Synergistic effect of bioregulators with pesticides and herbicides on improving growth, yield quality and crop resistance against pathogens and pests

S. P. Ponomarenko[1], Z. M. Hrytsaenko[2], V. A. Tsygankova[3], O.V. Babayants[4]

[1] National Enterprise Interdepartmental Science and Technology Center "Agrobiotech" of NAS and MES of Ukraine, Kiev
[2]Uman National University of Horticulture
[3]Institute of Bioorganic Chemistry & Petrochemistry, NAS of Ukraine, Kiev
[4]Selection & Genetic Institute – the National Centre of Seed-growing and Variety Study of NAAS of Ukraine, Odessa

Abstract

In the greenhouse experiments the antipathogenic activity of new polycomponent bioregulators Regoplant and Stimpo has been investigated at cultivation of different varieties of winter wheat and soya plants on the infectious backgrounds. The high biological efficiency against phytopathogens was obtained at preseeding treatment of plants and spraying of crops in vegetation period by Regoplant (up to 98 %) and Stimpo (up to 89 %) according to control (without treatment by bioregulators). Application of bioregulator Regoplant in combination with herbicides Herbitox or Grenadier for cultivation of sugar sorghum and winter wheat showed the best indexes of plant growth and yield quality. Obtained results, may be explained by synergistic effect of bioregulator Regoplant and herbicides, as well as bioprotective effect of biostimulator Regoplant against damaging action of herbicides on plant cells. In the molecular-genetic experiments the 2nd generation of the wheat plants (infected by pathogenic micromycetes of *Fusarium L.* genus without treatment by bioregulators) showed the increased resistance to pathogenic micromycetes of *Fusarium L.* genus. Using DOT-blot hybridization method the considerable difference in the degree of homology between mRNA of control wheat seedlings and small regulatory si/miRNA of experimental seedlings (obtained from seeds of the 1st generation, infected by pathogenic micromycetes of *Fusarium L.* genus and treated by bioregulators) was found. It is proposed that indicated difference connected with reprogramming of plant genome under impact of bioregulators - inductors of synthesis si/miRNA with antipathogenic properties.

Key words: bioregulators Regoplant and Stimpo, bioprotective effect against phytopathogens, herbicides Herbitox and Grenadier, degree of homology between si/miRNA with mRNA

Introduction

The elaboration of new progressive technologies for improving the productivity and quality of products is the main task for successful development of agriculture over the world. Many crops are susceptible to a wide range of diseases caused by an abundance of pathogens and parasites [Stanton J. and Stirlin G., 1997; Ponomarenko et al., 2010] that negative impact on the genetic potential, resulting in decrease in yield and quality due to mycotoxins present in crop production [Gao X. et al., 2006]. The worldwide spreading of diseases caused by parasitic nematodes leads to significant losses in the agricultural sector [Davis E.L. and Tylka G.L., 2000]. For example, as a result of the violation of crop rotation regimes in North and South America at the cultivation of soybean the threshold of spreading of soybean cyst nematode *Heterodera glycines* was increased by 2.4 times on the 84 % plantations, leading to the loss of hundreds millions dollars [Ponomarenko S. and al., 2016].

Unfortunately, the solution of this global problem requires a lot of efforts of scientists and manufacturers, and the improving of the crop quality requires a significant use of a wide range of chemical pesticides. Without prejudice to the safety requirements according to the manufacturer's guidelines, pesticides largely impair the realization of the genetic potential of cultivated plants, violate the soil microbial biodiversity, and damage the environment and the health of farmers and consumers. The pesticides and fungicides, their compositions and analogs lead to the development of resistant strains of pathogens and reduce the genetic potential of plant varieties and their hybrid varieties [Aktar M.W.et al., 2009].

The elaboration of the alternative environmentally friendly methods, which improve plant growth and development, comes out on top, in favor of this strategy suggest materials of the First World Congress on the use of biostimulators in agriculture (Strasbourg, France, November 2012) and the Second World Congress on the use of biostimulators in agriculture (Florence, Italy, November 2015) [Tsygankova V.A., Ponomarenko, S.P. et al., 2012; Jardin P., 2015]. Alternative directions include organic and integrated methods of agricultural technologies with biological control. The important interest is the strategy of induced resistance of plants using elicitors stimulating the immune system of plants, natural molecules mimicking the attack of a pathogen or danger states.

