during the last 20 years in the USA, Western Europe and Russia showed that the use of avermectine and abamectine reduced population of phytoparasitic nematodes on different crops [8], in particular tomatoes [9], banana [10], cotton plant [11], tobacco [12] and garlic [13]. The use of avermectines

against the root-knot nematodes *M. incognita, M. arenaria, M. javanica* is the most effective. It is known also about efficiency of preparations action against the stem nematode *Ditilenchus dipcasi*, root nematode *Rotylenchulus reniformis* and phytoparasite *Tylenchulus semipenetrans*. The cotton seed treatment with abamectine against root-knot nematodes, sugar beet – against beet nematode and corn – against *P. pratensis* [11, 14] is effective also.

In Ukraine over the last years the domestic antiparasitic preparations are used more widely. Avercom is most effective among them. It was created on the basis of avermectines – complex antiparasitic antibiotics (producer – ground streptomycete *Streptomyces avermitilis*) at the Institute of Microbiology and Virology NAS of Ukraine [15, 16]. It was found that this preparation promotes immuno-protecting properties of plants, forwards their growth and limits harmfulness of parasitic organisms – nematodes [15]. The conducted laboratory researches showed that in the high concentrations the Averkom caused 80 % death of *Meloidogyne incognita* larvae. Soil treatment with this preparation in laboratory conditions decreased nematode population by four times. [16].

The composition polyfunctional preparations Biogen, Stimpo and Regoplant are to new effective domestic preparations with nematocidal and antipathogenic action. They were created at the Institute of Bioorganic Chemistry and Petrochemistry NAS of Ukraine together with the State Enterprise Interdepartmental Science & Technology Center "Agrobiotech" NAS and MES of Ukraine. Bioprotective properties appeared because of the synergistic effect of root fungus-endophyte products in the culture *in vitro* of ginseng Panax Ginsed M. (mix of amino acids, carbohydrates, fatty acids, polysaccharides, phytohormones and microelements) and aversectines – complex antiparasitic macrolide antibiotics of soil streptomycete *Streptomyces avermitilis* [1]. Streptomycetes are known as producers of not only antibiotics but also such bioactive compounds which have phytoprotecting and growth stimulating action on plants. It is different hormones, vitamins, amino acids, carotenoids, enzymes, toxins and other matters which

influence on the growth processes of plants, namely, stimulate the seed germination and promote the productivity [15, 16].

Results of molecular-genetic research [17, 18] showed that these preparations considerably enhance plant tolerant to the different pathogens due to stimulation of the synthesis of cellular small regulatory RNA that participate in RNAi (RNA interference) process which is called posttranscriptional gene silencing (PTGS) in plants, animals and fungi [19-24]. Gene silencing mediated by either degradation or translation arrest of target RNA has roles in adaptive protection against viruses, genome defense against mobile DNA elements and developmental regulation of gene expression. Small regulatory si/miRNA of ~22-24-nt [19-24] carries out a leading role in silencing. They are generated from the predecessors – pre-miRNA of ~70 nucleotides (nt) and longer double-stranded RNA (dsRNA) molecules through their cleavage by RNase III endoribonuclease named Dicer.

The si/miRNA together with site-specific endo- and exonucleases block (silencing) translation of different classes mRNA of pathogens and parasites (having high level of homology) [19-32] or own cellular mRNA (with aberrant and defective structure or which expressed at high level during infection) [33] as well as enzymatic cleave jointly with the enzymes of RISC complex (RNA-induced silencing complex) these target mRNA molecules that results in their degradation.

The purpose of our work was determination of possibility of enhancement by polycomponent preparations Biogen, Stimpo and Regoplant of sugar beet and spring wheat immune-protective properties through increasing synthesis of small regulatory si/miRNA and, as a result, to achieve increased plant resistance to the plant-parasitic nematode, *Heterodera schachtii* and to pathogenic micromycete *Fusarium graminearum.*

In experiments we used the plants of sugar beet *Beta vulgaris L*. infected in the laboratory or in greenhouse conditions by sugar beet nematode *H. schachtii* and root-knot nematode *M. incognita*. We used also plants of spring wheat of Grizo variety infected by pathogenic micromycete *Fusarium graminearum*. In experiments for determination of variety sensitiveness to growth regulators we

used the varieties of winter wheat Yatran 60, Volodarka, Smuhlianka and Podolianka which were kindly given by an academician NAS of Ukraine V.V. Morgun. The experimental plants were processed by composition polycomponent preparations Regoplant, Stimpo, Biogen.

Experiments on the study of efficiency to decrease nematode population nematodes using growth regulators we conducted at first in the conditions of greenhouse. The plant seeds were treated with preparations Regoplant, Stimpo, Biogen where the concentration of Aversectine was 0.05; 0.1; 0.5; 1 and 10 μg/ml. Treated seeds were planted in diameter 10 cm pots with and the temperature of 25±5 °C. For the infection of plants we used the infectious larvae of cyst nematodes which were extracted from cysts. Cysts of nematodes were isolated from soil using decantation on sieves. Cysts were carried in the Oostenbrink's cups and filled by the saturated solution of zinc chloride [34]. The cyst incubation was carried out during 7 days at a room temperature. The larvae of nematodes were removed from a suspension with the use of a sieve with the diameter of screen mesh 25 μm.

