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Research Article

The Influence of Seed Treatment Before Sowing with the Biostimulator Reglalg on the Productivity of Different Wheat (*Triticum aestivum* L.) Varieties

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Abstract

One of the main grain crops in the world is wheat. In this regard, special attention is paid to the development of technologies to increase its productivity, which can be achieved, among other things, by the use of biologically active substances. The mechanism of action of these substances is to activate metabolic processes, which ultimately increases productivity, accelerates maturation, improves immunity, and makes it possible to induce in plants a complex non-specific resistance to many diseases and resistance to adverse environmental factors.

In the multi-year research, we studied the effect of treating the seeds of the hexaploid wheat varieties Moldova 5, Kuialnik, and Missia before sowing with the biostimulator (BS) Reglalg on the quality and quantity of the harvest. The obtained data demonstrate that treating the seeds before sowing with BS Reglalg solution ensured the tendency to increase the parameters that determine the productivity of autumn wheat; the twinning coefficient of the plants, the number of grains in the central spike, the number of productive spikes per square meter, and the weight of 1000 grains. In all the years of testing, the beneficial effects of treating wheat seeds before sowing with BS Reglalg contributed to the increase of the harvest, the level of which increased depending on the conditions of the year, the specific cultivation technology, and the biological characteristics of the variety. The surplus of harvest obtained, or calculated, from one hectare varying between 500 and 800 kilograms. Although the treatment of wheat seeds before sowing with BS Reglalg beneficially influenced the harvest quantity, the value of their quality indices was comparable to that of the yield obtained in the control variants.

Keywords: *Triticum aestivum* L; Biostimulator Reglalg; Varieties; Harvest; Gluten; Index Zelleny

Introduction

In recent years, the tendency to decrease the use of synthetic substances for plant protection has become increasingly clear since more and more data have appeared that demonstrate their harmful effects on the environment and the health of consumers [1]. Moreover, due to the suppression of symbiotic bacteria and species competing with dangerous organisms and the selection over the years of organisms more resistant to synthetic protective substances, their harmful effects on soil fertility become more and more aggravating. Because of this, soil productivity and the economic effectiveness of synthetic plant protection preparations gradually decreased [1,2].

The transition to organic agriculture is how to overcome the problems accumulated over the years. At the base of organic farming is holistic production management involving system elimination, synthetic fertilizers, and pesticides, plants modified by genetic engineering methods, and ionizing radiation [3]. During the period of transition from traditional to organic agriculture, it becomes essential to optimize the strategies of choosing the most suitable varieties and hybrids proposed for cultivation in the specific environmental conditions, the selection of genotypes adapted to these conditions, as well as the use of biostimulators to increase plants viability, the adaptive capacity and productivity of plants in situations with the increased risk of exposure to frost, heat, and drought temperatures during the vegetation period.

Based on the source of origin and composition, BS were classified into three groups: BS, containing humic substances, amino acids and substances extracted from seaweed [4,5]. The latter represent a natural and inexhaustible source of BS that can have different applications in agriculture. Unlike synthetic plant protection substances, preparations obtained from algae such as BS *Regallg* do not exert negative effects on the environment, therefore they are compatible with organic agriculture [6,7]. It has been shown that the use of seaweed extracts in agriculture can generate several benefits, including stimulation of root system growth, increased photosynthesis and resistance to abiotic stress factors. Therefore, BS contain substances whose function, when applied per plants or introduced into the rhizosphere, independent of the nutrient content of the product, stimulate the natural processes of increasing the vigor and resistance of plants to thermal stress factors, contributing to increase the amount and production quality [7-9].

In previous publications, for different hexaploid wheat genotypes, we have described newly developed methods for the rapid assessment of primary and adaptive resistance of plants during ontogenesis [7,10], as well as the possibility of their modification with the help of BS Reglalg [7,11]. In this article, we present the results of assessing the influence of seed treatment with BS Reglalg on the quantity and quality of the harvest obtained from different wheat varieties.

