

## **The effectiveness of biopreparations in soft wheat cultivation and the quality assessment of the grain by the digital x-ray imaging**

L.E. Kolesnikov<sup>1,\*</sup>, I.I. Novikova<sup>2</sup>, E.V. Popova<sup>2</sup>, N.S. Priyatkin<sup>3</sup>, E.V. Zuev<sup>4</sup>,  
Yu.R. Kolesnikova<sup>4</sup> and M.D. Solodyannikov<sup>1</sup>

<sup>1</sup>Saint-Petersburg State Agrarian University, Faculty of Agrotechnologies, Soil science and Ecology, Department of Plant Protection and Quarantine, Petersburgskoe Shosse (highway), 2, RU196601 St. Petersburg, Pushkin, Russia

<sup>2</sup>All-Russian Institute of Plant Protection (VIZR), Laboratory of microbiological plant protection, shosse Podbel'skogo highway, 3, RU196608 S. Petersburg, Pushkin, Russia

<sup>3</sup>Agrophysical Research Institute (ARI), plant biophysics division, Grazhdanskiy ave. 14, RU195220 St. Petersburg, Russia

<sup>4</sup>Federal Research Center N.I. Vavilov All-Russian Institute of Plant Genetic Resources (VIR), genetic resource of wheat department, plant introduction department, Bolshaya Morskaya street, 42–44, RU190000 St. Petersburg, Russia

\*Correspondence: kleon9@yandex.ru

**Abstract.** The main trend of modern crop production is the expansion of the use of plant protection solutions and technologies, that ensure not only effective management of the number of populations of harmful organisms, but also the production of environmentally safe agricultural products with minimal anthropogenic impact on agro-ecosystems. One of our priorities is to develop new environmentally sound polyfunctional biopreparations, that combine useful properties of microbial strains such as phytopathogen antagonists and chitosan compositions that increase disease resistance. The introspective analysis of the seed material quality realized with the non-destructive express techniques application was used for evaluation the effectiveness of the compositions' complex influence on plants. The research studies the effectiveness of the influence of *Bacillus subtilis* strains and chitosan compositions, including their combined effect, on soft wheat productivity and its damage by disease agents. Data on the quality assessment of the grain using microfocus x-ray radiography and gas-discharge visualization (electrophotography) are also provided. The complex of more than 20 structural elements of wheat productivity was analysed during the research. Assessment of the plant damage intensity according to the standard criterion - the disease development, and additional phytopathological indicators was carried out. The evaluation of the grains' introspective characteristics was carried out by the microfocus x-ray radiography techniques combined with the digital x-ray image analysis. It is shown that the biopreparation complexes, containing *B. subtilis* strains and Chitosan, have a combined biological activity manifested in the optimization of wheat plants' physiological state, increasing productivity, diseases resistance.

**Key words:** *Bacillus subtilis*, biopreparations, Chitosan, microfocus x-ray radiography, polyfunctional complex, soft wheat, wheat diseases, yield structure.

## INTRODUCTION

Wheat is one of the most important cereal crop that provides food for more than a half of the world's population (Reynolds et al., 2009; Falcone et al., 2019). One of the reasons for the decline in wheat productivity is the deterioration of the phytosanitary condition of the crops, that is highly dependent on the technologies of its cultivation.

Recently, the world has been most in demand for green farming strategies based on the use of natural alternatives to chemical plant protection products. Some alternative methods of dealing with common plant diseases are based on the use of natural compounds that not only induce resistance through the priming mechanism, but also help to uncover the complex mechanisms underlying the phenomenon of induced resistance. These include chitosans, oligogalacturonides, volatile organic compounds, azelaic and pipercolic acids and others (Aranega-Bou et al., 2014).

Chitosan is a natural polyaminosaccharide, which is a copolymer of glucosamine and acetylglucosamine, which has a number of valuable properties (nontoxicity, biocompatibility, hypoallergenicity, biodegradability) and widely used in agriculture to increase the resistance of plants to diseases (El Hadrami et al., 2010, Aranega-Bou et al., 2014). The organic polymers (chitosan and others) can be used as a prospective eco-friendly carrier for bacteria in the creation of immobilized preparative forms of microorganisms in biofertilizers (Shcherbakova et al., 2018). Chitosan has been shown to be able to efficiently induce phytochemicals in plants, is an effective elicitor, and serves as an alternative to plant genetic modification (Kim et al., 2005). In addition, according to existing data, chitosan affects the growth and productivity of plants (Reddy et al., 1999; Kim et al., 2005; Cho et al., 2008; Aranega-Bou et al., 2014). The treatment of wheat seeds with chitosan (2–8 mg mL<sup>-1</sup>) significantly improved seed germination to recommended seed certification standards (> 85%) and germination energy at concentrations > 4 mg mL<sup>-1</sup> in spring wheat varieties by combating *Fusarium graminearum* seed infection (Reddy et al., 1999). In a number of experiments with various host plants and pathogens (Iriti et al., 2010), it was shown that chitosan can directly activate systemic resistance or induce a more effective protective response to a call, depending on the dose, taking into account different cytotoxicity thresholds for each chitosan derivative and plants. (Iriti, & Faoro, F., 2009; Aranega-Bou et al., 2014). An important role in creating natural plant protection systems is played by microbial antagonists of plant pathogens. Compounds that are released from the cell wall of phytopathogens as a result of the action of antagonist hydrolases can function as resistance elicitors, causing protective reactions in a plant: phytoalexin synthesis, activation of hydrolytic enzymes, lignification, etc. (Sidorova et al., 2018). The group of strains of *Bacillus subtilis* is recognized as a powerful biocontrol tool, because it has suppressive activity against a wide range of phytopathogens due to its ability to produce many secondary metabolites of various chemical nature: cyclic lipopeptides, polypeptides, proteins and non-peptide compounds, among others (Stein, 2005; Sidorova et al., 2018). The ability of bacteria to synthesize compounds of a certain structure suggests the presence of a specific mechanism of action on a phytopathogenic object, and also explains the biological activity of a particular strain against specific microorganisms (Sidorova et al., 2018).