Nematodes in an amount 1 000 larvae on every pot were included in two weeks after sowing of the seed treated with regulators, and in two weeks the plants were excavated and the amount of larvae in roots was determined. As control we used plants obtained from the seeds which were not treated with growth regulators. The roots of sugar beets were carefully washed in flowing water. Then roots were dipped in solution of lactic acid, glycerin, aniline and distilled water on a few minutes, were warmed up 2 minutes in microwave and were dried out on air. Pieces of roots 1,5 cm were placed in homogenizer. The homogenized roots were transfer in cylinders with volume 150 ml and 100 ml of water, were carefully shake. Then we counted the amount of nematodes which penetrated in a root.

We studied the impact of seed treatment with growth regulators looking at percent of nematode larvae penetration in sugar beet roots.

Determination of lethal concentration LD_{50} and LD_{90} we conducted in laboratory conditions. We registered the death rate of beet and root-knot

nematodes on 2nd and 24th hour after treatment with preparations Regoplant and Stimpo in the concentrations of Aversectine 0.05; 0.1; 0.5; 1 and 10 μg/ml. The test was carried out on watch-glass in four reiterations. We brought 500 μl of testable concentration of growth regulators and 40 larvae of cyst or root-knot nematodes on every glass. The larvae of second age of cyst nematode were obtained by above-described method. For the selection of larvae of second age of root-knot nematode we used a filter screen with the pores 20 μm [35].

By means of method of molecular hybridization of mRNA from si/miRNA we looked up possibility of induction by growth regulators of synthesis of si/miRNA with nematocidal activity. With this purpose the seeds of sugar beet with high germinating capacity were sprouted in the Petri dishes on the nematode-free aquatic medium (control) and with the suspension of cysts wherefrom the larvae of nematodes (approximately on 5-7th day) appeared in the process of incubation at 23 °C. Composition preparations Regoplant, Stimpo, Biogen were added in parallel tests.

Analogical experiments conducted for the verification of antipathogenic action of small regulatory si/miRNA depending on the level of their synthesis. In these experiments the seeds of spring wheat varieties Grizo, not treated (control) and treated with polycomponent preparations Regoplant, Stimpo, Biogen, were sprouted in the Petri dishes at the temperatures 25±5 °C and infected by pathogenic micromycete *Fusarium graminearum*.

Isolation si/miRNA of high-purity from the tested plants was fulfilled by our developed and published early method [18] that has such stages:

1) Separation of total preparation RNA from plant cells [36-38]. The polymerity of separated total preparations RNA was analyzed using electrophoresis in 1.5 % agarose gel in presence of 7 M urea by the Locker's method [39] (gels were stained by solution of ethidium bromide before photographing of RNA fractions in ultraviolet).

2) Division of poly(A)$^+$mRNA (mRNA) and poly(A)$^-$mRNA on oligo(dT)-cellulose column with the purpose of the further use of poly(A)$^+$mRNA for testing

of functional activity of si/miRNA in wheat germ cell-free systems of protein synthesis [36, 38];

3) Precipitation of high molecular poly(A)$^-$mRNA from an eluate was executed by 10 % solution of polyethylene glycol (mol. mass 8000) from 0.5 M of NaCl, and si/miRNA – by 96 % ethanol at -22 °C during the day; from a column the poly(A)$^+$mRNA was precipitated by 2-3 volumes of buffer of such composition: 10 мМ tris-HCl (pH 7.5), 1 мМ of EDTA, 0.05% of DDS-Na [40, 41], and after an elution from a column the poly(A)$^+$mRNA were precipitated by ethanol.

4) Molecular hybridization in solution 2xSSC of low-molecular si/miRNA with fraction of poly(A)$^+$mRNA.

5) Transferring of hybrid molecules poly(A)$^+$mRNA with si/miRNA on oligo(dT)-cellulose column with a next elution from a column by a buffer mentioned in a point 3.

6) Temperature (95 °C) denaturizing of the hybrid molecules poly(A)$^+$mRNA with si/miRNA .

7) Separation of poly(A)$^+$mRNA from si/miRNA by fractionation on oligo(dT)-cellulose column.

8) The repeated precipitate of si/miRNA by 96 % ethanol and verification of separated si/miRNA purity by 15 % polyacrylamide gel electrophoresis (PAGE).

Species-specific silencing activity of si/miRNA was determined in wheat germ cell-free systems of protein synthesis [38].

For hybridization si/miRNA, extracted from the experimental plants, with mRNA of control plants, si/miRNA was intensively marked in vivo by ^{33}P using $Na_2HP_{33}O_4$ [36]. For verification si/miRNA inhibitory activity in the wheat embryo cell-free systems of protein synthesis the unmarked si/miRNA was used [38].

DOT-blot hybridization ^{32}PcDNA with mRNA. Preparations mRNA from experimental (treated with growth regulators) wheat plants were exposed to DOT-blot hybridization with cDNA of control plants with the purpose of determination

of homology percent mRNA populations from experimental and control plants [40, 41].