Materials and Methods

We used in the research three varieties of Moldova 5, Missia, and Kuialnik of winter wheat cultivated in 2015-2017 in the Experimental Field of the Institute of Genetic, Physiology and Protection of Plants in the Chisinau area (Moldova). We carried out wheat sowing, cultivation, and harvesting per generally accepted technologies [12].

The experiments were located on rectangular plots with an area of 9 m^2 each (length-9 m, width -1 m) placed in a block, in three randomized repetitions for each variant, and bordered by protection plots. We divided the seeds of the genotypes included in the research into the control and experimental variants. Before sowing the grains, we sprinkled seeds with 10 milliliters of water (control) or BS Reglalg diluted with water in a 1:200 (experiment) per 1 kilogram. Of grains, shade during 12 hours. We sowed the seeds of all varieties in the second half of October, mechanized at a depth of 6 cm. We provided phenological observations during plant growth and development. During the vegetation period, we provided phenological observations on the growth processes (emergence, pre-twinning, twinning, emergence of adventitious roots) and plant development (earring and grain formation phases - milk maturity, wax, and full maturity).

During the wheat harvest, the plants were collected from 1 m^2 in three repetitions from each control plot and the experimental variant during the wheat harvest. The plants from each square meter, bundled together, were brought to the laboratory, where the ears were detached. Next, we bundled the plants from each square meter and detached the ears in the laboratory; we counted and weighed each bundle's ears. After this, we calculated the number of spikelets and grains in the ear, determining the average number of spikelets per ear, grains per spikelet, or ear. From the wheat seeds collected from the ears of each sample, we separately randomized examples of 1000 grains, we weighed them. Based on these results, we calculated the expected yield per hectare (Rh) both for the plants in the control variant and for those in the experimental variant according to the formula [13]

Rh= Nrs * Nrb * MMB / 10 000, q/ha,

where: Nrs-the number of ears per m^2, Nrb-the number of grains in the ear, MMB-mass per 1000 grains.

The protein sedimentation rate is one of the most essential parameters in assessing flour quality. We obtained the data on the protein content and the hardness of the wheat grains by performing the Zeleny sedimentation index test, expressed by the volume (ml) of the sediment obtained from the flour suspension in a 6% acetic acid solution.

To determine the sedimentation index in the seed samples collected from the productive ears of the control and the experimental variant, in the first stage, the pieces intended for analysis were separated from impurities by manual sieving, using a sieve with a mesh size of 25 mm x 3 mm. We ground the samples consisting of 30 g of seeds using a Retsch GM 200 mill, passed the flour through a sieve with a mesh size of 300 µm, and for further analysis, 3 g of flour from each sample, weighed with an accuracy of ± 0.01g, transferred to sedimentation tubes, adding 50 ml of bromine-phenol blue solution in a concentration of 0.0004%. We shook the stoppered boxes manually by moving the cylinder horizontally for 5 seconds, placed the boxes for stirring in an electric stirrer for 5 minutes, added 25 ml of 6% acetic acid, and supplementary stirring for another 5 minutes. Later, after 15 minutes of keeping the tubes still, we controlled the recorded value of the sedimentation index (ml). The volume of sediment in the cylinder expressed in milliliters represents the Zeleny index. We present the results sedimentation index as the means of three determinations according to the requirements described in [14].

For determining the wet and dry gluten content, a 25g portion of flour, obtained from the 50 g of grains of each control or experimental grounded in a Retsch GM 200 laboratory mill, we inserted without loss in a porcelain mortar. We added 12,5 ml of sodium chloride with a concentration of 2%, then kneaded the mixture for 3 - 4 minutes until obtaining a homogeneous dough; then covered the dough with cellophane film for 15 minutes and washed it on the surface of a thick silk sieve with the 2% sodium chloride solution. The temperature of the dough preparation and washing solution we maintained at 18 - 20ºC, and considered the washing finished when the drops that flowed when squeezing the gluten mixed with iodine solution (cutaneous) in the concentration of 5% did not turn blue (they did not contain starch). At this point, only the purified gluten remained on the surface of the sieve. At this point, only the purified gluten remained on the surface of the sieve. For each sample, the entire washing operation took about 30 minutes. To remove the excess solution from the wet gluten, we squeezed the gluten mass by hand until it started to stick to the fingers, then placed the gluten directly on the platen of the balance and weighed, with an accuracy of ± 0.0001 g, calculating the mean from three gluten extraction from 25g of flour, calculated according to the following formula