The National Enterprise Interdepartmental Science and Technology Center "Agrobiotech" of NAS and MES of Ukraine jointly with the Uman National University of Horticulture conducted researches during 15 years of the more than 50 herbicides created by leading chemical companies (Bayer crop science (Germany), Du Pont International Operations (Switzerland), Nufarm GmbH & Co kG (Austria), Dow Agro Sciences GmbH (Austria), Syngenta International AG (Switzerland)) on the growth and development of the major agricultural crops. Researches were conducted with different norms of application of herbicides (40, 60, 80, 100 %) separately without plant growth regulators and with application of bioregulators created in the National Enterprise Interdepartmental Science and Technology Center "Agrobiotech" of NAS and MES of Ukraine [Ponomarenko S. et al., 2016]. In recent years, the studies of a number of August company herbicides (Russia): Fabian – for soybean growing, Herbitox – for sorghum growing, with different rates of application, both individually and jointly with Ukrainian bioregulators of third generation of "Agrobiotech" company were conducted.

The mutagenic effect of the herbicide Triflan on the germinated seeds of wheat, corn, peas was found by scientists of the Institute of Plant Physiology and Genetics, National Academy of Sciences of Ukraine in the 2001 year [Ponomarenko S. et al., 2016]. It was also shown that bioregulators reduced the mutagenic effect of herbicide by 60 % and activated the oxidative-recovery systems of plants, resulting in oxidation of residues of herbicides in the crop production and in the plant rhizosphere. We found out a recovery of biodiversity in the rhizosphere: bacteria - by 40 %, micromycetes - by 32 %, and these factors have contributed to the realization of plant genetic potential [Ponomarenko S. et al., 2016]. Obviously, that bioprotective action of these bioregulators is based on the synergistic effect of metabolic products (i.e. amino acids, carbohydrates, fatty acids, polysaccharides, phytohormones and microelements) of cultivated *in vitro* culture of fungus-endophyte of root of ginseng *Panax ginseng* L. and antiparasitic macrolide antibiotics - aversectines produced by soil streptomycete *Streptomyces avermitilis* [Ponomarenko et al., 2010].

Our molecular-genetic researches showed that these bioregulators considerably increase plant immune resistance to the different pathogens due to their stimulation action on the synthesis in the plant cells small regulatory si/miRNA that participates in RNAi-process or posttranscriptional gene silencing (PTGS) in plants [Tsygankova V.A. et al., 2014]. Gene silencing, which is mediated by either degradation or translation arrest of target mRNA, has an important role in adaptive protection of plant against viruses, in genome defense against mobile DNA elements and regulation of gene expression during plant ontogenesis [Vaucheret, 2006].

Considering our previously obtained results, the great theoretical and practical interest is the possibility of application of bioregulators in combination with pesticides and herbicides in agricultural practice for decrease their toxic effect on plant cells and improving of plant growth and resistance to pathogens and pests.

The aim of our work is comparative analysis of effectiveness of application of bioregulators Regoplant and Stimpo separately or in combination with standard insecticides and herbicides Herbitox and Grenadier for improving growth, yields quality and crop resistance against various pathogens and pests.

Materials and methods

In the greenhouse experiments we compared efficiency of PGRs in combination with standard insecticides in controlling ground beetle *Zabrus tenebrioides*, turnip moth *Scotia segetum* and *Chloropidae spp.,* and wheat nematode *Anguina tritici.* The experiments on comparative efficiency of bioregulators and standard insecticides were conducted in pots of 25 cm x 25 cm size; each experiment was replicated four times. Soil samples were contaminated separately by nematode, ground beetle, and turnip moth. We used winter wheat var. Dalnytska and var. Knop, and soybean var. Arcadia Odeska. Treated by bioregulators seeds of 50 plants were studied in the experiments. The bioprotective effect of bioregulators was tested according determination of the amount of damaged plantlets (in pcs/m^2) and biological efficiency (in %).