Thus, the researchers showed the influence of two strains of *B. fortis* IAGS162 and *B. subtilis* IAGS174, which not only provided maximum control of *Fusarium* wilt of

tomatoes, but also significantly increased the growth and yield of plant fruits in the field (Akram et al., 2013). The effect of six strains of *Bacillus pumilus* INR7, *B. megaterium* P2, *B. subtilis* GB03, *B. subtilis* S, *B. subtilis* AS and *B. subtilis* BS and four native strains of *Achromobacter* sp. B124, *Pseudomonas geniculata* B19, *Serratia marcescens* B29 and *B. simplex* B21 to suppress all wheat diseases by inducing plant defense mechanisms. Most treatments were equally effective against all diseases, both when watering the soil and when spraying plants. Bacterial strains not only suppressed diseases, but also enhanced plant growth, whereas when treated with chemical inductors, it decreased (Jasem et al., 2018). According to some studies, *B. subtilis* is preventive and has curative properties in the early stages of the yellow rust development. The pathogen's urediniospore germination significantly inhibited by spraying *Bacillus subtilis* strain E1R-j on leaf surface before inoculation (Li et al., 2013). The possibilities for the biological control of yellow rust was investigated by winter wheat treatments with the biological products that contained *Bacillus* spp., *Pseudomonas aurantiaca*, *Brevibacillus* spp., *Acinetobacter* spp., and chitosan. However, after two years of researches, the results were not convincing (Feodorova-Fedotova et al., 2019). The effect of preliminary inoculation of *Triticum aestivum* seeds with endophytic *B. subtilis* 26D strain cells on the growth of shoots and the activity of antioxidant enzymes when exposed to heavy metals - cadmium and lead was established. Inoculation of seeds with *B. subtilis* bacteria cells contributed to better growth of wheat plants in the soil, a decrease in the intensity of lipid peroxidation in plant tissues, both with the combined action of metals and with separate action. The protective effect of *B. subtilis* 26D was due to the biological activity of bacteria that produce a large number of biologically active substances (Smirnova et al., 2016). Inoculation of plant seeds with bacteria contributed to a decrease in the metal content in shoots (Kuramshina et al., 2016). Under conditions of moisture deficiency in plants inoculated with *B. subtilis*, an increase in mycorrhization was found, it was revealed that *B. subtilis* bacteria reduced stress caused by drought in plants (Kuramshina et al., 2015).

One of the highest priority areas in the practice of ecologic adaptive agriculture today is the use of multifunctional biological products in order to obtain high-quality agricultural products. Biological products, such as chitosan and its derivatives, which are a combination of microbes antagonists of pathogens with activators of plant disease resistance, can be promising plant protection products. The high protective effect of complex biologics is associated with a combination of the antagonistic properties of the microbe antagonist with the ability of chitosan, together with biologically active substances, to stimulate the mechanisms of phytoimmunity (Kolesnikov et al., 2018).

The soft-ray microfocuss radiography method has been successfully used for many years in Russia (Arkhipov et al., 2019) and in other countries (Burg et al., 1995; Gomes-Junior et al., 2012; Silva et al., 2013). Since 1976, the method has been included in international and national standards, primarily for assessing of grain infestation and damage by pests. It can be used to detect various structural defects of seeds, such as fracturing, enzymomycosis depletion (EMIS), internal germination, hidden pest population, damage by the bug 'harmful turtle', mechanical injuries and defects of the germ, empty seed. The methodology can be used for evaluation of seeds of various densities and sizes (Priyatkin et al., 2018). Developing and application of new software for seed images' automatic analysis is promising direction of modern scientific and practical elaborations. In particular, the measurement of morphometric as well as optical

parameters of radiographs that characterize the endosperm density degree can be performed, for example, by the use of software for images' morphometric analysis produced by Argussoft LLC, Saint Petersburg, Russia (Priyatkin et al., 2018).

The purpose of the work is to justify the prospect of using multifunctional preparations based on strains of microorganisms antagonists of pathogens and activators of plant disease resistance - chitosan complexes in the cultivation of common wheat.

## MATERIALS AND METHODS

The location of the research - Laboratory of Microbiological Plant Protection VIZR, Department of Plant Protection and Quarantine, St. Petersburg State Agrarian University, Plant Biophysics Sector of Agrophysical Research Institute.

The experimental studies were implemented in the experimental field of Pushkin laboratories of the Federal State Budgetary Institution of Higher Education FIC N.I. Vavilov All-Russian Institute of Plant Genetic Resources (VIR). The plant material of the study was the spring soft wheat variety Leningradskaya 6, k-64900, which was provided for the research by the VIR wheat genetic resources department.