Gluten = (m_1/m) x100%,

where: m_1-a mass of gluten remaining after whipping, g; m-a mass of the flour sample taken for analysis, g.

We note that the difference between the data obtained in the three parallel determinations of gluten in 100 g of flour did not exceed 2 g [15]. Additionally, from the obtained samples, we separated portions with a mass equal to 4 g each, shaped in a spherical form, and immersed in containers with three dm^3 of water at a temperature of +18-+20ºC after 15 minutes of immersion, removed water droplets from the spherical gluten globules and evaluate the quality of the gluten, placed with the base in the center of the column under the punch of the autograph ИДК-1М (РУСЬКРАНСНАБ, Belgorod). After pressing the gluten globule for 30 seconds, evaluate the extensibility properties of the gluten by reading the value indicated on the screen. Thus, after the rheological properties, expressing them in conventional units (mm). The gluten quality group depended on the significance of the obtained values [15].

Statistical data analysis, graphical, tabular, and textual representation performed using Statistica 12, Microsoft Word and Microsoft Excel programs. The values obtained for all the parameters for the seedlings of the control group were considered reference values, which reported the results of the determinations carried out in the experimental groups, and each experiment we repeated at least three times. Mean values (*M*), standard deviation (*SD*), the coefficient of variation (*CV*) and statistically significant differences (*p*) between variants were determined [16].

Results and Discussions

Initially, we tested the influence of different concentrations of the BS Reglalg solution on the following productivity indices

- The number of grains in the ear;
- The average mass of caryopses in the ear of the main plant;
- The total weight of caryopses in ears from one seed;
- The mean weight of 1000 caryopses.

As in the case of the influence on the morphological parameters and plants' adaptation to the action of extreme positive or negative temperatures, the optimal concentration for treating the wheat seeds was obtained when BS Reglalg is diluted with water in the ratio of 1/200. In the research devoted to determining the BS Reglalg influence on the productivity indices of different hexaploid wheat varieties, we chose this concentration of the biostimulator.

We included the effect of BS Reglalg on the parameters that characterize the productivity of wheat plants in table 1. The data contained in this table demonstrate that the treatment of seeds with BS Reglalg had a beneficial influence on the values of all productivity indices

- The average number of ears per m2
- The number of caryopses in the ear
- The average mass of caryopses per ear
- The weight of 1000 caryopses

Likewise, the number of productive spikes increased significantly because the autumn twinning was higher in the plants of these variants. Thus, for example, in 2016, for the Moldova 5 and Kuialnik varieties, this increase compared to the control was 9,6 and 10,1%. At the same time, in all experiments, we recorded the increased mass of caryopses in the spikes of experimental plants by 3,1-6% compared to that of the control variants. Although under the influence of BS Reglalg, the number of caryopses in the ear increased significantly, the mass of 1000 grains also tended to be higher than in the control variants, up to 2,7% (in 2015). The obtained data demonstrate that BS Reglalg beneficially influenced the development of physiological and biochemical processes in the experimental plants, which ensured the increase of the average mass even with an increased number of grains in the ear.

In 2016, for each of the three wheat varieties Moldova 5, Kuialnik and Missia, there was a difference in yield of 7, 8 and 5 centner per hectare (centner/ha), respectively, in favor of plants whose seeds were treated with BS Reglalg. If the harvest in 2017 was higher than in 2016, then the increase in yield due to the influence of BS Reglalg, on the contrary, was higher in 2016 and amounted to 9.8-17.1%. Data on the yield and yield growth of the Moldova 5 variety in 2017 did not actually change compared to 2015. In 2017, the yield of the Missia variety was the highest, but the difference between the experimental samples and the control was only 4.4%. In 2015 - 2017, the highest yield increase was observed in varieties Moldova 5 and Kuyalnik (17.1 and 16%), which indicates a positive effect of BS Reglalg on these varieties.

