The article was published in Journal: «Physiology and biochemistry of cultivated plants» 45 (2) 2013 P. 138 – 147. (In Ukr.).

STIMULATION OF PLANT IMMUNE PROTECTION AGAINST PATHOGENIC FUNGI, INSECTS AND NEMATODES WITH GROWTH REGULATORS

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In the conditions of field experiments during three years the bioprotective antipathogenic activity of new polyfunctional plant growth regulators (PGRs) Regoplant and Stimpo on infectious background cultivation of different sorts of winter and spring wheat, barley, soya and corn had been investigated. The best physiological indexes of plant productivity and resistance to phytopathogens were received at double treatment of plants by PGRs Stimpo and Regoplant at seed treatment and crop spraying during vegetation period. PGRs promoted additional yield safety more than by 60 % to control. In experiences *in vivo* it is established the effect of inheritance of resistance to the pathogenic organisms of the second plant generation (which was not treated with PGRs on an infectious background) obtained from seeds of first generation plants (which was treated with PGRs on an infectious background). Using DOT-blot hybridization method the high level of homology between immune-protective small regulatory si/miRNA and mRNA of the 1st and 2nd plant generation and lower level of homology in relation to control plants were established that testifies to existence of mechanisms reprogramming genome of plant cells under PGRs action.

Keywords: plant resistance to pathogens, growth regulators, small regulatory si/miRNA, method DOT-blot hybridization, heterosis-like effect of regulators' action.

Non- chemical crop protection is important component of sustainable crop production. The development of such compounds is based on achievements of modern microbiology, mycology, biotechnology, soil science and plant protection. The long-term research and wide practical application of created in the National Enterprise Interdepartmental Science & Technology Center «Agrobiotech», NAS and MES of Ukraine of new polycomponent preparations Biogen, Stimpo and Regoplant showed that these preparations match with economical and ecological demands of modern agriculture. These biostimulators have bioprotective and regulatory effects that are achieved by synergistic action of metabolism products (mixtures of amino acids, carbohydrates, fatty acids, polysaccharides, phytohormones and microelements of root fungus-endophyte products of ginseng Panax Ginsed M as well as of soil streptomycete *Streptomyces avermitilis* metabolites [2] with phytostimulating, antiparasitic and antipathogenic action.

In our molecular-genetic experiments we have showed that positive effects of the above mentioned PGRs exists in quantitative and qualitative changes in gene expression as a consequence of plant cell genome reprogramming by PGRs [1, 3-5, 14]. Research of features and sequences of these genetic processes changes became priority in our fundamental research in the development of new perspective PGRs. We have found also [6] that these preparations considerably enhanced plant resistance 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) found out in plants, animals and fungi [8, 9, 15]. Gene silencing is a process resulting in degradation or blocking of translation of molecules-targets mRNA. It is very important for plant adaptation and resistance to the viruses, in genome protection against the mobile DNA-elements, as well as in the ontogenetic regulation of gene expression. Small regulatory si/miRNA with size of 22-

24 nt [8, 9] have a leading role in silencing: together with site-specific endo- and exonucleases of RISC complex (RNA-induced silencing complex) block (silencing) translation of variable with imperfect structure cellular mRNA as well as mRNA of pathogens and vermin, or enzymatic cleave these target mRNA molecules that results in their degradation [7, 10, 11, 13].

The purpose of our work was determination the possibility of the above-mentioned composition preparations to increase synthesis of endogenous small regulatory si/miRNA as basic constituents of the plant immune system.

Methods

In experiments on research of PGRs efficiency we treated seeds of winter wheat, barley, soybean and corn. The growth regulators Stimpo and Regoplant were used for winter wheat, barley, corn and soybean seed treatment, and Stimpo – for crop spraying of winter wheat and barley at the stage of the end of tillering, soybean– at the budding stage and corn – at the stage of 7-9 leaves.