For the synthesis of single-stranded cDNA was applied the buffer solution containing: 100 mM tris-HCl (pH 8.3) at 42 °C, 10 mM $MgCl_2$, 140 mM KCl, 100 µg/ml oligo$(dt)_{12-18}$ primer, 2 mM methylmercuric hydroxide, 20 mM ß-mercaptoethanol, 1 mM vanadyl-ribonucleoside complexes, or 0.5 units/mkl of RNase, 1 mM solution of the all four dNTP, 100 µg/ml poly$(A)^+$ RNA, 400-800 units/ml of reverse transcriptase and [α-^{32}P]-dCTP (800 Ci/mM) [36, 40, 41].

For transferring ^{32}PcDNA on the filters it was dissolved in a concentration 20 µg/ml in a buffer of 0.3 M NaCl – 0.03 M sodium citrate, pH 7.0, (2XSSC) and for hybridization 1 ml of solution [^{32}P]-cDNA was applied on the modified and activated cellulose filters (Whatman 50, 2-aminophenyl-thioether paper which forms covalent connections with applied DNA or RNA, as distinct from ordinary cellulose or nitrocellulose filters which form hydrogen connections with DNA or RNA). It allows avoiding the loss of nucleic acids during washing of filters [40, 42].

For hybridization on the filters cDNA with mRNA were contained in small bottles for determination of radio-activity filled with solution of 2XSSC with tenfold surplus of mRNA relatively to cDNA [40, 41]. In every test the filters with DNA from a genetically remote source for control on specificity of hybridization were contained in separate small bottles, and also filters which did not contained DNA, with the purpose of size estimation of heterospecific RNA per-sorption on material of filters. Small bottles with filters were tightly closed and placed in a thermostat for hybridization.

Hybridization was conducted at 66 °C, however during work with nucleic acids from different sources we took into account, that the molecules of RNA with more stable secondary structure (due to content of greater amount of GC pairs than can be in total preparation of mRNA) needed higher temperatures. An optimal temperature and maximal level of hybridization was selected measuring the level

of mRNA hybridization accordingly with homologous and heterologous cDNA. Hybridization was compared in different time intervals during twenty-four hours.

After hybridization completion the small bottles with filters were cooled, filters were taken out and washed in a crater from every side by 50 ml 2XSSC. After washing the filters were placed in solution 2XSSC that contained RNase in a concentration 20 μg/мl. After incubation with RNase at a room temperature during 1 hour the filters were again washed by solution 2XSSC, then by ethyl alcohol. Radio-activity of samples was determined on filter glasses Millipore AP-15 in toluene scintillator in the scintillation counter LS 100C of the firm Beckman. The statistical processing of the obtained data was conducted by dispersive (Student) and correlation-regressive methods.

It was found in the conducted laboratory, greenhouse and field experiments that the complex polycomponent preparations Regoplant and Stimpo considerably promote plant stability to the dangerous parasites – nematodes (cyst and root-knot) and pathogenic fungus (*Fusarium myxomycete)* [43, 44].

Fig. 1 shows images with infected by cyst nematode *H. schachtii* sugar beet plants *Beta vulgaris L.* and also adult females and cysts of this nematode in the field condition.

Greenhouse experiment results showed (Table 1) that seed treatment with growth regulators Regoplant and Stimpo inhibite penetration of juveniles of beet nematode in the root of sugar beet in the first month of crop vegetation. The amount of nematode larvae which obtained in a root under action of Stimpo diminished by 67.8 %, and Regoplant – by 72.68 %. The increase of concentration of Aversectine caused the decrease of larvae which penetrated in the root of sugar beet in the case of treatment with two preparations ($P<0.05$). The maximal (80 %) in relation to control decline of larvae amount in the roots of sugar beet was looked in the concentration 5 μg/ml.

***A* –** sugar beet plants infected by nematodes *H. schachtii*

***B* –** sugar beet roots damaged by nematodes

C – white and brown cysts of nematodes

Fig.1. Sugar beet plants *Beta vulgaris L.* and them damaged roots infected by cyst nematode *H. schachtii* (A and B) and white and brown cysts of nematodes (C).

Seeds' treatment with Biogen maximal diminished of larvae amount which penetrated in a root was 81 % with the concentration of preparation 1 µg/ml. It is found that the further increase of this preparations' concentration (up to 10 µg/ml) did not influence on penetration of nematode larvae in a root. In the case of plant treatment with preparations in concentration, which exceeded 10 µg/ml, the further decrease of larvae penetrated in the root of this crop did not happened (Fig. 2).

Table 1

Efficiency of sugar beet seed treatment with growth regulators against the beet nematode H. schachtii

Preparation	Concentration, µg/ml	Decrease of penetration of larvae in a root comparatively to control, %
Stimpo	0.05	49.75
	0.5	70
	1	71
	5	80
	10	69
Regoplant	0.05	52
	0.5	69.7
	1	81.5
	5	80.25
	10	79.75

