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Increase of plant resistance to diseases, pests and stresses with new biostimulants

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Abstract

In the greenhouse experiments the antipathogenic activity of new polycomponent plant growth regulators (PGRs) Regoplant, Stimpo and Radostim had been investigated at cultivation of different varieties of winter, soya and corn plants on infectious backgrounds.The best 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 %), less bioprotective efficiency showed Radostim (up to 74 %) according to control (without treatment by PGRs). At 2nd generation of the wheat and chickpea plants (infected by pathogenic micromycetes of *Fusarium L.* genus without treatment by PGRs) the increased resistance to pathogenic micromycetes of *Fusarium L.* genus was set also. Using DOT-blot hybridization method the considerable difference between mRNA of control wheat and chickpea seedlings and small regulatory si/miRNA of experimental seedlings (obtained from seeds of the 1st generation of plants, infected by pathogenic micromycetes of *Fusarium L.* genus and treated by PGRs) was found. It is proposed that indicated difference connected with partial reprogramming genome of plant cells under the impact of PGRs - inductors of synthesis si/miRNA with antipathogenic properties.

INTRODUCTION

Development of economically feasible and environmentally friendly agricultural technologies able to provide stability of agricultural ecosystems, to promote wide use of biocontrol, and to guarantee improvement of quality, is one of the challenges of modern agriculture. Pests (insects, mites, and nematodes), diseases (bacteria, viruses, fungi, nematodes), and weeds cause a significant yield reduction in agricultural production worldwide. According to the Food Agricultural Organization (FAO), the global annual crop losses due to pests, diseases, and weeds reached 20-25 % [Stevens and May, 2009]. 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, and white butterflies belong to the most widespread and dangerous pests that cause significant reduction of yields of important agricultural crops such as corn, wheat, barley, soybean, and rape. The problem of plant protection against widespread fungi (e.g., *Fusarium spp.*,

Fusarium graminearum, Fusarium oxysporum Ciceriae, Cercospora spp., Ascohyta spp., Perronospora spp., Blumeria spp., Pucinia spp., Sclerotinia spp., and *Verticillim spp.*); bacterial (*Pseudomonas spp*.), viral (*Potyvirus spp.*) and phytoparasitic nematodes (such as *Heterodera schachtii*, *Meloidogyne incognita*, *Globodera rostochiensis*, *Ditilenchus dipcasi*, *Rotylenchulus reniformis*, *Tylenchulus semipenetrans*) and other diseases is also economically important [Stevens and May, 2009]. Non-chemical crop protection is important component of sustainable crop production. Development of such compounds was based on achievements in modern microbiology, mycology, biotechnology, soil science, and plant protection. Long-term research and wide practical application of Stimpo, Radostim, Regoplant and Biolan - the new polycomponent biostimulants developed in the National Enterprise Interdepartmental Science & Technology Center «Agrobiotech», Natl. Acad. of Sci. and Min. of Ed., Sport and Sci.of Ukraine, showed that these plant growth regulators (PGRs) match with economical and environmental demands of modern agriculture. These PGRs 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 [Ponomarenko et al., 2010] with phytostimulating, antiparasitic and antipathogenic effect. In our molecular-genetic experiments, we have showed that positive effects of the above-mentioned PGRs were revealed in quantitative and qualitative changes in gene expression as a consequence of plant cell genome reprogramming by PGRs [Tsygankova et al., 2010; Tsygankova et al., 2011]. We have found also [Tsygankova, Ponomarenko et al., 2012; Tsygankova, Stefanovska et al., 2012] that these PGRs significantly enhanced plant resistance to different pathogens due to stimulation of the synthesis of cellular small regulatory si/miRNA that participate in RNAi (RNA interference) process. This process called posttranscriptional gene silencing (PTGS) was found in plants, animals, and fungi [Filipowicz et al., 2005; Bakhetia et al, 2005; Katiyar-Agarwal, 2006]. Small regulatory si/miRNA with 22-24 nt size plays a leading role in silencing: together with site-specific endo- and exonucleases of RNA-induced silencing complex (RISC), it blocks (silences) translation of variable cell mRNA with imperfect structure as well as mRNA of pathogens and parasites, or enzymatically cleaves these target mRNA molecules causing its degradation [Vaucheret, 2006; Voinnet, 2008; Zhang, 2007]. The purpose of our work was verifying the possibility of increasing of the plant resistance to pathogens and parasites by above mentioned PGRs and determination of genetic mechanisms of these PGRs' post-action (i.e. effect of inheritance of increased by PGRs plant resistance to pathogenic and parasitic organisms).