The field experiments were carried out during 3 years (2010-2012) in the Selection & Genetic Institute – the National Centre of Seed-growing and Variety Study of NAAS of Ukraine (Odessa) in the department of phytopathology and entomology with the additional technical assistance of the Research and Production Company "Fungi" in the field site on artificial infectious backgrounds and on the natural invasion background. The second experimental ground was in the research farm on the south of the Odesa region. Plots were of 10-30m^2area, accounting experimental plants – 25, replications of experiments - 4, complete randomization with frequent control (without preparations). We used the varieties of winter wheat Dalnytska, soybean– Arcadia Odeska, corn hybrid - Kobza MV.
Seeding rate – 4.5 million seeds per 1 hectare. Date of sowing was 27.09.2010. Soils on the experimental field were southern chernozem contenting humus in account 3.2%. General amount of fallouts over a period of September 2010 – June 2011 was 521.8 mm.

We compared efficacy of PGRs with standard insecticides to control Ground beetle *Zabrus tenebrioides,* Turnip Moth *Scotia segetum, Cloropidae spp. and* wheat nematode *Anguina tritici* The greenhouse experiments of comparative efficacy of PGR and standard insecticides conducted in pots of 25 cm x 25 cm, each experiment was replicated four times. Soil probes were contaminated separately by nematode, ground beetle; turnip moth. We used the varieties of winter wheat Dalnytska, soybean – Arcadia Odeska, corn hybrid - Kobza MV. Seeds in a box were sown after treatment with preparation. In experiments were used 50 plants. We studied of efficacy base on two indicators: 1 – Amount of damaged plantlets, pcs./m^2; 2 – Biological efficiency, %

The impact of the PGRs on stability, productivity, quality of obtained seeds, as well as on plant resistance to the infection was studied. Experiments were conducted on the artificial infectious backgrounds diseases of wheat rot and mildew caused with such pathogens for wheat as *Mucor spp., Rhizopus spp., Aspergillus spp., Penicillium spp., Trichothecium roseum*. Efficiency against pathogens onthe lowlevel of spores, i. e. 0.1 g of spores per 1 kg of seeds and at high levelof spores, i. e. 1 g of spores per 1 kg of seeds.

Results and discussions

At the Selection & Genetic Institute – the National Centre of Seed-growing and Variety Study of NAAS of Ukraine (Odessa) the wide efficacy tests of PGRs produced by the Center "Agrobiotech has been carrying out since 2003.Researches carried out on the fields of this institute in the field infectious site and in the working conditions of agricultural enterprises of Odesa region. The main task of these investigations is determination of new PGRs impact on the sowing quality of seeds, productivity and resistance to the phytopathogenes of the varieties of winter and spring wheat, winter and spring barley, soybean, corn created by the way of selection. Many years phytopathologists, virologists, entomologists search am efficient control methods of the viral infections on cereal crops. The creation of resistant ant tolerant varieties for viruses and their transmitters is the most reliable method. However this way is long-term process and not always it can be successful. It has been proved that plants have no genetically fixed resistance to the most harmful virus of barley yellow dwarf which can result in the loss up to 60 % of winter wheat yield. That is why the search of another way of problem decision is necessary.

Obtained results of positive impact PGRs' on all physiologic signs of plant are presented in a Table 1. It will be seen that in the conditions of standard seeds treatment with PGRs, really, their impact on growth processes, on the elements of yield structure and productivity is sufficiently different in comparison with control variant. A higher positive dynamics is found at the application of Stimpo and Regoplant – newest innovative preparations which take the registration tests.

The positive impact of preparation on all the physiologic indexes was shown in the variant of the crops praying by preparation Stimpo. There is a reliable additional yield. The best indexes are shown in a variant when PGRs is used twice – at the seed pre - sowing treatment and crop spraying during vegetation. It is shown that preparations with the bioprotective effect Stimpo and Regoplant application provided significantly different, 60% of yield increase compared to control. Other preparations demonstrated high efficiency too.