We believe that these effects are due to the beneficial effect of BS Reglalg on the anatomical characteristics (decrease in the length of the epicotyl), increase in the constitutive and adaptive resistance of plants to extreme temperatures. In total, these effects ensured an increase in the vitality of plants, an increase in the photosynthetic activity of leaves throughout the entire growing season.

We determined the wet gluten content and the sedimentation index of the flour, thus evaluating the effect of BS Reglalg on the quality of the crop. Analyzing the data presented in table 2 regarding the crude gluten content in the flour obtained from the caryopsis of the wheat varieties studied, we note that the content of this component is significantly different depending on the variant.

Variety	**Variants**	**Average number of ears per m^2, *M ± SD***	**The number medium of caryopsis per ear, *M ± SD***	**Average mass of caryopses per ear, *M(g) ± SD***	**Mass of 1000 caryopes, *M(g) ± SD***	**Recalculate Harvest, *M(centner/ha) ± SD***	**CV**
Plant productivity indices in 2015							
Moldova 5	Control	426 ± 51	37,8 ± 2,0	1,39 ± 0,09	36,8 ± 0,2	59 ± 0,29	10,1
	Reglalg 1/200	447 ± 25	38,1 ± 1,5	1,44 ± 0,11	37,8 ± 0,3	64 ± 0,34	9,8
	p	0,3	0,67	0,3	0,3	0,14	
	δ	4,9	0,8	3,6	2,7	8,5	
Plant productivity indices in 2016							
Moldova 5	Control	353 ± 52	32,3 ± 1,7	1,17 ± 0,09	36,3 ± 1,0	41 ± 0,49	13,1
	Reglalg 1/200	387 ± 38	33,7 ± 1,1	1,24 ± 0,03	36,7 ± 0,5	48 ± 0,31	9,3
	p	0,179	0,088	0,089	0,289	0,025	
	δ	9,6	4,3	6	1,1	17,1	
Kuialnik	Control	366 ± 39	37,1 ± 1,0	1,38 ± 0,05	37,2 ± 0.8	50 ± 0,46	9,1
	Reglalg 1/200	403 ± 45	38,0 ± 1,4	1,44 ± 0,09	37,8 ± 0,7	58 ± 0,60	10,4
	p	0,087	0,143	0,108	0,164	0,011	
	δ	10,1	2,4	4,4	1,6	16	
Missia	Control	407 ± 21	34,0 ± 1,4	1,28 ± 0,08	36,7 ± 0,5	51 ± 0,19	4,1
	Reglalg 1/200	425 ± 25	35,2 ± 2,2	1,32 ± 0,09	37,3 ± 0,8	56 ± 0,42	6,0
	p	1,00	0,65	0,06	0,65	0,41	
	δ	4,4	3,5	3,1	1,6	9,8	
Plant productivity indices in 2017							
Moldova 5	Control	392 ± 25	38,6 ± 11,0	1,52 ± 0,11	39,5 ± 4,0	60 ± 0,26	8,2
	Reglalg 1/200	425 ± 22	39,0 ± 12,1	1,54 ± 0,14	39,6 ± 4,1	65 ± 0,38	8,4
	p	0,004	0,563	0,696	0,915	0,049	
	δ	8,4	1,0	1,3	0,3	8,3	
Missia	Control	343 ± 19	43,1 ± 11,0	1,99 ± 0,09	45,6 ± 4,5	68 ± 0,22	5,9
	Reglalg 1/200	350 ± 21	44,8 ± 11,4	2,04 ± 0,12	45,7 ± 4,9	71 ± 0,25	8,1
	p	0,53	0,21	0,14	0,57	0,24	
	δ	2,0	3,9	2,5	0,2	4,4	

Table 1: The productivity indices of different wheat varieties plants, developed from the seeds treated with water or with a solution of BS Reglalg diluted with water in a ratio of 1/200 and cultivated on the experimental field of the IGFPP in 2015-2017.