The effect of bioregulators on stability, productivity, and quality of obtained seeds, as well as on soybean resistance to pathogenic and parasitic organisms was determined. The experiments with wheat and soybean rot and mildew diseases caused by pathogens, such as *Mucor spp., Rhizopus spp., Aspergillus spp., Penicillium spp.,* and *Trichothecium roseum*, were conducted on the artificial infectious backgrounds. Efficiency against pathogens was studied at low level of spores, i.e., 0.1 g of spores per 1 kg of seeds, and at high level of spores, i. e., 1 g of spores per 1 kg of seeds.

The results were compared with the effect of modern pesticides produced by leading agrochemical companies such as Alpha-Cypermethrin insecticide, Yunta Quadro insecto-fungicide, Lamardor, Selest Top, Imidacloprid, Terios and Microplant micronutrients.

We research also biological effect of bioregulator Regoplant and different doses of herbicide Grenadier and herbicide Herbitox on increase of growth and yield quality of winter wheat var. Knop and sugar sorghum var. Sylosne 42 of Ukrainian selection. It is a mid-ripening, high-performance, resistant to lodging, abscission, drought and diseases variety. This variety is recommended for cultivation in the forest steppe area, its vegetation period is 124 days. Seeds were kindly provided by the Institute of Bioenergy Crops and Sugar Beet NAASU. Cultivation of sugar sorghum var. Sylosne 42 was conducted on the experimental field of the Department of Biology of the Uman NUH, soil – the typical podzolic black soil, area – 0.73 ha. Cultivation of winter wheat var. Knop was conducted on the experimental field of the Selection & Genetic Institute – the National Centre of Seed-growing and Variety Study of NAAS of Ukraine (Odessa).

In the laboratory experiments we studied also a post-effect of bioregulators Regoplant and Stimpo on the second generation of wheat plants var. Lastivka and Princess Olga. These plants were not treated by bioregulators; however, they were obtained from seeds of the first generation of wheat plants that were infected by pathogenic micromycetes *Fusarium graminearum* and were treated by bioregulators (experimental plants) or not treated by bioregulators (control plants). Specificity of bioregulators post-effect was determined according to: 1) integral indexes of growth and development of control and experimental 7-day old seedlings; 2) molecular-genetic indexes: the difference in the degree of homology (according to % hybridization) between the basic constituents of plant immune system - si/miRNA isolated from experimental 7-day old seedlings and mRNA of control seedlings using DOT-blot hybridization method [Maniatis, 1982]. Small regulatory si/miRNA was isolated from experimental seedlings by our elaborated method [Tsygankova, et al., 2012].

Results

We tested bioprotective properties of bioregulators Regoplant and Stimpo at cultivation of winter wheat and soybean on the infectious backgrounds. Use of bioregulators in combination with chemical pesticides caused increase of plant resistance against different pathogens and pests. Plant growth regulators reduced phytotoxicity of chemical pesticides and stimulated immune protective reactions of plants. As a result, commercial grain yield and seed material quality were improved. The bioprotective effect of bioregulators was sufficiently high at their use on crops infected by nematodes, ground beetle, turnip moth, and chloropid flies hat (Table 1). Bioprotective effect of bioregulators did not exceed an effect achieved at the use of insecto-fungicides, e.g.,Yunta Quadro and Selest Top. However, the level of efficiency shown by Regoplant and Stimpo against wheat nematode, ground beetle, and turnip moth, and chloropid flies was just set as high taking into account their economic effect and environmental safety.

We studied also Regoplant and Stimpo bioprotective effect on soybean plants infected by dangerous pathogens of rot and mildew of soybean (Table 2). We found that bioregulators have a positive effect on growth and development of soybean seeds. They reduced the infection impact on seed development and commodity grain quality.

The results of the cultivation of sugar sorghum, var. Sylosne 42 proved the feasibility of application of bioregulator with bioprotective effect Regoplant for presowing seed treatment at the rate of 250 ml/t and joint use of herbicide Herbitox (1 l/ha) and Regoplant (50 ml/ha). The most significant result is obtained by presowing seed treatment with Regoplant and crop spraying with Regoplant and Herbitox (Tables 3-5).

The analogical results were obtained at the cultivation of winter wheat var. Knop, which was treated during germination and vegetation period by bioregulator Regoplant and herbicide Grenadier (Fig. 1).