During 2010-2012 we have conducted testing bioprotective effect of new PGRs on the infected backgrounds cultivation of winter wheat, barley, soybean, corn in comparison to use modern pesticides of leading companies "Bayer Crop Science AG", Germany", insecticides Alpha-cypermethrin ("Syngenta", Switzerland), insecto-fungicides Yunta Quadro (active substance clothianidin or tebuconazol, or prothiconazol), Lamardor (active substance tebuconazol), Selest Top (active substance thiametoxam or fludioxonil, or difenoconazole), Imidacloprid, micronutrients Terios and Microplant. Using PGRs along with chemical pesticides caused increase of in plant resistance to different types diseases cause by microbial pathogens. Plant Growth regulators reduce the phytotoxicity of chemical protectants and stimulate immune reactions of plants. As a result the improvement of commercial grain yield and seed material quality. It was found at the study of PGRs impact on nematodes, ground beetle, turnip moth, chloropid flies hat their bioprotective effect is sufficiently high (Table 2). PGRs did not exceed an effect exposed by using such insecto-fungicides, as Yunta Quadro and Selest

Top. However, level of efficiency which is shown by Regoplant against wheat nematode, Regoplant – against ground beetle, Regoplant – against turnip moth, and all the investigated PGRs against chloropid flies was justified economically and ecologically. Therefore, we consider perspective the use of preparations Regoplant and Stimpo for prevention and control of mentioned above harmful soil in habited pests, wheat nematode and cereal flies.

Regoplant and Stimpo showed also antipathogenic activity against the causative agents of rot and mildew (Table 3). However, the use of these preparations as alternative of chemical pesticides we do not consider as reliable, especially on the high infectious background. On the low infectious backgrounds the PGRs' application is fully possible, taking into account a level of their potential efficiency.The test was carried out in laboratory conditions on artificially infected by pathogens seeds of variety Odeska semidwarf.

We studied also PGRs' impact on the vegetation of soybean and corn. Regoplant and Stimpo positively impact on the growth processes of soybean (Table 4) and corn plants (Table 5).

In the laboratory experiments the soybean seeds were artificially infected by causative agents of the most economically meaningful and harmful soybean diseases. We used soybean variety– Arcadia Odeska. In investigations of comparison efficiency of Regoplant and Stimpo with chemical protectants we found that on the artificial infection background of soybean by the dangerous seed pathogens the sufficient PGRs' efficiency appears. Thus, using these preparations as protectants we may to expect positive impact on soybean seed enhancement (Table 6).

Table 7 shows the results of PGRs' efficiency against the causative agents of rot and mildew of corn. Seeds of corn hybrid Kobza MV were artificially infected by causative agents of most economically meaningful and harmful diseases of corn. In the test variants the seeds were treated with preparations. In the tests of efficiency of Regoplant and Stimpo on diminishing of infection impact and on the growth processes of seed material we found that PGRs positively impact on growth and development of corn seeds. They diminish infection impact on seed development, reduce the infectious loading on commodity grain etc. We consider perspective usage Regoplant and Stimpo in the technology of corn cultivation in the well-developed farms of Ukraine.

In the experiments *in vivo* the PGRs' post-action –the effect of inheritance of plant resistance to the pathogenic organisms of the second plant generation (which was not treated with PGRs on an infectious background) obtained from seeds of first generation plant (which was treated with PGRs on an infectious background) was studied too. We found that plants of the second generation which was not treated with PGRs keep a high viability and productivity, what near to those which were obtained on the plants of first generation which was treated with PGRs on an infectious background. The molecular-genetic analysis using DOT-blot hybridization method [12] showed high level of homology between immuno-protective small regulatory si/miRNA and mRNA of experimental plants of first and second plant generations and more low level of homology in relation to control plants. This effect we called "quasi-heterosis". It was found that PGRs Biolan, Biosil and Stimpo substantially increased growth properties and productivity of heterosis plants as well as resistance to parasitic and pathogenic organisms. We concluded that basic mechanism of these PGRs in plant cells consists in almost twofold increasing of synthesis (abundance) small regulatory si/miRNAs which have antipathogenic and antiparasitic properties. Based on the tests of PGRs Regoplant and Stimpo conducted in the field and laboratory conditions we could recommend these preparations for registration fro using at cereal crops.