The experimental design provided for the following options:

- without treatments (control);
- 'Vitaplan, CL' - the culture liquid of *B. subtilis* strain VKM B-2604D and *B. subtilis* strain VKM B-2605D at a ratio of 1: 1 with a titer of living cells and spores / g *B. subtilis* - 1010;
  - 'Vitaplan, CL + 0.1% Chitosan' (chitosan salicylate). Chitosan salicylate was added to the concentration of 0.1% to the Vitaplan culture fluid diluted with distilled water 10 times (cell titer 1010 CFU mL<sup>-1</sup>). To obtain chitosan salicylate, chitosan with a mM of 60 kDa obtained by oxidative degradation (Muzzarelli Riccardo, 1977) from chitosan with a molecular mass of 150 kDa and a degree of deacetylation of 85% (Bioprogress, RF) was used. Based on it, a chitosan derivative was synthesized: Chit + SC, containing ion-bound fragments of salicylic acid (SC), comprising 25%.
  - 'Vitaplan CL + colloidal chitin (0.1%)' During the deep cultivation of Vitaplan, 0.1% of colloidal chitin was added to the standard nutrient medium (calculated on the dry weight of chitin). Then the culture fluid with a titer of 1011 CFU mL<sup>-1</sup> was diluted 10 times. The preparation of colloidal chitin was implemented according to the method of Roberts & Selitrennikoff (1988) from chitin with mM 100 kDa (by dissolving chitin in concentrated hydrochloric acid and subsequent precipitation of colloidal chitosan with acetone).
  - 'Vitaplan CL + colloidal chitin (0.1%) + 0.1% Chitosan' (chitosan salicylate). During the deep cultivation of Vitaplan, 0.1% of colloidal chitin (calculated on the dry weight of chitin) was added to the standard nutrient medium, the titer of the culture fluid was 1011 CFU mL<sup>-1</sup>. Then the culture fluid was diluted 10 times and 0.1% chitosan salicylate was added.
  - 'Vitaplan, WP' (standard), containing cells of the strains of *Bacillus subtilis* VKM B-2604D and *B. subtilis* VKM B-2605D. The *B. subtilis* strain VKM-2604D synthesizes antibiotics of various structures (the polypeptide antibiotic from the bacteriocin group and the polyene antibiotic), and the *B. subtilis* strain VKM B-2605D forms a polypeptide close to bacillillin and hexaene antibiotics, one of which is assigned to the subgroup of mediocidin.

In the experiment the seeds of spring soft wheat Leningradka 6, k-64900 were treated with the biopreparations before sowing and vegetating plants later were sprayed three times.

During the tillering stage of wheat, the number, length, and weight of the roots (main germinal root, germinal and coleoptile roots) originating from the epicotyl were determined. The number and length of the nodal roots of wheat were taken into account. The calculation of the phases of ontogenesis of soft wheat during processing with biological products was implemented on the Eukarpia (Zadox) scale.

When examining the wheat yield structure, the data on field germination, productive and total bushiness, plant height, spike length, number of spikelets per spike, number of grains per spike, spike weight, and the 1,000 grains weight were analyzed. The weight of the vegetative part of plants and the area of flag and pre-flag leaves were determined.

The degree of damage to plants by root rot was assessed in the field on a generally accepted scale.

The intensity of wheat damage by the pathogen of powdery mildew (*Blumeria graminis* Speer.) was taken into account during the phases of tillering of wheat, going into the tube, ripening (milk ripeness of grain) according to the indicators: the conditional value of the development of the disease, the number and area of spots with plaque. The brown rust development (*Puccinia recondita* Rob. Ex Desm. F. Sp. Tritici Eriks.) on flag leaves was taken into account during the stem extension stage and milk stage, according to the standard criterion - the disease development and additional parameters: the pustules number, the pustule area. The intensity of the defeat of the samples by wheat leaf blotch (*Stagonospora nodorum* Castell. et Germano) was determined in the phases of milk and wax ripeness of grain according to the conditional development of the disease on the flag and pre-flag leaf surface in accordance with the James scale. The intensity of wheat damage by yellow rust was determined both by using a generally accepted indicator - the conditional intensity of the pathogen development (according to the Manners scale), and by using additional parameters: the number of pustules (total per sheet), the number of strips with pustules, the length of strips with pustules, the area of the pustule and their numbers in the band (Kolesnikov et al., 2018).

