THE INFLUENCE OF BIOSTIMULANT *REGLALG* ON PLANTS RESISTANCE TO ABIOTIC AND BIOTIC STRESS FACTORS

Dascaliuc Alexandru, Voineac Vasile, Ralea Tudor, Jelev Natalia

Institute of Genetics, Physiology and Plants Protection

Rezumat

Preparatele obținute prin extragerea substanțelor biologic active din diferite organisme, atunci când sunt aplicate pe plante, influențează creșterea și răspunsul lor la factorii de stres abiotic și biotic. Acțiunea lor este diferită de cea a substanţelor nutritive și a preparatelor pentru protecția plantelor. Pentru a le distinge de alte preparate, a fost propus termenul biostimulator. În acest articol sunt incluse câteva exemple ale efectelor biostimulatorului *Reglalg*, extras din biomasa algelor din *Spirogyra*, asupra rezistenței plantelor de grâu și viță de vie la factorii de stres abiotic și biotic.

Cuvinte cheie: biostimulator, alge, grâu, viță de vie, factori de stres, germinare, rezistenţă.

Depus la redacție 14 decembrie 2018

Adresa pentru corespondenţă: Dascaliuc Alexandru, Institutul de Genetică, Fiziologie şi Protecţie a Plantelor, str. Pădurii, 20, MD-2002 Chişinău; e-mail: dascalica@yahoo.com; tel.: (+373) 67504121

Introduction

Plants respond to the action of abiotic and biotic stress with sophisticated innate reactions induced by the signals being modified plant-derived or invading organism derived molecules [4]. Perception of these signals induces the changes in the expression of genes that are implicated in plants adaptation to stress conditions. Substances that induce increased resistance to abiotic and biotic stressors have been called adaptogens or protectors; those that induce resistance toward non-adapted pathogens also are known as "*defense elicitors*" [5]. Treating of plants in the absence of stress factors with extracts containing such signals, or components inducing their productions by plants themselves, can promote plant resistance to imminent action of stress in the future. Activation of plants growth and resistance to stress can be realized by the application of various substances, physiological effects of which are distinct from that of fertilizers and products for plant protection. It was proposed to unite them under a common term "*biostimulant*". Among numerous definitions of biostimulant, we mention only the European Union *model definition*: "*A plant biostimulant is any substance or microorganism, in the form in which it is supplied to the user, applied to plants, seeds or the root environment with the intention to stimulate natural processes of plants benefiting nutrient use efficiency and/or tolerance to abiotic stress, regardless of its nutrients content, or any combination of such substances and/or microorganisms intended for this use"* [7].

Products from algae, due to the presence of a series of compounds that promote plants growth and adaptation to stress conditions, are widely used in agriculture and are defined as biostimulants [10]. Here we provide information about the effects of *Reglalg* [6], the biostimulant extracted from freshwater algae of genus Spirogyra, applied for processing the seeds of various winter wheat varieties before sowing and vine plants during the growing season. The influence of these procedures on the plants yields quantity and quality, as well as on their tolerance to abiotic and biotic stress was appreciated.

Materials and methods

In our study, the varieties of winter wheat Moldova 5, Albidium 114, Misia, and Cuialnic were used. The plants of the experimental variants were obtained from the seeds sprayed with solution of the biostimulant *Reglalg* diluted with water in specific ratios, while those of control variants - from the seeds sprayed with water. In order to appreciate the genotype resistance to frost, at the initial stage of germination (before emergence of the central root) seeds were exposed to different doses of shock with negative temperature *(SNT)*, realized by incubating them in the air thermostat with specified temperature (-8, -9. -10, -11, -12, and -13°C) during 8 hours. Parameters of germination were determined after incubation the seeds on the wet filter paper, in the air thermostat, in the dark, at temperature 25°C and relative air humidity 75-85%. The control (sprinkled with water) and experimental (sprinkled with solution of *Reglalg*) seeds were also sowed in the field conditions. The parameters of plants resistance to *SNT* were determined after passing the first and second phase of hardening [3].

For appreciating the influence of biostimulant *Reglalg* on disease susceptibility, productivity and quality of vine grapes harvest, the plants of cultivar Pinot Noir where treated with the 0, 5 liters of *Reglalg* diluted with 500 liters of water and sprayed per

1 hectare of plantation in the three periods of vegetation: 1 - 10 days before flowering; 2 - immediately after flowering, and 3 - 10 days thereafter. The experiments were held in three variants: 1 - plants were treated 12 times per vegetation according the accepted standard scheme of fungicides application; 2 - according accepted standard scheme of fungicides use plus application of *Reglalg*; 3 - treating plants with half of the accepted in standard scheme of fungicides plus application of *Reglalg*. In order to appreciate the quality of the harvest, in the grape we have determined the content of sugars [8], (ГОСТ 27198-87) and organic acids (ГОСТ 25555.0-82).