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Table 1

PGRs and their impact on the productivity and elements of harvest structure (2010-2012)

Variant	Stooling coefficient	Density of productive stems pcs./m^2	Amount of grains in 1 ear, pcs.	Weight of 1ear, g	Weight of1000 grains, g	Yield		
						c/ha	± toControl c/ha	± to Control%
1	2	3	4	5	6	7	8	9
Control, water 15 l/t	1.55	532	35	1.25	35.7	31.6		
Pre-sowing seed treatment								
Stimpo, 25 ml/t	2.43	605	43	2.04	47.4	44.2	+12.6	+40
Regoplant, 250 ml/t	2.91	661	47	2.16	46.0	45.1	+13.5	+43
Terios, 1 l/t	2.45	658	41	2.01	42.7	41.1	+9.5	+30
Pre-sowing seed treatment + crop spraying								
Regoplant, 250 ml/t + Stimpo, 20 ml/ha	2.90	662	46	2.19	47.6	49.8	+18.0	+57
Terios, 1 l/t +Microplant, 1.5 l/ha	2.44	656	40	2.12	53.0	47.1	+15.3	+48
LSD $_{0.05}$*	0.39	15	2.1	0.49	1.3	1.6	0.75	

Note: *average data of 3-year experiments*

*the least substantial difference

1 – Amount of the infected plants;

2 – Biological efficiency, %.

Table 2

Efficiency of PGRs' bioprotective effect in comparisonwith traditional chemical preparations – seedprotectants against wheat nematode, ground beetle, turnip moth, chloropid flies(2010 - 2012)

Variant	Norm of used preparation,l/t	Wheat nematode		Ground beetle		Turnip moth		Chloropid flies	
		1	2	1	2	1	2	1	2
Control		42.5		36.6		15.2		39.4	
Stimpo	0. 025	22.5	47	14.6	60	9.9	35	17.1	57
Regoplant	0.25	5.4	87	6.8	81	5.9	61	10.1	74
Yunta Quadro	0.15	3.5	92	0.1	100	0	100	2.1	95
Selest Top	0.2	4.1	90	1.9	95	0	100	2.8	93
Imidacloprid	1.0	17.9	58	1.1	97	0.6	96	2.1	95
Alpha-cypermethrin	0.5	29.9	30	9.6	74	4.5	70	7.1	82
$LSD_{0.05}$*		1.1		0.9		0.8		2.3	

***Note**: average data of 3-year experiments*

*the least substantial difference

1 – Amount of the infected plants;

2 – Biological efficiency, %.

Table 3

PGRs efficiency in comparison withseed protectants against the causative agents of wheat rot and mildew (variety Odeska semidwarf, 2010-2012)

Variant	Norm of used preparati-on, l/t	Fusarium sp.		Altemaria sp.		Bipolaris sorokiniana		Complex of storage fungi*		Bacillus sp.**	
		1	2	1	2	1	2	1	2	1	2
Control		18.5		21.5		13.5		45.5		9.5	
Stimpo	0.025	8.5	54	10.5	47	4.5	67	16.5	64	1.5	16
Radostim	0.25	6.5	65	9.0	58	9.0	33	19.5	57	2.5	74
Regoplant	0.25	4.0	78	5.0	77	3.5	74	11.0	76	1.0	89
Lamardor	0.2	0.5	97	0	100	0	100	0	100	1.5	84
Yunta Quadro	1.5	0.5	97	0	100	0	100	0	100	1.5	16
Microplant	1.5	19.5	-5	12.5	42	7.5	44	34.5	24	6.5	32
LSD $_{0.05}$***		0.4		0.9		0.6		4.2		3.2	

***Note**: average data of 3-year experiments*

1 – Amount of the infected plants;

2 – Biological efficiency, %.

* Fungi *Mucor spp., Rhizopus spp., Aspergillus spp., Penicillium spp., Trichothecium roseum*.