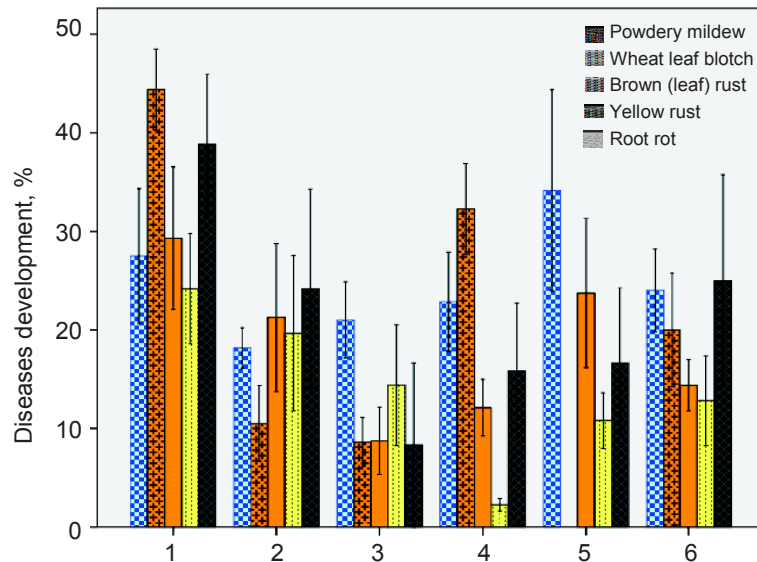
X-ray analysis was performed using a mobile X-ray diagnostic unit PRDU-02 and the Argus-BIO software, according to the method described previously (Kolesnikov et al., 2019).

Descriptive statistics methods were used to assess the biological effectiveness of biological products and multifunctional complexes (based on standard errors of the mean  $\pm$  SEM, 95% confidence intervals, and Student t-test), factor analysis, and Spearman rank correlation calculation. Statistical analysis was performed in SPSS 21.0, Excel 2016.

## RESULTS AND DISCUSSION

In the control variant, the following development of pathogens was recorded on cultivar Leningradskaya 6, k-64900 experiment (without treatment): helminthosporious root rot -  $38.9 \pm 7.1\%$  (2016–2019 -  $39.4 \pm 3.1\%$ ) yellow rust -  $24.2 \pm 5.6\%$  (2016–2019 -  $11.2 \pm 2.4\%$ ), leaf blotch -  $47.0 \pm 15.4\%$  (2016–2019 -  $41.4 \pm 4.2\%$ ); brown rust -  $29.3 \pm 7.2\%$  (2016–2019 -  $20.9 \pm 4.2\%$ ), powdery mildew -  $18.2 \pm 2.1\%$  (2016–2019 -  $18.8 \pm 4.8\%$ ). When applying multifunctional complexes on soft wheat: 'Vitaplan, CL + 0.1% Chitosan' and 'Vitaplan, CL + colloidal chitin (0.1%) + 0.1%

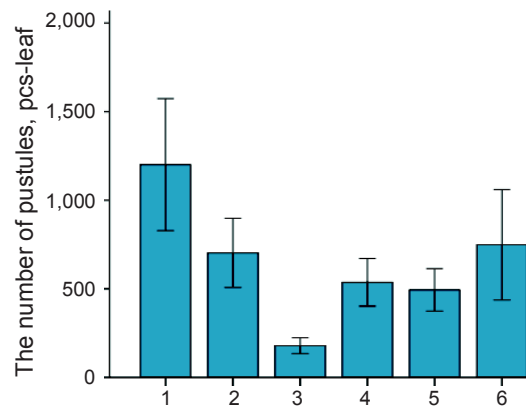
Chitosan', a statistically significant decrease ( $P < 0.05$ ) development of root rot - by 22–31%, leaf blotch - by 41–47% (Fig. 1).



**Figure 1.** Change in the intensity of the development of wheat diseases with the use of biological products and multifunctional complexes in comparison with the control. 2019 year. Explanation: 1 – Control (water); 2 – Vitaplan, CL; 3 – Vitaplan, CL + 0.1% Chitosan; 4 – Vitaplan, WP; 5 – Vitaplan, CL + colloidal chitin (0.1%) + 0.1% Chitosan; 6 – Vitaplan, CL + colloidal chitin (0.1%) (95% confidence intervals for means).

According to the literature data, it is known that chitosan induces the synthesis of various phytoalexins that can start-up plant phytoimmunity reactions and suppress infections (Gamzazade et al., 1999). At the same time, any activity in the polymer (fungicidal, antibacterial, growth-stimulating) can be increased by its chemical modification with appropriate biologically active substances (BAS).

The use of the Vitaplan, CL + 0.1% Chitosan complex reduced wheat damage by brown rust by 20.6%, and the Vitaplan, CL + colloidal chitin (0.1%) + 0.1% Chitosan complex reduced yellow rust by 13.4%. On the flag leaves of wheat in the indicated experimental variants, the decrease in the total number of yellow rust pustules (Fig. 2) - by 37.6% and 55.3%



**Figure 2.** Change in the number of pustules of wheat rust species when using biological products and multifunctional complexes compared to the control. 2019 year.

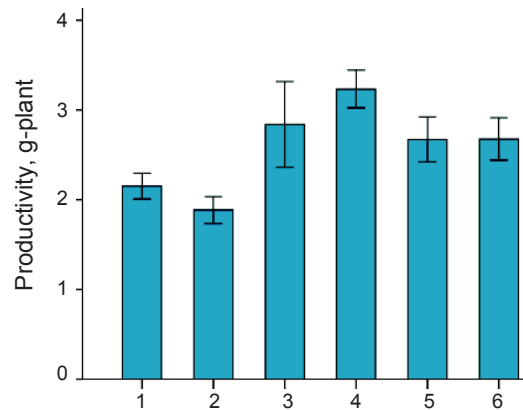
Explanation: 1 – Control (water); 2 – Vitaplan, CL; 3 – Vitaplan, WP; 4 – Vitaplan, CL + colloidal chitin (0.1%) + 0.1% Chitosan; 5 – Vitaplan, CL + colloidal chitin (0.1%); 6 – Vitaplan, CL + 0.1% Chitosan (95% confidence intervals for means).