In 2016 the lowest crude gluten content was characteristic for the grains of the Missia variety and the highest-for those of the Moldova 5 variety. The data obtained demonstrate that the differences between the crude gluten content values in the control version and the experimental version of each wheat variety are not statistically significant ($p > 0.05$), and the maximum value of the percentage deviation is respectively only 1.8 and 4.2% for the wet and dry gluten content characteristics. Only in 2017 did the importance of these characteristics increase, as did the percentage deviation (up to 6.7 and 10.7%), and *p* took a value less than 0,05. From the data of Moldova 5 variety obtained in 2015, it follows that with increasing the dose of BS Reglalg, its effect also increased, reaching the maximum of which diluted with water at a ratio of 1/200.

Variety	Variant	Wet and dry gluten content, %		Gluten deformation index, c.u.	index Zelleny, ml
Gluten content and quality in wheat flour obtained in the 2015 harvest					
Moldova 5	Martor	26 ± 0,19	9,1 ± 0,20	70 ± 3,12	45,5 ± 1,99
	Reglalg 1/800	26 ± 0,15	9,5 ± 0,19	68 ± 2,18	46,1 ± 1,10
	p	0,05	0,24	0,45	0,65
	δ	0	4,4	2,9	1,3
	Reglalg 1/400	27 ± 0,21	9,8 ± 0,24	65 ± 3,34	48,8 ± 1,77
	p	0,03	0,14	0,17	0,08
	δ	3,9	7,7	7,1	7,3
	Reglalg 1/200	28 ± 0,12	10,5 ± 0,16	60 ± 2,53	49,2 ± 2,33
	p	0,01	0,02	0,01	0,11
	δ	7,7	15,4	14,3	8,1
Gluten content and quality in wheat flour obtained in the 2016 harvest					
Moldova 5	Martor	29,2 ± 0,15	11,9 ± 0,34	65 ± 2,12	49,1 ± 1,10
	Reglalg 1/200	29,2 ± 0,20	12,4 ± 0,23	63 ± 3,53	51,9 ± 1,77
	p	0,844	0,188	0,505	0,093
	δ	0	4,2	3,1	5,7
Kuialnik	Martor	28,3 ± 0,25	11,0 ± 0,43	70 ± 4,24	43,0 ± 1,33
	Reglalg 1/200	28,8 ± 0,62	11,4 ± 0,29	65 ± 2,82	45,6 ± 1,77
	p	0,225	0,208	0,082	0,160
	δ	1,8	3,6	7,1	6,1
Missia	Martor	27,3 ± 0,18	10,6 ± 0,67	73 ± 2,12	42,7 ± 1,55
	Reglalg 1/200	27,4 ± 0,16	11,0 ± 0,56	70 ± 1,41	48,6 ± 1,99
	p	0,303	0,570	0,211	0,014
	δ	0,4	3,8	4,1	13,8
Gluten content and quality in wheat flour obtained in the 2017 harvest					
Moldova 5	Martor	31,7 ± 0,10	12,1 ± 0,10	65 ± 1,57	42,2 ± 2,21
	Reglalg 1/200	32,9 ± 0,15	13,4 ± 0,05	64 ± 2,41	44,5 ± 3,31
	p	8,13E-04	4,52E-05	5,19E-01	2,52E-01
	δ	3,8	10,7	1,5	5,5
Missia	Martor	29,8 ± 0,15	11,2 ± 0,04	74 ± 1,00	39,8 ± 1,10
	Reglalg 1/200	31,8 ± 0,10	12,0 ± 0,03	69 ± 1,00	42,2 ± 2,21
	p	4,86E-05	9,34E-06	1,83E-03	0,082
	δ	6,7	7,1	6,8	6,0

Table 2: Flour quality indices from the grains of different wheat varieties collected from plants obtained from seeds treated before sowing with water or with various solution doses of BS Reglalg.