Our laboratory experiments demonstrated the inheritance of wheat plants resistance to pathogenic organisms. We found that plants of the 2nd generation which were not treated with bioregulators, maintain high viability which is similar to the results obtained on the 1st generation of plants treated with bioregulators on infectious background. The molecular-genetic analysis using DOT-blot method hybridization si/miRNA with mRNA populations showed high degree of homology between immune-protective small regulatory si/miRNA and mRNA of experimental plants and lower degree of homology according to mRNA of control plants (Table 6). We called this effect "quasi-heterosis". It was found that Regoplant and Stimpo strongly increased growth rate of heterosis plants as well as resistance to pathogenic organisms. We concluded that principal mechanism of these bioregulators in plant cells includes almost twofold increasing of the synthesis (abundance) of small regulatory si/miRNA, which has antipathogenic properties.

Discussion

It is known that plant growth is the result of the different processes such as cell differentiation and development, and formation of tissue, organ and whole organism; these basic processes are controlled by genetic program of plant growth and development [Tsygankova V.A., 2015]. The plant cell endogenous system of coordination and self-regulation includes a variety of endogenous regulatory systems, among which an important role belongs to physiologically active compounds such as phytohormones [Adam G., 2012; Tsygankova V.A., 2015]. It is not surprising, taking account diverse impact of phytohormones on plant growth and development, improving of yield and product quality and increase of plant resistance to unfavorable environmental factors [Pieterse C.M.J. et al., 2012; Denancé N. et al., 2013].

Today there are thousands of new synthesized compounds, although only a few from them are used in the agricultural practice as plant growth regulators (PGRs) [Gianfagna T.J., 1987; Basra A.S., 2000]. Even such classes of recently identified substances as brassinosteroids (BR) which also belong to phytohormones are not widely used because their manufacture is not organized [Adam G., 2012] The worldwide use of PGRs is restricted due to insufficient knowledge of the mechanisms of their regulatory action, taking into account the biological characteristics of plants and high selectivity of action of PGRs that have different structure. Unfortunately, the empirical approach is used often to the selection and application of growth regulators on various plant crops, without regard biological characteristics of each plant and without consideration of the changes in metabolic processes that occur in plant cells under PGRs influence, as well as the dependence of PGRs effects on plant growth from the environmental factors and so on.

Our long-term and multiple studies show that exogenous PGRs of different nature have a significant influence on many metabolic processes in plant cells due to their impact on the genetic and phytohormonal levels of regulation, however, depending on genotype of the rate of change of these processes is different [Tsygankova, Ponomarenko et al., 2011]. Considering the results of our previous research and discussion in the literature about the nature of the relationship between exogenous PGRs with the endogenous regulatory system, our attention for the last years was paid to the search for new growth regulators that possess the ability to the direct changes in plant growth and development, increasing their resistance to unfavorable factors environment.

In the present work the bioprotective effect of new bioregulators Regoplant and Stimpo has been investigated at cultivation of different varieties of winter and soya plants on infectious backgrounds. It was found that preseeding treatment of plants and spraying of crops in vegetation period by Regoplant and Stimpo increased plant viability and resistance to different phytopathogens. The highest bioprotective activity against phytopathogens was obtained at preseeding treatment of plants by Regoplant (up to 98 %) and Stimpo (up to 89 %) bioregulators according to control (without treatment by bioregulators).

Comparative study of effectiveness of using of bioregulator Regoplant separately or in combination with herbicide Herbitox for cultivation of sugar sorghum showed that the best results were obtained at the presowing seed treatment with Regoplant and crop spraying with Regoplant and Herbitox. The analogical results were obtained at the cultivation of winter wheat var. Knop, which was treated during germination and vegetation period by bioregulator Regoplant and herbicide Grenadier. Obviously, these facts are as a result of the synergistic effect of bioregulator Regoplant and herbicide Herbitox or herbicide Grenadier, as well as bioprotective effect Regoplant on decrease of toxic effect of herbicides on plant cells. This technology can be recommended to farmers as Ukrainian innovative project.

In the laboratory experiments it was shown that the seedlings of the second generation of wheat plants (that were grown on infectious background without treatment by bioregulators), obtained from the seed of the first generation of plants (that were grown on infectious background and were treated by bioregulators Regoplant and Stimpo), show high viability and resistance to pathogenic micromycetes of *Fusarium L.* genus due to integral indexes of growth and development comparatively with control seedlings. The obtained molecular-genetic indexes testify that at the phase forming of seeds there is observed the reprogramming genome of plant embryos under influence of bioregulators through the way of activation of expression of genes encoding antipathogenic si/miRNA.