Note. $LSD_{0.05}$ (least substantial difference) – 55

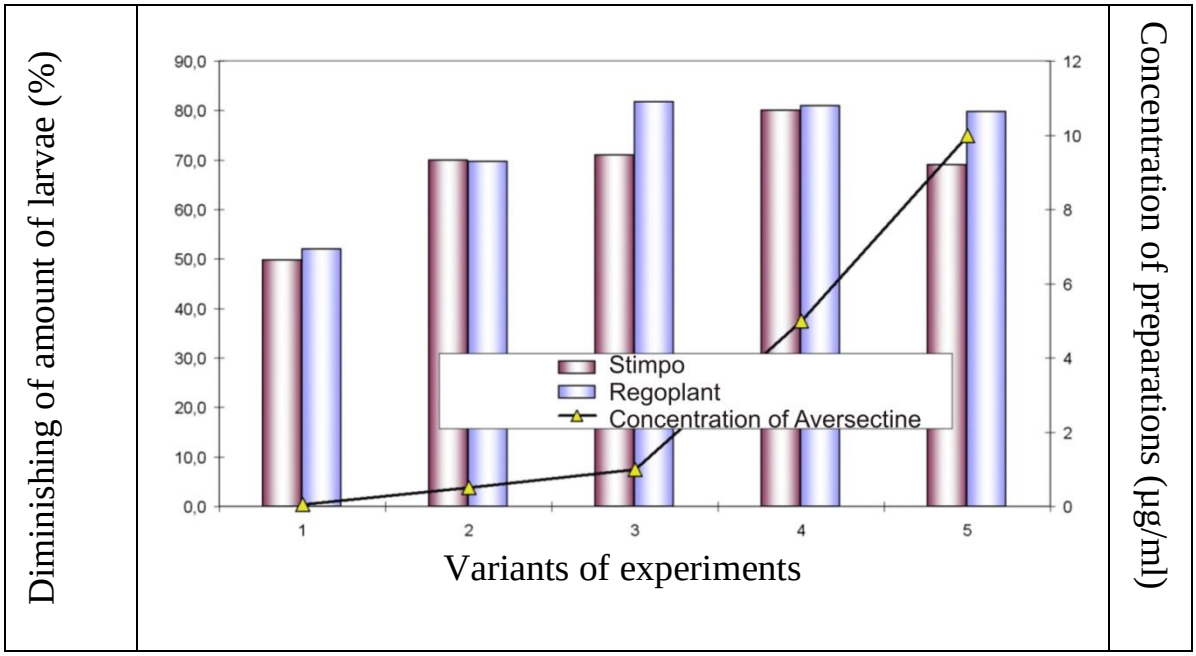


Fig. 2. Impact of sugar beet seed treatment with growth regulators with the different concentration of Aversectine on penetration of larvae of beet nematode at the beginning of crop vegetation

The results of study of growth regulators influence in different concentrations on two types of nematodes and determination of lethal

concentrations LD_{50} and LD_{90} of these preparations are shown in the Table 1 and also on Fig. 3 and 4. There is a difference between operating of regulators Stimpo and Regoplant on beet and root-knot nematodes. Regoplant predetermines 50 % death rate of larvae of root-knot nematode in 2 hours in a concentration by 30 % less than Stimpo, and in 24 hours – twice less. More significant difference in results was obtained for a beet nematode. In 2 hours 50 % of larvae perished in the concentrations of growth regulator Regoplant by 80 % less than regulator Stimpo, and in 24 hours – six fold less.

90 % death rate of both types of nematodes was observed for growth regulators Stimpo and Regoplant only in 24 hours (Table 2). A lethal concentration LD_{90} for a beet nematode was by 80 % less than for root-knot. Regoplant caused 90 % death rate of root-knot nematode larvae in twice less concentration than Stimpo. On a beet nematode the 90 % death rate is registered in application approximately of identical concentration of preparations.

Thus, the results of experiments conducted in greenhouse testify that under the action of growth regulators Regoplant and Stimpo the risk of penetration and accumulation of nematode larvae in the root of sugar beet at the beginning of crop vegetation is decreased significantly.

Table 2

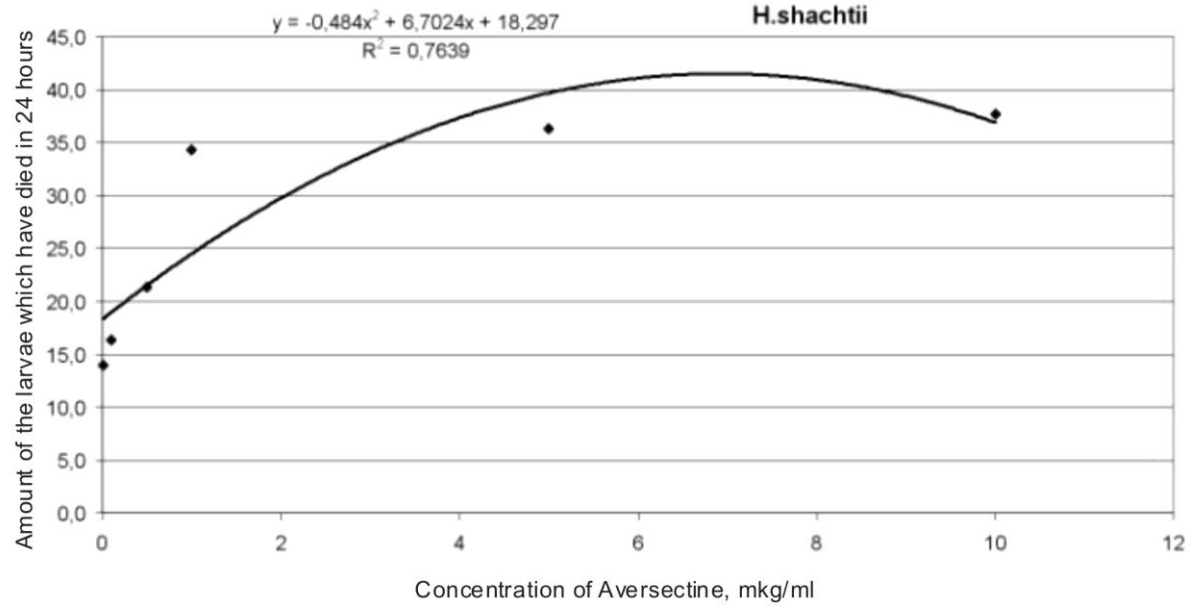
Concentration LD_{50} and LD_{90} of growth regulators for sugar beet and root-knot nematodes