(the number of bands by 37.8% and 34.9%) was determined; brown rust - by 20.6% and 5.6%, respectively.

An important indicator that characterizes the wheat resistance or susceptibility to types of rust is the area of the pustule. To the greatest extent, the area of yellow rust pustule decreased in comparison with the control in the experimental variant: 'Vitaplan, CL + colloidal chitin (0.1%) + 0.1% Chitosan' - by 22.8%. No statistically significant effect of biological products on the area of wheat rust pustule was revealed.

A significant decrease in the development of major wheat diseases has affected its potential yield. In the experiment, where the Vitaplan, CL + colloidal chitin (0.1%) + 0.1% Chitosan multifunctional complex was used), the wheat yield statistically significantly increased by 50.3% compared to the control (Fig. 3). The use of the multifunctional complex 'Vitaplan, CL + 0.1% Chitosan' increased the yield of wheat by 24.4%. According to researchers, chitosan inhibits the phytopathogens development: it suppresses the growth of fungal mycelium spores, has antiviral activity, and in addition, it stimulates seed germination, plants growth and development, and increases their productivity (Gamzazade et al., 1999; Yakushkina & Bakhtenko, 2005).

Numerous experimental data have been published on the ability of useful microorganisms of the rhizo- and phyllosphere to synthesize metabolites that affect the plants resistance and growth and have signaling and hormonal functions. Thus, natural growth regulators are auxins, gibberellins, cytokinins, abscisic (ABC), salicylic, and jasmonic acids (Forchetti et al., 2007; Dodd et al., 2010; Sivasakthi et al., 2013; Kudoyarova et al., 2014). Many strains of bacteria of the genera *Bacillus*, *Azospirillum*, *Pseudomonas*, etc. have the ability to synthesize auxins, which leads to stimulation the root system development, and, as a result, to more active absorption of water and nutrients by plants. These processes together increase the plants resistance to diseases and allow them to pass faster the development stages, when they are most susceptible to pathogens (Kumar et al., 2012). It was revealed that gibberellin can synthesize by many strains of the genus *Bacillus* bacteria (Kilian et al., 2000). The *Bacillus*, *Rhizobium*, *Arthrobacter*, *Azotobacter*, *Azospirillum*, *Pseudomonas* bacteria can produce cytokinins. For example, the chlorophyll and cytokinins content increased in plants when they were inoculated with cytokinin-producing strains of *B. subtilis*, which subsequently caused an increase in the biomass of the root system and the vegetative part (Cohen et al., 2009).



**Figure 3.** The potential yield of soft wheat when using biological products and multifunctional complexes. 2019 year.

Explanation: 1 – Control (water); 2 – Vitaplan, CL; 3 – Vitaplan, WP; 4 – Vitaplan, CL + colloidal chitin (0.1%) + 0.1% Chitosan; 5 – Vitaplan, CL + colloidal chitin (0.1%); 6 – Vitaplan, CL + 0.1% Chitosan (95% confidence intervals for means).

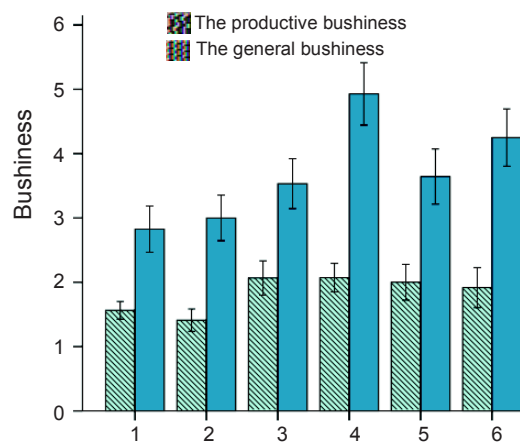
When using the multifunctional complex ‘Vitaplan, CL + 0.1% Chitosan’, a significant increase in the length of nodal roots was noted by 49.2%, and the total bushiness - by 50.4%. In the experiment, where the Vitaplan, CL + colloidal chitin (0.1%) + 0.1% Chitosan complex was used, a significant increase in productive bushiness - by 32.3%, total bushiness - by 74.4% was revealed (Fig. 4), spike weight - by 66.8%, as well as root lengths by 30.9%, number of nodal roots - by 25%, length of nodal roots - by 18.6%.

Plant growth and formation processes are regulated by a certain proportion of phytohormones. At the same time, each morphogenetic process does not require its own hormone; from 2-3 substances can be created many different proportions. In this way the direction and rate of growth, the appearance of each organ will depend on the certain proportion. (Yakushkina & Bakhtenko, 2005).

The relationship between the elements of wheat productivity when using the multifunctional complexes ‘Vitaplan, CL + colloidal chitin (0.1%) + 0.1% Chitosan’ and ‘Vitaplan, CL and 0.1% Chitosan’ were studied by factor analysis. The cumulative percentage of variance in the measurements of productivity indicators, due, in particular, to factors F1 and F2, was 54% and 49.7%, respectively. To simplify the interpretation of factors, the varimax rotation method is used.