The statistical analysis was realized by determining the values of the mean, standard deviation of mean, and the significance of differences between variants with the level $p < 0.05$ [1].

Results and discussions

The data shown in fig. 1 demonstrates that after exposure to *SNT,* the level of inhibition the seeds germination was different for wheat varieties included in research. The seeds primary resistance to *SNT* of the variety Albidium 114 was higher than that of the seeds of other three varieties. We can also point out that the level of germination of the seeds pre-treated before applying *SNT* with the solution of *Reglalg* was about 20% higher than that of those pre-treated with water. Thus, independently of the primary reaction to *SNT*, the protective effect of seeds pre-treating with the solution of *Reglalg* was approximately the same for all wheat varieties included in research. These data demonstrate that in the embryos of the seeds treated with the solution of the biostimulant *Reglalg*, even at the germination stage, are induced the processes that ensure the increasing their resistance to negative temperatures, independently of wheat genotype.

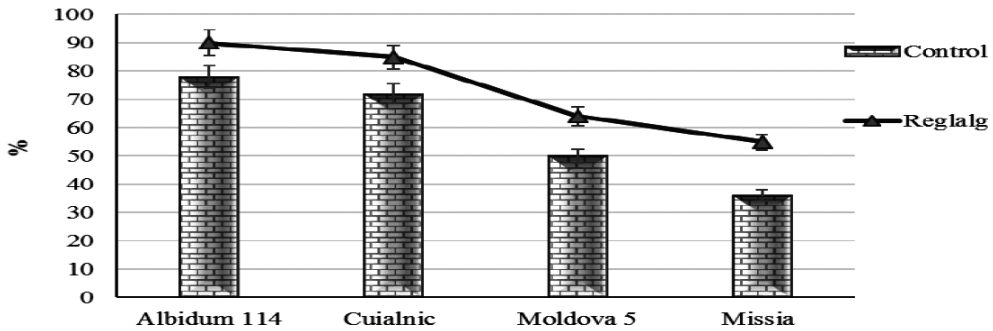

Figure 1. The percentage of wheat varieties Albidum 114, Cuialnic, Moldova 5, and Missia seeds germination, which prior to exposure with the *SNT* during 8 hours, at -7°C, were imbibed during five minutes in water (Control), or in solution of the *Reglalg,* diluted with water in ratio 1 : 200, (Reglalg).

The included in fig. 2 data shows that after the first and second phases of hardening the control variants of Moldova 5 plants were more resistant to exposure with the *SNT* than those of the variety Missia. After finishing the both phases of hardening, the resistance to *SNT* of the plants obtained from the seeds treated before sowing with the solution of the biostimulant *Reglalg* (experimental variants) was significantly higher than that of the control variants. Comparing the beneficial effects of *Reglalg* on the resistance to *SNT* of the plants of both varieties, we can observe that stimulation of hardening

to frost of the Missia plants was more pronounced than that of the variety Moldova 5. Thus, the beneficial effect of *Reglalg* on winter hardening was more pronounced at the plants of the variety that is less resistant to the *SNT*. The previously obtained data demonstrated that at the plants grown from the seeds, treated before sowing with the solution of *Reglalg*, the length of the epicotyl decreases by 1,0-2,5 cm [3]. Due to this, their secondary roots and the tillering node were formed in the wetter layers of soil, and in the winters without snow the soil temperature at the level of tillering node of experimental plants is 2-6°C higher [9] than the temperature of exposure of the tillering node of the control plants. This suggests that the beneficial effect of treating wheat seeds before sowing with the biostimulant *Reglalg* is assured not only due increased plants resistance to frost, but also by avoiding the deleterious effects of winter frosts, spring and summer droughts.