Preparation	**Root cyst nematode *H. Schachtii***				**Root-knot nematode *M. incognita***			
	LD_{50}(µg/ml)		LD_{90}(µg/ml)		LD_{50}(µg/ml)		LD_{90}(µg/ml)	
	2 hours	24 hours	2 hours	24 hours	2 hours	24 hours	2 hours	24 hours
Stimpo	3.9	0.6	–	4	1.9	1	–	7
Regoplant	2.8	0.1	–	3.9	2	0.5	–	3.8

Note. *Averaged results of 3 experiments*

Results of laboratory experiments testify that a root-knot nematode is more susceptible to the action of growth regulators in a first period after treatment with

these regulators, whereas sugar beet nematode becomes more susceptible to the regulators action in a later period (Fig. 3 and Fig. 4).



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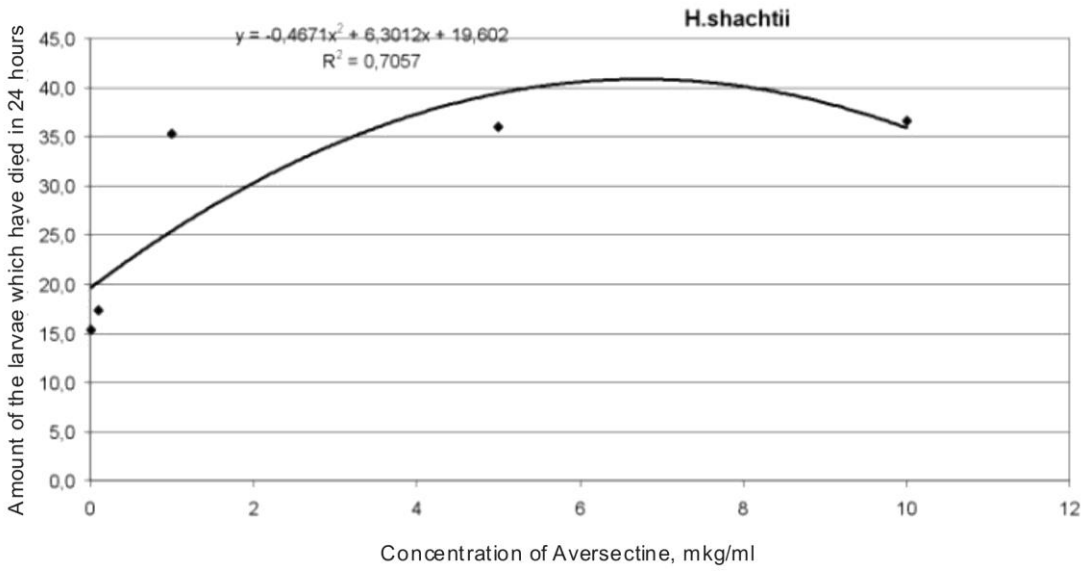

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Fig. 3. Impact of growth regulators on the larvae of beet nematode in 2 hours after treatment with regulators: 1 – Stimpo; 2 – Regoplant