It was shown that six indicators turned out to be the most sensitive to the action of the multifunctional complex ‘Vitaplan, CL + colloidal chitin (0.1%) + 0.1% Chitosan’: plant phase, number and length of roots, plant height, productive bushiness, flag area leaf, and when applying ‘Vitaplan, CL and 0.1% Chitosan’ - five indicators: root length, number of nodal roots, productive and total bushiness, root weight (described by F1). Between the values of these indicators revealed strong positive correlation. In addition (factor F2), ‘Vitaplan, CL + colloidal chitin (0.1%) + 0.1% Chitosan’ had some effect on the spike weight, the number of spikelets per spike, the number of grains per spike, the weight of grains in one spike, and ‘Vitaplan, CL and 0.1% Chitosan’ - on spike weight, number of spikelets per spike, weight of grains of one spike and the 1,000 grains weight. Moreover, the number of spikelets per spike correlated, in particular, with the 1,000 grains weight.

X-ray analysis data are presented in Fig. 5. It was found that the area of the x-ray projection and the length of the projection of the grains was significantly increased with the use of the multifunctional complexes Vitaplan CL + 0.1% Chitosan and Vitaplan CL + colloidal chitin (0.1%). It was also revealed that the use of multifunctional complexes significantly affected the values of the ‘X-ray projection elongation’ in the

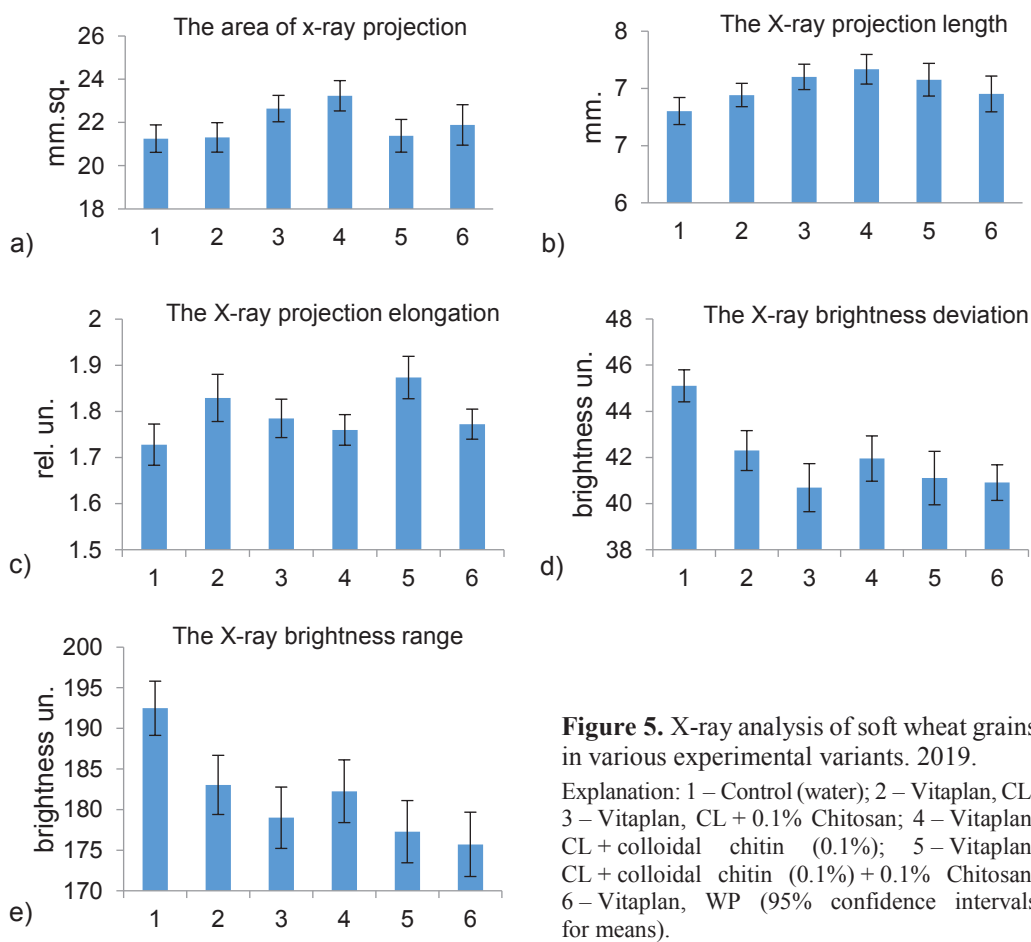


**Figure 4.** Change in the productive and general bushiness of soft wheat when using biological products and multifunctional complexes. 2019 year.

Explanation: 1 – Control (water); 2 – Vitaplan, CL; 3 – Vitaplan, WP; 4 – Vitaplan, CL + colloidal chitin (0.1%) + 0.1% Chitosan; 5 – Vitaplan, CL + colloidal chitin (0.1%); 6 – Vitaplan, CL + 0.1% Chitosan (95% confidence intervals for means).