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 PGRs 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 of Dalnytska cultivar, soybean of Arcadia Odeska cultivar, and hybrid corn of Kobza MV cultivar. Seeds were sown in a box after treatment with PGRs. 50 plants were studied in the experiments on determination of the amount of damaged plantlets (in pcs/m^2) and biological efficiency (in %).

The effect of PGRs on stability, productivity, and quality of obtained seeds, as well as on wheat, soybean and corn plants' resistance to infections was determined. The experiments with wheat, soybean and corn 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.

In laboratory experiments we studied also a post-effect of PGRs on the second generation of wheat plants of 2 cultivars: Lastivka and Princess Olga and chickpea *Cicer arietinum L* plants of 2 cultivars: Rosanna and Triumph. These plants were not treated by PGRs; however, they were obtained from seeds of the first generation of wheat and chickpea plants that were infected by pathogenic micromycetes *Fusarium graminearum* and *Fusarium oxysporum f. ciceris* and were not treated by PGRs (control plants) and treated with PGRs (experimental plants). In our experiments we used control and experimental seeds (obtained from the first generation of control and experimental plants) that were sprouted in Petri-dishes on a filter paper wetted by distilled water (without adding PGRs) on an infectious background (at the presence of pathogenic micromycetes *Fusarium graminearum* and *Fusarium oxysporum f. ciceris*). Specificity of PGRs post-effect was determined according to: 1) integral indices of growth and development of control and experimental 7-day old seedlings; 2) molecular-genetic indices: the difference in the level of homology between the basic constituents of plant immune system - si/miRNA isolated from experimental 7-day old seedlings to mRNA of control seedlings. Small regulatory si/miRNA was isolated from experimental seedlings by our elaborated method [Tsygankova, Andrusevych, 2011].

RESULTS AND DISCUSSION

We tested bioprotective properties of new PGRs at cultivation of winter wheat, soybean, and corn on the infected backgrounds. 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. Use of PGRs in combination with chemical pesticides caused increase of plant resistance against different diseases caused by microbial pathogens. Plant growth regulators reduced phytotoxicity of chemical protectants and stimulated immune reactions of plants. As a result, commercial grain yield and seed material quality improved. The bioprotective effect of PGRs was found sufficiently high at their use on crops infected by nematodes, ground beetle, turnip moth, and chloropid flies hat (Table 1). PGRs effect 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 against wheat nematode, ground beetle, and turnip moth, and by all the PGRs studied against chloropid flies was just set as high taking into account their economic effect and environmental safety.

Regoplant and Stimpo showed also antipathogenic activity against causative agents of wheat (Odeska semidwarf cultivar) rot and mildew (Table 2). However, we do not consider the use of these PGRs as alternative to chemical pesticides reliable, especially on the highly infected background. On the low infected backgrounds, the PGRs application is quite possible, taking into account the level of their potential efficiency.

We studied also Regoplant and Stimpo bioprotective effect on soybean and corn plants infected by dangerous pathogens of soybean and causative agents of rot and mildew of corn (Table 3 and Table 4). We found that PGRs has a positive effect on

growth and development of soybean and corn seeds. They reduced the infection impact on seed development and commodity grain quality.