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Tables

Table 1. Bioprotective effect of bioregulators and seed protectants against phytopathogens*

Experiment	Application dose, L/t	Wheat nematode		Ground beetle		Turnip moth		Chloropid flies	
		Amount of infected plants	Biological efficiency, %	Amount of infected plants	Biological efficiency, %	Amount of infected plants	Biological efficiency, %	Amount of infected plants	Biological efficiency, %
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	

* All experiments were replicated four times. Total amount of plants in each experiment was 50.

** $LSD_{0.05}$ – Least substantial Difference

Table 2. Bioregulators efficiency against the causative agents of rot and mildew of soybean*

Experiment	Application dose, L/t	*Fusarium sp.*		*Botrytis cynerea*		*Alternaria spp.*		Complex of storage fungi**	
		Amount of infected seeds	Bio-efficiency, %	Amount of infected seeds	Bio-efficiency, %	Amount of infected seeds	Bio-efficiency, %	Amount of infected seeds	Bio-efficiency, %
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	

*All experiments were replicated four times. Total amount of plants in each experiment was 50. ** Fungi *Mucor spp., Rhizopus spp., Aspergillus spp., Penicillium spp., Trichothecium roseum*.

Table 3. The dynamics of leaf surface area during the application of different doses of herbicide Herbitox and different ways of use of growth regulator Regoplant

Variant	Tillering		Ear emergence		Milky-wax ripeness		Content of chlorophylls	
	Leaf area on 1 plant, cm^2	%	Leaf area on 1 plant, cm^2	%	Leaf area on 1 plant, cm^2	%	Sum of chlorophylls *a and b*	%
Control	44.1	100.0	367.6	100.0	426.5	100.0	3.719	100.0
Herbitox 1.0 l/ha	51.6	117.0	385.9	105.0	464.9	109.0	3.89	104.6
Background + Herbitox 1.0 l/ha + Regoplant 50 ml/ha	**64.6**	**146.5**	**460.6**	**125.3**	**542.5**	**127.2**	**5.106**	**137.3**
LSD_{05}	1.78	-	3.29	-	4.42	-	4.42	-

Table 4. Weed infestation

Variant	Number of weeds	Weight of weeds	Killing of weeds, %	
	Pcs./m^2	g/m^2	by quantity	by weight
Control	32	135.1	0	0
Herbitox 1.0 l/ha	13.4	83.9	58.1	37.9
Background + Herbitox 1.0 l/ha + Regoplant 50 ml/ha	**4.3**	**16.5**	**86.6**	**87.8**
LSD_{05}	0.78	-	-	-

Table 5. Impact of growth bioregulator Regoplant and herbicide Herbitox on yield of sorghum green mass and grain

Variant	Yield of green mass		Grain yield		
	t/ha	%	t/ha	± to control	%
Control	32.46	100.0	3.52	-	-
Herbitox 1.0 l/ha	33.18	102.2	3.86	+0.34	+9.7
Herbitox 1.0 l/ha + Regoplant 50 ml/ha	34.77	107.1	4.14	+0.62	+17.6
Background + Herbitox + 1.0 l/ha + Regoplant 50 ml/ha **Full technology**	**38.73**	**119.3**	**4.60**	**+1.08**	**+30.7**
LSD_{05}	0.313	-	0.187		-

Table 6. The degree of hybridization between si/miRNA with mRNA of wheat plants*

Plant	Cultivar	Hybridization, %		
		Control	Regoplant	Stimpo
Wheat	Lastivka	98 ± 1.4	82±1.6 (≈16%)	86±1.2 (≈12%)
	Princess Olga	98 ± 1.6	84±1.4 (≈14%)	88±0.96(≈10%)

*All experiments were conducted in triplicate. Significant differences from control values, $p < 0.05$.

Fig. 1. Impact of the bioprotective plant growth regulator Regoplant (ISTC Agrobiotech) and herbicide Grenadier (China) on the yield of winter wheat (var. Knop) in comparison with the different systems of basic cultivation. Institute of Agriculture (Odessa)