‘Vitaplan, CL’ and ‘Vitaplan, CL + colloidal chitin (0.1%) + 0.1% Chitosan’ options, in other cases we can talk about the tendency of increase of this indicator. Thus, in a number of test variants, the seeds became larger, the shape of the seeds also changed - they became more elongated. The greatest impact of the multifunctional complexes was noted on the brightness parameters of X-ray diffraction patterns of the grains. In all cases, in comparison with the control, a statistically significant decrease in the values of the mean square deviation of brightness (the largest decrease in the ‘Vitaplan CL + 0.1% Chitosan’ variant) and the Interval of brightness (the largest decrease in the ‘Vitaplan WP’) was found. This fact may indicate a more optically uniform x-ray projection of the caryopsis, and possibly a reduced percentage of latent defects, manifested in the form of various kinds of blackouts on the x-ray.



## CONCLUSIONS

Thus, as a result of the studies, a high biological effectiveness of multifunctional biological products was noted, which manifested itself in a decrease in the intensity of diseases and an increase in the productivity of wheat. It is shown that the *B. subtilis* strains contained as part of the Vitaplan biopreparation and the ‘Vitaplan, QL + Chitosan II’

and 'Vitaplan, CS + Chitosan II' biopreparation complexes have a combined biological activity associated with the synthesis of various BAS and manifested in the optimization of wheat plants' physiological state, increasing productivity, diseases resistance and reducing diseases incidence. The use of the multifunctional complex 'Vitaplan, CL + 0.1% Chitosan' statistically significantly reduced the value of 5 phytopathological indicators (development of root rot, spots with powdery mildew, development and number of brown rust pustules, number of yellow rust strips), and 'Vitaplan, CL + colloidal chitin (0.1%) + 0.1% Chitosan' - 3 indicators (root rot development, development and area of yellow rust pustule. Use of the multifunctional complex 'Vitaplan CL + colloidal chitin (0.1 %) + 0.1% Chitosan' to the greatest extent *UV* increased potential yield of wheat (by 50.3%), which was mainly due to an increase in productive bushiness of plants (by 32.3%). It was shown by the method of x-ray analysis of digital x-ray images that the use of multifunctional complexes to one degree or another influenced the characteristics of grain x-ray (dimensional indicators, shape indicators, as well as brightness indicators). The digital x-ray image analysis has shown that the implementation of polyfunctional complex has increased the average brightness of the digital x-ray images of the grain and the modification of their size and density. It was revealed that the area of the x-ray projection and the length of the projection of the grains was significantly increased with the use of the multifunctional complexes Vitaplan CL + 0.1% Chitosan and Vitaplan CL + colloidal chitin (0.1%). It was also revealed that the use of multifunctional complexes significantly affected the values of the 'X-ray projection elongation' in the 'Vitaplan, CL' and 'Vitaplan, CL + colloidal chitin (0.1%) + 0.1% Chitosan' options, in other cases we can talk about the tendency of increase of this indicator. Thus, in a number of test variants, the seeds became larger, the shape of the seeds also changed - they became more elongated. The greatest impact of the multifunctional complexes was noted on the brightness parameters of X-ray diffraction patterns of the grains. In all cases, in comparison with the control, a statistically significant decrease in the values of the mean square deviation of brightness (the largest decrease in the 'Vitaplan CL + 0.1% Chitosan' variant) and the Interval of brightness (the largest decrease in the 'Vitaplan WP') was found. This fact may indicate a more optically uniform x-ray projection of the caryopsis, and possibly a reduced percentage of latent defects, manifested in the form of various kinds of blackouts on the x-ray. To establish a relationship between the indicators of x-ray of grains and their sowing qualities, a further test for the energy of germination is promising germination, germination and additional growth indicators (root and sprout length). The results of the work can be used to develop environmentally friendly technologies for wheat cultivation and optimize the phytosanitary condition of crops.

ACKNOWLEDGEMENTS. The research was performed within the framework of the State Task according to the theme plan of VIR, Project No. 0662-2019-0006 'Search For and Viability Maintenance, and Disclosing the Potential of Hereditary Variation in the Global Collection of Cereal and Groat Crops at VIR for the Development of an Optimized Genebank and Its Sustainable Utilization in Plant Breeding and Crop Production'.