Our laboratory experiments demonstrated the inheritance of wheat and chickpea plants resistance to pathogenic organisms. We found that plants of the 2nd generation which were not treated with PGRs, maintain high viability which is close to the results obtained on the 1st generation of plants treated with PGRs on infected background (Fig.1 and Fig. 2) [Tsygankova, 2012]. The molecular-genetic analysis by the DOT-blot method hybridization si/miRNA with mRNA populations [Tsygankova, 2010; Maniatis, 1982] showed high level of homology between immuno-protective small regulatory si/miRNA and mRNA of experimental plants and lower level of homology in respect to mRNA of control plants (Table 5). We called this effect "quasi-heterosis". It was found that Regoplant, Stimpo and Biolan strongly increased growth rate of heterosis plants as well as resistance to pathogenic organisms. We concluded that principal mechanism of these PGRs in plant cells includes almost twofold increasing of the synthesis (abundance) of small regulatory si/miRNA, which has antipathogenic properties.

CONCLUSIONS

In the greenhouse experiments the bioprotective activity of new PGRs: Regoplant, Stimpo and Radostim had been investigated at cultivation of different varieties of winter, soya and corn plants on infected backgrounds. It was set that preseeding treatment of plants and spraying of crops in vegetation period by PGRs Regoplant, Stimpo and Radostim 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 %), less bioprotective efficiency showed Radostim (up to 74 %) according to control (without treatment by PGRs). In the laboratory experiments was shown that the seedlings of the 2nd generation of wheat and chickpea plants (that were grown on infected background without treatment by PGRs), obtained from the seed of the first generation of plants (that were grown on infected background and were treated by PGRs: Regoplant, Stimpo and Biolan), show high viability and resistance to pathogenic micromycetes of *Fusarium L.* genus due to integral indexes of germination and growth comparatively with control seedlings. The molecular-genetic indexes - % homology si/miRNA of experimental plants to mRNA of control plants testify that at embryogenesis during forming of plant seeds there is observed reprogramming genome of plant seed embryos through the way of “switching on” genes of the antipathogenic si/miRNA which synthesis is induced under PGRs’ action.

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Tables

Table 1. Bioprotective effect of PGRs 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. Bioprotective effect of PGRs and seed protectants against wheat rot and mildew*

Experiment	Application dose, L/t	Fusarium sp.		Altemaria sp.		Bipolaris sorokiniana		Complex of storage fungi**		Bacillus sp. ***	
		Amount of damaged ears	Bio-efficiency, %	Amount of damaged ears	Bio-efficiency, %	Amount of damaged ears	Bio-efficiency, %	Amount of damaged ears	Bio-efficiency, %	Amount of damaged ears	Bio-efficiency, %
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	

*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.* *** Causative agents of bacterial rot

Table 3. PGRs 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 4. PGRs' efficiency against the causative agents of rot and mildew of corn*

Variant	Application dose, L/t	*Fusarium sp.*		*Alternaria sp.*		*Nigrospora sp.*		Complex of storage fungi**	
		Amount of infected seeds	Biological efficiency, %	Amount of infected seeds	Bio-efficiency, %	Amount of infected seeds	Bio-efficiency, %	Amount of infected seeds	Bio-efficiency, %
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	

Applica-tion 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 5. The hybridization levels of si/miRNA with mRNA of wheat and chickpea plants*

Plant	Cultivar	Hybridization, %			
		Control	Biolan	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%)
Chickpea	Rosanna	98 ± 1.2	86 ± 1.6 (≈12%)	78±1.4 (≈20%)	88±0.98 (≈10%)
	Triumph	98 ± 1.4	82 ± 1.8 (≈16%)	80±1.6 (≈18%)	76±2.42(≈22%)