** Causative agents of bacterial rot

***the least substantial difference

Table 4

PGRs and their impact on the productivity and elements of structure of harvest of soy (2010 - 2012)

Variant	Germi-nation energy, %	Germina-tion capacity, %	Amount of flowers on1 cluster,pcs.	Amount of beans on 1 plant,pcs.	Weight of 1000 seeds, g	Yield		
						c/ha	± toControlc/ha	±toControl%
1	2	3	4	5	6	7	8	9
Control, water 15 l/t	72	67	7-8	40-42	154.5	14.8		
Presowing seed treatment								
Stimpo, 25 ml/t	74	79	12-13	44-46	159.9	17.4	+2.6	17.5
Regoplant, 250 ml/t	76	81	14-15	52-54	167.1	19.9	+5.1	34.5
Terios, 1 l/t	74	81	14-15	61-62	159.9	17.9	+3.1	20.9
Presowing seed treatment + crop spraying								
Regoplant, 250 ml/t + Stimpo, 20 ml/ha	69	65	6-7	38-40	149.9	14.9		

Terios, 1 l/t +Microplant, 1.5l/ha	76	80	14-16	52-54	174.1	26.9	+12.0	80.5
Control, water 15l/t	74	80	14-15	61-62	171.1	22.6	+7.7	51.7
LSD $_{0.05}$*	1.7	1.9	1.6	2.8	1.5	1.4		

Note: *average data of 3-year experiments*

*the least substantial difference

Table 5

PGRs' impact on the productivity and elements of harvest structure of corn (2010-2012)

1. Variant	2. Germination energy, %	3. Germination capacity, %	4. Amount of cobs on 1 plant	5. Weight of 1000 seeds, g	6. Yield, cwt/ha	± to Control	
						c/ha	%
1	2	3	4	5	6	7	8
Control, water 15 l/t	67	68	1.1-1.6	256.1	22.6		
Seedpresowing treatment							
Stimpo, 25 ml/t	74	94	2.4-2.6	298.4	36.9	+14.3	63.2
Regoplant, 250 ml/t	76	96	2.6-2.8	301.1	37.9	+15.3	67.7
Seedpresowing treatment + of crop sprinkling							
Regoplant, 250 ml/t+ Stimpo, 20 ml/ha	76	96	2.6-2.8	309.7	39.9	+17.8	80.5
LSD $_{0.05}$*	1.7	1.9	0.8	1.3	0.9		

***Note**: average data of 3-year experiments*

*the least substantial difference

Table 6

PGRs' efficiency in comparison with other preparations – seed protectants against the causative agents of rot and mildew of soybean (2010-2012)

Variant	Norm of used preparations, l/t	Fusarium sp.		Botrytis cynerea		Alternaria spp.		Complex of storage fungi*	
		1	2	1	2	1	2	1	2
Control		43.6		29.3		9.7		32.4	
Stimpo	0.025	12.8	71	8.1	72	1.1	89	8.9	73
Regoplant	0.25	1.8	96	0.6	98	0.5	95	4.5	86
Lamardor	0.2	1.5	96	0	100	0	100	0	100
Yunta Quadro	1.5	0	100	0	100	0	100	0	100
LSD $_{0.05}$**		0.8		0.7		0.6		1.4	

***Note**: average data of 3-year experiments*

1 – Amount of the infected seeds;

2 – Biological efficiency, %

* fungi*Mucor spp., Rhizopus spp., Aspergillus spp., Penicillium spp., Trichothecium roseum*.

**the least substantial difference

Table 7

PGRs' efficiency in comparison with other preparations – seed protectors against the causative agents of rot and mildew of corn (2010-2012)

Variant	Norm of used prepara-tions, l/t	Fusarium sp.		Alternaria sp.		Nigrospora sp.		Complex of storage fungi*	
		1	2	1	2	1	2	1	2
Control		21.8		12.9		11.7		65.4	
Stimpo	0.025	10.3	53	2.6	80	4.6	61	27.1	59
Regoplant	0.25	9.1	58	0.7	95	0.6	95	13.6	79
Lamardor	0.2	0	100	0	100	0	100	0	100
Yunta Quadro	1.5	0	100	0	100	0	100	0	100
LSD $_{0.05}$**		0.5		0.8		0.6		3.1	

***Note**: average data of 3-year experiments*

1 – Amount of the infected seeds;

2 – Biological efficiency, %

* – Fungi *Mucor spp., Rhizopus spp., Aspergillus spp., Penicillium spp., Trichothecium roseum.*

**the least substantial difference