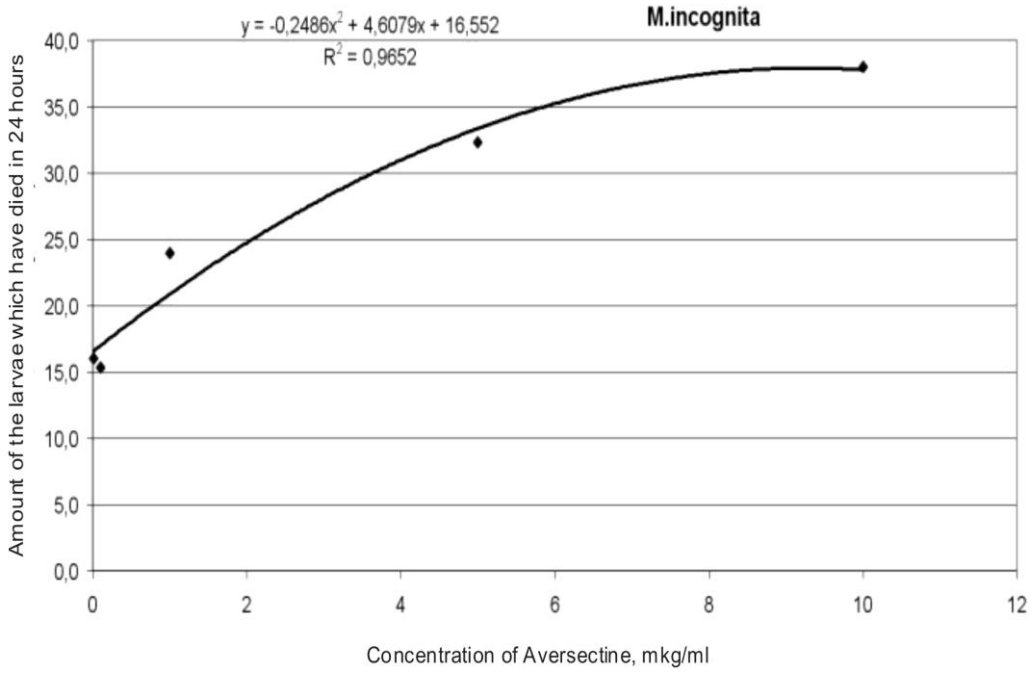

Fig. 4. Impact of growth regulators on the larvae of root-knot nematode in 24 hours after treatment with regulators: 1 – Stimpo; 2 – Regoplant

On the basis of data obtained in laboratory and greenhouse condition we can conclude that Regoplant has more clearly expressed nematocidal effect than Stimpo.

The morpho-physiological indexes of growth and development of sugar beet sprouts, which confirm high efficiency of action of composition preparations against the cyst nematode *H. schachtii*, have been obtained *in vitro* experiments.

Fig. 5 shows 5-day sugar beet sprouts growing on the infectious background (in the presence of *H. schachtii* larvae). Sprouts were obtained from seeds treated with Stimpo in a concentration 5 μg/ml (A) – test and untreated with growth regulator seeds (B) – control. The test plants under action of growth regulator Stimpo have a strong growth and development on an infectious background, while the control plants perish on a 5^{th} day after germination.

A **B**

Fig. 5. 5-day sugar beet sprouts grown on an infectious background (in the presence of larvae of cyst nematode *H. schachtii*):
A – test plants obtained from seeds treated with Stimpo (concentration 5 μg/ml);
B – plants obtained from untreated seeds (control).

Conducting molecular-genetic experiments on the study of action mechanism of composition preparations, we proceed from the premise that the affection of organism by different types of pathogens or vermin induces the synthesis of specific to their structure mRNA the pool of si/miRNA, and that the growth regulators stimulate the synthesis of si/miRNA, thereby the plant immunity rises [43, 44]. The answers for these questions can assist development of new generation of growth regulators with properties of the selective activating of synthesis of si/miRNA specific to mRNA of various pathogen or parasite.

Results of polyacrylamide gel electrophoresis (Fig. 6) testify that the obtained preparations of si/miRNA of high-purity had size 21-25 nucleotides that conform to the classic parameters of these types of RNA.

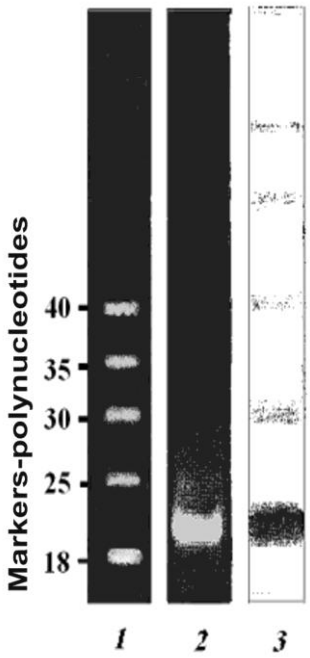

Fig. 6. Polyacrylamide gel electrophoresis of si/miRNA from sugar beet sprouts. Marker polynucleotides (numbers are mark length in nucleotides) and preparation si/miRNA on the paths of gel (1 and 2) were saturated by ethidium bromide; radioautograph of ^{33}P marked si/miRNA from gel (path 3)

Data in a Table 3 testify to the considerable increase of synthesis of cellular si/miRNA after infecting of sugar beet plants by the larvae of nematodes, and conversely about the considerable increase of synthesis of nematocidal si/miRNA under influence of composition preparations Stimpo and Regoplant and, as a result, diminishing of plant cell affection by nematodes under action of these preparations.

Table 3

Level (%) differences on the hybridization level of cytoplasmic P^{33}-mRNA populations with homologous si/miRNA from the plants of sugar beet treated with polycomponent composition regulators and nematode *H. schachtii* in relation to control plants (as control we used % of P^{33}-mRNA hybridization with homologous si/miRNA from plants that were not treated with regulators and nematodes)

Name of regulator	**Control**	**A percent of Aversectines to the regulators**	**Variants of experiments**	
			Growth regulators	Growth regulators + nematodes
Biogen (Emistim C + Aversectines)	98*	0.2	96±0.54**(2%)	86±0.46**(12%)
		2.5	94±0.72** (4%)	88±0.58** (10%)
		5.0	91±0.66**(7%)	92±0.62**(6%)
Stimpo (Biolan + Aversectines)		0.2	97±0.58**(1%)	82±0.64**(16%)
		2.5	93±0.84**(5%)	84±0.72**(14%)
		5.0	92±0.62**(6%)	87±0.68**(11%)
Regoplant (Radostim + Aversectines)		0.2	94±0.38**(4%)	86±0.48**(12%)
		2.5	92±0.73**(6%)	88±0.52**(10%)
		5.0	88±0.68**(10%)	90±0.38**(8%)

Notes.