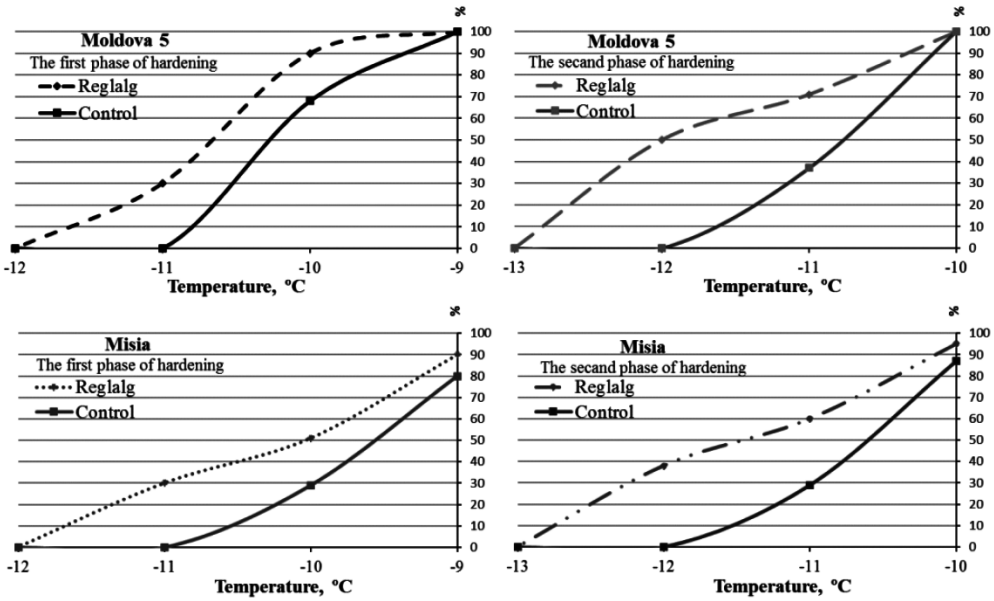

Figure 2. The initiation of secondary roots by the plants of wheat varieties Moldova 5 and Missia, obtained from seeds treated before sowing with water (Control), or solution of the *Reglalg* (Reglalg), and in the winter, after the first and second phase of hardening, were exposed to the *SNT* during 8 hours at the temperatures indicated on the abscissa.

The data about the influence of biostimulant *Reglalg* on the indexes of productivity of wheat varieties plants Moldova 5 and Missia are included in the table 1. They indicate that the pre-sowing seeds treatment with the solution of the biostimulant *Reglalg* has positive, but statistically non-significant effect, on almost all indexes of productivity, which ultimately resulted in a statistically significant increasing of the both variety level of yield. Being more resistant to the extreme temperatures compared with the plants of control variants, the experimental plants were more vigorous during all period of vegetation, what in the final assured their higher productivity. The above data can be explained by the capacity of the plants from experimental variants to maintain their homeostasis in the larger intervals of environmental conditions [2].

Table 1. The indexes of the productivity of wheat plants, varieties Moldova 5 and Missia, obtained from the control seeds and seeds treated before sowing with the solution of the biostimulant *Reglalg*.

Variety	Variants	Spikes per m²	Grains per spikelet	Number of grain per spike	Weight of spike (g)	Weight of 1000 grains(g)	Grams per square meter
Moldova 5	Control	392±*25*	2,78±0,30	38,6 ±*11,0*	2,39±*0,31*	39,5±*4,0*	598
	Reglalg	430±*22*	2,82±0,32	39,0 ±*12,1*	2,43±*0,34*	39,6±*4,1*	664
Misia	Control	343±*19*	2,90±0,45	43,1 ±11,*0*	3,09±*0,41*	45,6±*4,5*	674
	Reglalg	350±*21*	2,98±0,43	44,8±*11,4*	3,19±*0,42*	45,7±*4,9*	717

The effectiveness of vine plants protection was tested by their multiple treatments during vegetation with the solutions containing the recommended complexes of fungicides, separately, or in combination with the biostimulant *Reglalg*. The results are summarized in table 2. They indicate that the application of *Reglalg*, when compared with that of prototype preparation Immunocitofit, has superior protective effects on vine grape from the mildew and gray rot. The effectiveness of vine plants protection was tested by repeated plants treatment by vegetation with the solutions containing the recommended complexes of fungicides, separately and in combination with the biostimulant *Reglalg*.

Table 2. The influence of treating the vine plants, cultivar Pinot Noir, with the solutions of biostimulant *Reglalg* on the mildew and gray rot spreading and development, and as well on the biological efficiency of grape protection.

Variant	Mildew			Gray rot		
	Spreading, %	Develop-ment, %	Biological efficiency,%	Spreading, %	Develop-ment, %	Biological efficiency, %
Fungicides	16,6	1,8	63,5	66,6	27,9	11,0
Fungicides + Immunocitofit	9,3	1,3	73,1	55.6	20,4	34,9
Fungicides + *Reglalg*	8,2	1,2	75,9	34,2	11,8	62,3

Similar researches were provided by us with the grape vine plants of variety Cabernet, the protection of which was carried out without the use of synthetic fungicides, in accordance with the requirements of bio-dynamics agriculture. In this case, for plants protection the only *Bordeaux* mixture and sulfur-containing preparations were used. The beneficial effects of treating plants with biostimulant *Reglalg* was manifested in this case also. Its influence on the values of indices of grapes protection was similar to those determined for Pinot Noir grapes. During the entire period of vegetation, the effectiveness of photosynthesis by leaves of plants treated with *Reglalg* was 10-20% higher and in autumn the senescence of the leaves were delayed by 1-2 weeks, compared with those of the control plants.