Crude gluten deformation index values from each cultivar's control and the experimental variant were comparable, ranging between 60 and 74 conventional units, indicating a good caryopses quality.

The sedimentation index of flour is one of the most important parameters that characterize flour quality [14]. We determined the given parameter according to Zeleny's method based on the ability of proteins to swell in weak solutions of organic acids. The quality index was significantly higher for the flour from the caryopses of the Moldova 5 variety compared to the flour obtained from the other two wheat varieties (2016). At the same time, the sedimentation index was higher by 5.7-13.8%, which indicates its superior quality. In 2017, for the Moldova 5 and Missia varieties, the values of these parameters in both the control and experimental variants decreased compared to 2016. For the Moldova 5 variety, a decrease in these values was also observed compared to 2015. However, in the years 2015 - 2017, compared to the other two wheat varieties, it had the highest levels for the protein sedimentation index parameter, which characterizes the quality of the standard autumn wheat flour. For the wheat variety Missia, the decrease (more than twice) of the mentioned index was characteristic, as well as a percentage deviation from 13.8% (the year 2016) to 6.0% (the year 2017).

Tests under production conditions, in various farms of the Republic of Moldova, on a total area of about 220 hectares, showed that the yield obtained after seed treatment before sowing with BS Reglalg increased by 5-8.5 centner/ha compared to the yield obtained with control fields (Table 3).

District, Household and year	Variety	Variant	Surface, ha	Harvest (centner/ha)	Surplus (centner/ha)
IGFPP-on the Experimental Scientific Base, year 2015	Moldova 5	Control	0,0842	57,3	8,5
		Reglalg	0,0842	65,8	
Sângerei district (Rădoaia village), Agricultural Production Cooperative "Colina agrară", Rădoianul Prim SRL, year 2015	Moldova 5	Control	20	36,0	6,0
		Reglalg	180	42,0	
Taraclia district (Balabanu village), "Izvoraş" peasant farm, year 2016	Kuialnik	Control	10	37,0	5,0
		Reglalg	10	42,0	
Experimental Fields of the IGPP, 2016	Moldova 5	Control	0,0135	41,2	6,6
		Reglalg		47,8	
	Kuialnik	Control		50,7	7,1
		Reglalg		57,8	
	Lăutari	Control		50,3	5,7
		Reglalg		56,0	

Table 3: The harvest of plants of different varieties of wheat, obtained from seeds treated with water or with a solution of BS Reglalg (1/200) and grown on the production fields of various agricultural households in the districts of the Republic of Moldova.

The previously presented data demonstrated that the treatment of winter wheat seeds before sowing with a solution of BS Reglalg ensures the increase of primary resistance [11], vigor, and the ability of the plants to adapt to the action of frost in winter, heat, and drought in summer [7]. The effects mentioned benefits, in the end, were also manifested on the quality and quantity of the harvest. Thanks to the treatment of the seeds with BS Reglalg, the harvest volume increased and manifested. The harvest volume increased due to the twinning coefficient and the number of grains in the ear, especially in the primary ear. Due to the greater vigor, the plants of the experimental variants demonstrated the ability to maintain at a higher level the processes that ensure the accumulation of reserve substances in the grains; to form a more significant number of productive brothers, as well as a higher number of grains in the central ear.

Conclusions

Treating the seeds before sowing with the solution of BS Reglalg ensured the increase of the values of the parameters that determine the productivity of the autumn wheat; the coefficient of the twinning of the plants, the number of grains in the main ear, and the mass of 1000 grains.

In all years of testing, the effects of wheat seed treatment before sowing, the solution of BS Reglalg ensured the increase of the harvest, but the level of its increase was influenced by the conditions of the year and the specifics of the variety.

Although the treatment of wheat seeds before sowing with the solution of BS Reglalg beneficially influenced the harvested quantity, the value of their quality indices was comparable to that of the yield obtained in the control variants.

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