** – Presence of significant differences, $P < 0.05$, n=3.

* – Presence of significant differences in control, $P < 0.05$, n=3.

The level (%) of differences was studied by means of method of Dot-blot hybridization. In experiments we used 7 days sugar beet sprouts. Solution of each composition preparations at volume 20 μl was added in the test samples (Petri dishes). Practically all the beet sprouts treated with nematodes without growth regulators perished on the 5^{th} day of incubation.

Physiologic and morphogenic indexes of growth and development (energy of seed germination, sprout density, speed of growth, length and volume of root system and above-ground part, stability of plant sprouts to drowning) were investigated in the conducted laboratory experiments on determination of variety-specific action of composition preparations with bioprotective properties (Biogen, Stimpo, Regoplant) on the different varieties of winter wheat (Yatran 60, Volodarka, Smuhlianka and Podolianka).

Determination of percent of homology in populations of cytoplasmic mRNA-transcripts where genome realizes the program of synthesis of structural and functional elements, processes of plant growth and development during their ontogenesis, by the method of DOT-blot hybridization of P^{33}-cDNA of one variety with mRNA of all other varieties showed significant differences in population compositions ("spectrums"), and also difference in the changes of population indexes of mRNA under action of composition growth regulators (Table 4).

Obtained results on the differences in the integral physiologic and morphogenic indexes of wheat sprouts of different varieties together with the noted molecular-genetic differences, and also before found cardinal varietal differences in correlation (balance) of phytohormones and under action of growth regulators [1], testify to the presence possibly irreversible processes of genome reprogramming in test varieties, and also reverse processes under action of external regulator factors (on the different mechanisms of both processes). One of possibilities of the educed varietal differences can be regrouping (reprogramming) of genes and exception from action (for example, as a result of mutation during variety creation) of active genes which functioned in the initial paternal forms of plants and including of earlier nonactive but near on the function genes in multigene families or superfamilies of genes, where every member (variant) of family some differs on the nucleotide sequence in regulatory, coding and noncoding areas in their structures, has the different settings in adaptation processes and regulated by different factors [37].

Table 4

Level (%) of differences of population descriptions of cytoplasmic mRNA of different winter wheat varieties, which were treated and not treated with growth regulators with bioprotective properties

Wheat varieties	Percent of cDNA hybridization of wheat Yatran 60 with homologous control and heterologous experimental mRNA	Growth regulators								
		Biogen (Emistim C + Aversectines)			Stimpo (Biolan + Aversectines)			Regoplant (Radostim + Aversectines)		
		0,2	2,5	5,0	0,2	2,5	5,0	0,2	2,5	5,0
Yatran 60	98±0.63*	96± 0.48** (2%)	94± 0.44** (4%)	91± 0.54** (7%)	97± 0.62** (1%)	96± 0.46** (2%)	94± 0.72** (4%)	96± 0.42** (2%)	93±0.46** (5%)	91±0.76** (7%)
Volodarka	89±0.56** (11%)	97±0.82** (1%)	86±0.78** (12%)	88±0.47** (10%)	90±0.48** (8%)	86± 0.66** (12%)	84± 0.54** (14%)	86± 0.58** (12%)	84± 0.98** (14%)	82±0.8** (16%)
Smuhlianka	91±0.52** (9%)	88±0.96** (10%)	87±0.63** (11%)	82±0.43** (16%)	88± 0.56** (10%)	90± 0.54** (8%)	86±0.48** (12%)	87±0.76** (11%)	88±0.84** (10%)	84±0.78** (14%)
Podolianka	83± 0.78** (15%)	82± 0.65** (16%)	79± 0. 74** (19%)	78± 0.86** (20%)	77± 0.68** (21%)	81± 0.53** (17%)	76± 0.84** (22%)	80± 0.68** (18%)	77± 0.82** (21%)	75±0.96** (23%)

Notes.

** – Significance of differences against control, $P<0.05$, n=3.

The level (%) of differences was studied by means of method of Dot-blot hybridization. 7 days wheat sprouts were used in experiments. 20 mkl solutions of each composition growth regulators was added in the test samples (Petri dishes). Digital values 0.2; 2.5; 5.0 are % of Aversectines in relation to the growth regulators.

Such possibility is confirmed by results on the level of polypeptide biosynthesis oppression in the cell-free systems of protein synthesis by small regulatory RNA (si/miRNA) isolated from the different wheat varieties (Fig. 7). The cumulative result will serve as basis for further determination of molecular-genetic

mechanisms of differences in the sensitiveness of different winter wheat varieties to the plant growth regulators.