Enhanced vigor of experimental plants was also manifested in the spring of the next year. The leaves of the experimental plants have appeared earlier and were growing faster than those of the control plants were. The above-mentioned data suggests that under the influence of the biostimulant *Reglalg* increase grape vine plants vigor and their ability to tolerate abiotic and biotic stress factors.

Table 3. The influence of per vegetation treatment of vine plants, variety Pinot Noir, with the biostimulant *Reglalg* on the shoots growth and on the quantity and quality of the grape.

Variant	Shoots length (cm)	Grape mass (g)	Weight of 100 grape berries (g)	Volume of 100 grape berries (ml)	Sugar content in juice (g/dm^3)	Acidity of the juice (g/dm^3)	Expected yield (kg/bush)
Control	191,9	175,0	147,5	140,0	19,9	13,0	4,6
Fungicides	202,4	275,0	152,0	150,0	17,8	10,2	7,4
Fungicides + Immunocitofit	217,5	290,0	164,7	160,0	18,2	11,5	7,9
Fungicides + Reglalg	222,4	300,0	174,0	170,0	20,6	8,2	8,3

Data about the effects of treating grape vines plants with the preparation *Reglalg* three times per vegetation on the annual growth of shoots and different parameters of harvest are given in the table 3. They clearly demonstrate that the use of *Reglalg* has had beneficial effects on the growth of shoots and as well on all parameters that characterize the quantity and quality of the grape.

Conclusions

1. Treating the wheat seeds before sowing with the solution of the biostimulant Reglalg assure the increasing of their primary resistance to frost and, as well, improve plants adaptation to the winter frosts that in the end ensures the increasing of the crop yield.

2. Treatment of vine plants during vegetation with the solutions of the preparation Reglalg stimulates the shoots growth, decrease the level of grapes infection with the mildew and gray rot, and assure the increasing the plants yield.

3. In general, the complex and standardized composition of the biostimulant Reglalg ensures the initiation in plants of specific biochemical processes which, in the case of imminent action of the stressors, are accelerating specific adaptations, thus contributing to the reduction of the stress-related damages and in final to increasing plants productivity.

4. Considering the lack of Reglalg toxicity and the possibility of halving the use of fungicides for vine plants protection, the implementation of this biostimulant can facilitate the technologies of vine plants cultivation according to the requirements of the organic agriculture.

References

1. *Clewer A., Scarisbrick D*. Practical statistics and experimental design for plant crop science // Chichester, New York: John Wiley & Sons, LTD, 2001. 332 p.

2. *Dascaliuc A., Ivanova R., Arpentin Gh*. Systemic approach in determining the role of bioactive compounds. In Pierce G., Mizin V., Omelchenco A. eds. Advanced Bioactive Compounds Countering the Effects of Radiological, Chemical and Biological Agents, Strategies to counter biological damage; Series: NATO Science for Peace and Security Series A: Chemistry and Biology. Dordrecht, Springer. 2013. P. 121-131.

3. *Jelev N*. Diminuarea impactului factorilor de stres abiotic asupra plantelor de Triticum aestivum L. prin utilizarea regulatorului natural de creştere *Reglalg* // Buletinul Academiei de Ştiinţe a Moldovei, Ştiinţele vieţii. 2016. Vol 330, Nr.3. P.72-79.

4. *Noctor G., Gomez L., Vanacker H., Foyer C.* Interactions between biosynthesis, compartmentation and transport in the control of glutathione homeostasis and signaling // J. Exp. Bot., 2002, v. 53, no. 372, P. 1283–1304.

5. *Thakur M., Sohal B.* Role of elicitors in inducing resistance in plants against pathogen infection: a review // ISRN Biochemistry, Volume 2013, Article ID 762412, P. 1-10.

6. The Certification AA No.0448 for utilization in the Republic of Moldova agriculture of the stimulator of growth *Reglalg1,* The State Center for Certification of Chemical and Biological Means of Plant Protection and Growth Regulators, 12 February 2003 **.**

7. *Traon D., Amat L., Zotz F., du Jardin P. A* Legal Framework for Plant Biostimulants and Agronomic Fertiliser Additives in the EU. Report for the European Commission: Enterprise & Industry Directorate – General. January 2014. 133 p.

8. *Митракова С., Трошин Л.* Оценка сортов винограда для производства сока //Научный журнал КубГАУ [Электронный ресурс]. – Краснодар: КубГАУ, 2009. –№ 06 (50).

9. *Пономарев В.И.* Повышение зимостойкости озимой пшеницы. М.: Рос-сельхозиздат, 1975. 316 с.

10. *Сиренко Л., Козицкая В.* Биологически активные вещества водорослей и качество воды //Киев: Наук. думка, 1988. 256 с.