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

Figures

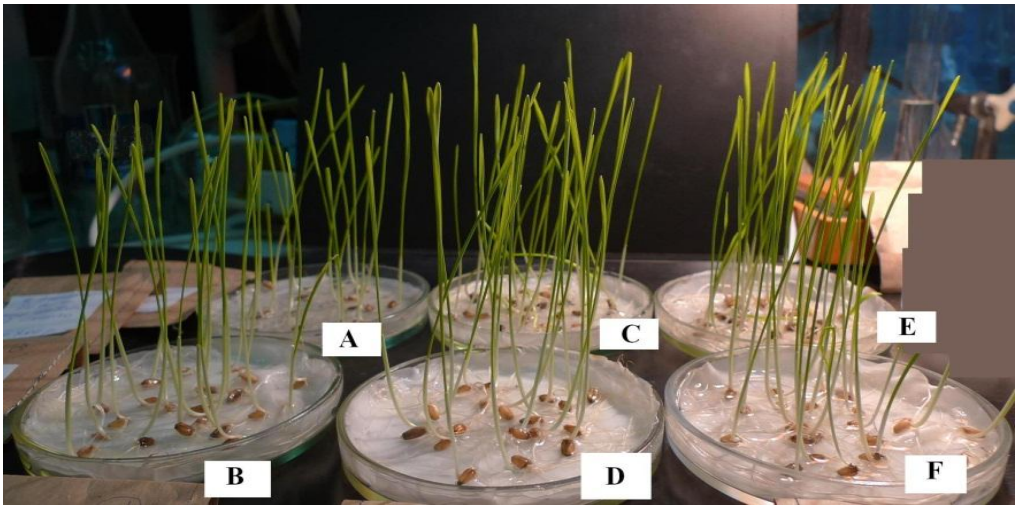

Fig.1. The 7-day old seedlings of wheat plants (Lastivka cultivar): seedlings, obtained from seeds of control plants with no PGR treatment (A and B); and treated with Stimpo (C and D) and Regoplant (E and F).

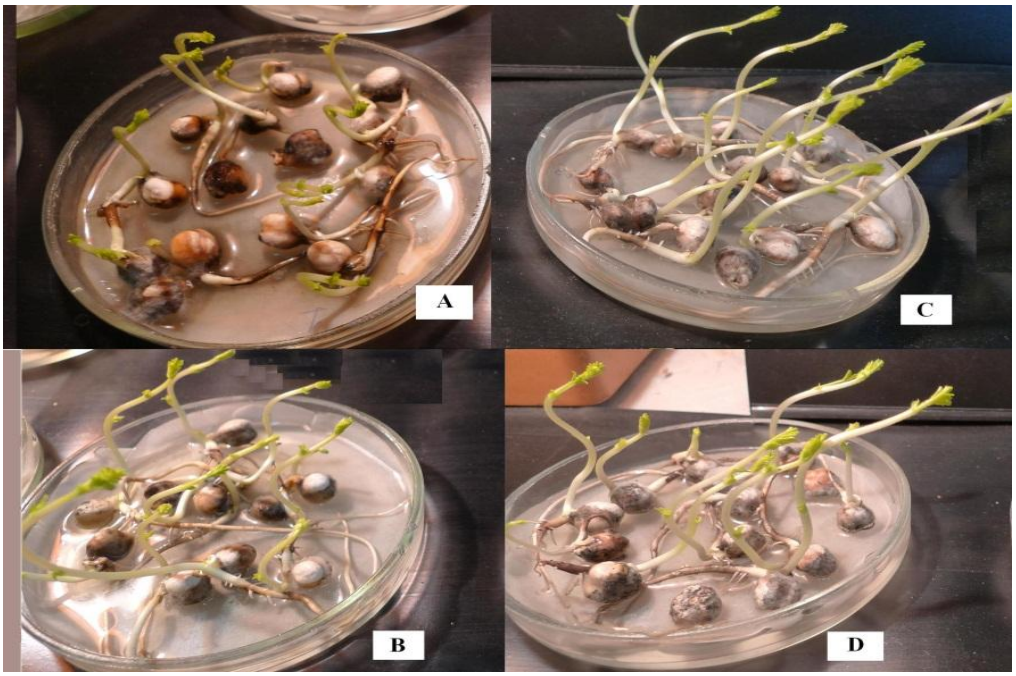

Fig.2. The 7-day old seedlings of *Cicer arietinum* L. (Triumph cultivar): seedlings, obtained from seeds of control plants with no PGR treatment (A); and treated with Stimpo (B), Regoplant (C), and Biolan (D).