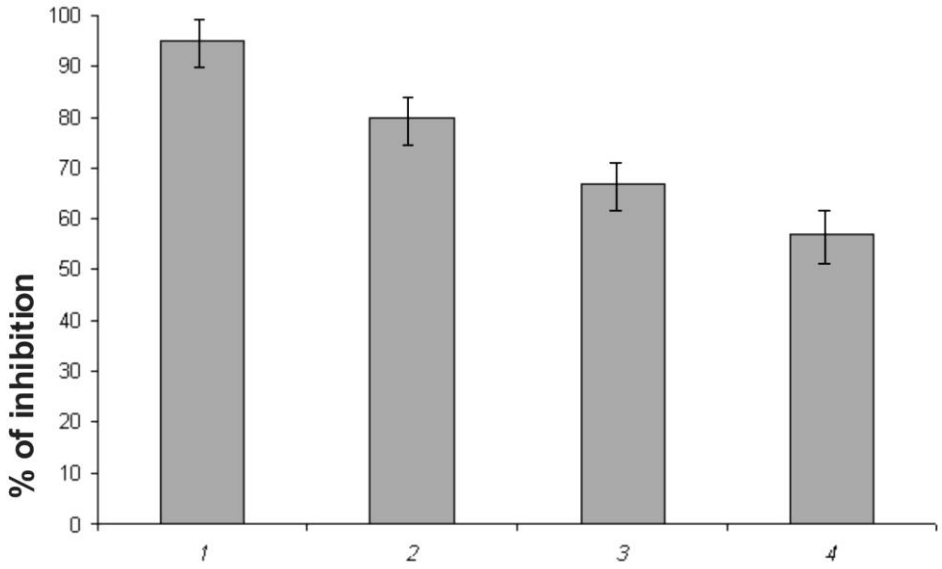

Fig. 7. Percent of inhibition in the wheat embryo cell-free systems of protein synthesis of polypeptide biosynthesis on the mRNA template from the cells of winter wheat of Yatran 60 variety by means of small regulatory RNA (si/miRNA), isolated from the cells of wheat different varieties:
Yatran 60 (1); Volodarka (2); Smuhlianka (3) and Podolianka (4)

Dependence of the structure of varietal specificity of si/miRNA inhibitory activity on the processes of mRNA translation of varieties is observed.

In the experiments on the study of molecular-genetic mechanisms of antipathogenic action of growth regulators we took into account obtained earlier in the field conditions data [1, 17] about the considerable strengthening of protective properties of different varieties of spring wheat to pathogenic micromycete *Fusarium graminearum*.

In laboratory experiments conducted on treated with growth regulators Biogen, Stimpo, Regoplant plants of spring wheat varieties Grizo the results which testify to the significant increase of stability of these plants (~ to 35-70 %) to pathogenic micromycete *Fusarium graminearum* were obtained. By the method of DOT-blot hybridization of preparations of cytoplasmic mRNA (through cDNA)

and previous determination of hormonal composition of control and test plants [17] it is found that the increase of the productivity and crop stability to the pests is related to the substantial changes of population descriptions (sets) of mRNA and small regulatory si/miRNA, possibly, due to the selective switching of gene activity in the corresponding gene multifamilies (Table 5).

Therefore, by means of molecular-genetic method of Dot-blot hybridization we for the first time found a difference in population descriptions of si/miRNA between control sugar beet sprouts and plants treated with composition preparations – growth regulators with bioprotective properties, and also plants treated with regulators on artificially created by the nematode *H. schachtii* the infected background. Results testify to existence of the flexible system of plant cell genome reprogramming under the action of different external regulatory factors.

Table 5

Level (%) hybridization of cytoplasmic P^{33}-mRNA populations with homologous si/miRNA from spring wheat sprouts variety Grizo that were grown from seeds treated with growth regulators and micromycete *Fusarium graminearum*, in relation to control (1-month) plants (as control we used % of hybridization of P^{33}-mRNA with homologous si/miRNA from plants not infected by pathogenic micromycete *Fusarium graminearum* and micromycete *Fusarium oxysporum*)

Regulator	**Control**	**% of Aversectines to regulators**	**Variants of experiments**	
			Plants treated with growth regulators	**Plants infected and treated with growth regulators**
Biogen (Emistim C + Aversectines)	98*	0.2	95±0.54**(3%)	82±0.46**(18%)
		2.5	92±0.72** (6%)	88±0.58** (10%)
		5.0	91±0.66**(7%)	92±0.62**(6%)
Stimpo (Biolan + Aversectines)		0.2	97±0.58**(1%)	80±0.64**(18%)
		2.5	93±0.84**(5%)	89±0.72**(9%)
		5.0	90±0.62**(8%)	90±0.68**(8%)
Regoplant (Radostim + Aversectines)		0.2	94±0.38**(4%)	86±0.48**(12%)
		2.5	89±0.73**(9%)	90±0.52**(8%)
		5.0	87±0.68**(11%)	92±0.38**(6%)

Notes.

** – Presence of significant differences, $P < 0.05$, n=3.

* – Presence of significant differences in control, $P < 0.05$, n=3.
The level (%) of differences was studied by means of method of Dot-blot hybridization. In experiments we used 2-month plants of spring wheat of Grizo variety grown from seeds that was not treated and treated with growth regulators on an infectious background with micromycete *Fusarium graminearum*.

Substantial changes in population descriptions of si/miRNA of spring wheat sprouts of Grizo variety are educed in the determination of percent of si/miRNA homology to mRNA in adult plants grown from seeds treated with growth regulators, not infected and infected by micromycete *Fusarium graminearum*. In this case we observed changes depended on the concentration of growth regulators.

Conclusions. Thus, we found population differences dependent on growth regulator doze, varietal differences of cytoplasmic mRNA of wheat plant by the method of Dot - blot hybridization and with the use of the wheat embryo cell - free system of protein synthesis, which can be explained by genetic nature of different stability of plant varieties to biotic and abiotic external factors.

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