## REFERENCES

- Akram, W., Anjum, T., Ali, B. & Ahmad, A. 2013. Screening of native bacillus strains to induce systemic resistance in tomato plants against fusarium wilt in split root system and its field applications. *International journal of agriculture and biology* **15**, 1289–1294.
- Aranega-Bou, P., de la O Leyva, M., Finiti, I., García-Agustín, P. & González-Bosch, C. 2014. Priming of plant resistance by natural compounds. Hexanoic acid as a model. *Frontiers in plant science* **5**, 488. doi: 10.3389/fpls.2014.00488
- Arkhipov, M.V., Priyatkin, N.S., Guskova, L.P., Potrakhov, N.N., Gryaznov, A.Y., Bessonov, V.B., Obodovskii, A.V. & Staroverov, N.E. 2019. X-Ray computer methods for studying the structural integrity of seeds and their importance in modern seed science. *Technical Physics. The Russian Journal of Applied Physics* **64**(4), 582–592. doi: 10.1134/S1063784219040030
- Burg, W.J. van der, Jalink, H., Zwol, R.A. van, Aartse, J.W. & Bino, R.J. 1995. Non-destructive seed evaluation with impact measurements and X-ray analysis. *Acta Horticulturae* **362**, 149–157.
- Cho, M.H., No, H.K. & Prinyawiwatkul, W. 2008. Chitosan treatments affect growth and selected quality of sunflower sprouts. *Journal of food science* **73**, 70–77. doi: 10.1111/j.1750-3841.2007.00607.x
- Cohen, A.C., Travaglia, C.N., Bottini, R. & Piccoli, P.N. 2009. Participation of abscisic acid and gibberellins produced by endophytic *Azospirillum* in the alleviation of drought effects in maize. *Botany* **87**, 455–462. doi: 10.1139/B09-023
- Dodd, I.C., Zinovkina, N.Y., Safronova, V.I. & Belimov, A.A. 2010. Rhizobacterial mediation of plant hormone status. *Annals of Applied Biology* **157**, 361–379. doi: 10.1111/j1744-7348.2010.00439.x
- El Hadrami, A., Adam, L.R., El Hadrami, I. & Daayf, F. 2010. Chitosan in Plant Protection. *Marine Drugs* **8**(4), 968–987. doi: 10.3390/md8040968
- Falcone, G., Stillitano, T., Montemurro, F., De Luca, A.I., Gulisano, G. & Strano, A. 2019. Environmental and economic assessment of sustainability in Mediterranean wheat production. *Agronomy Research* **17**(1), 60–76. <https://doi.org/10.15159/AR.19.011>
- Feodorova-Fedotova, L., Bankina, B. & Strazdina, V. 2019. Possibilities for the biological control of yellow rust (*Puccinia striiformis* f. sp. *tritici*) in winter wheat in Latvia in 2017–2018. *Agronomy Research* **17**(3), 716–724. <https://doi.org/10.15159/AR.19.137>
- Forchetti, G., Masciarelli, O., Alemano, S., Alvarez, D. & Abdala, G. 2007. Endophytic bacteria in sunflower (*Helianthus annuus* L.): isolation, characterization, and production of jasmonates and abscisic acid in culture medium. *Applied Microbiology and Biotechnology* **76**(5), 1145–1152. (doi: 10.1007/s00253-007-1077-7)
- Gamzazade, A.I., Ismailov, E.Ya. & Tyuterev, S.L. 1999. New modification of plant disease resistance inductor and growth regulator. In: *Materials of the V all-Russian conference "New perspectives in the study of chitin and chitosan"*. Moscow-Shchelkovo, pp. 83–87 (in Russian).
- Gomes-Junior, F.G., Yagushi, J.T., Belini, U.L., Cicero, S.M. & Tomazello-Filho, M. 2012. X-ray densitometry to assess internal seed morphology and quality. *Seed Science and Technology* **40**(1), 102–107.
- Iriti, M. & Faoro, F. 2009. Chitosan as a MAMP, searching for a PRR. *Plant signaling and behavior* **4**, 66–68. doi: 10.1016/j.plaphy.2008.08.002
- Iriti, M., Giulia, C., Sara, V., Ilaria, M., Soave, C., Fico, G. & Faoro, F. 2010. Chitosan-induced ethylene-independent resistance does not reduce crop yield in bean. *Biological Control* **54**, 241–247. doi: 10.1016/j.biocontrol.2010.05.012

- Jasem, A.M., Sharifi, R. & Abbasi, S. 2018. Induced systemic resistance to wheat take-all disease by probiotic bacteria. *Journal of Plant Protection Research* **58**(3), 304–310. <https://doi.org/10.24425/jppr.2018.124639>
- Kilian, M., Steiner, U., Krebs, B., Junge, H., Schmeiedeknecht, G. & Hain, R. 2000. FZ B 24® *Bacillus subtilis* – mode of action of microbial agent enhancing plant vitality. *Pflanzenschutz-Nachrichten Bayer* **1**, 72–93.
- Kim, H.J., Chen, F., Wang, X. & Rajapakse, N.C. 2005. Effect of chitosan on the biological properties of sweet basil (*Ocimum basilicum* L.). *Journal of agricultural and food chemistry* **53**, 3696–3701. doi: 10.1021/jf0480804
- Kolesnikov, L.E., Popova, E.V., Novikova, I.I., Priyatkin, N.S., Arkhipov, M.V., Kolesnikova, Yu.R., Potrakhov, N.N., van Duijn, B. & Gusarenko, A.S. 2019. Multifunctional biologics which combine microbial anti-fungal strains with chitosan improve soft wheat (*Triticum aestivum* L.) yield and grain quality. *Sel'skokhozyaistvennaya Biologiya [Agricultural Biology]* **54**(5), 1024–1040 (in Russian). doi: 10.15389/agrobiology.2019.5.1024eng
- Kolesnikov, L.E., Novikova, I.I., Surin, V.G., Popova, E.V., Priyatkin, N.S. & Kolesnikova, Yu.R. 2018. *Applied Biochemistry and Microbiology* **54**, 540–546 (in Russian). <https://doi.org/10.1134/S0003683818050083>
- Kudoyarova, G.R., Melentiev, A.I., Martynenko, E.V., Timergalina, L.N., Arkhipova, T.N., Shendel, G.V., Kuz'mina, L.Y., Dodd, I.C. & Veselov, S.Y. 2014. Cytokinin producing bacteria stimulate amino acid deposition by wheat roots. *Plant Physiology and Biochemistry* **83**, 285–291. doi: 10.1016/j.plaphy.2014.08.015
- Kumar, P., Dubey, R.C. & Maheshwari, D.K. 2012. *Bacillus* strains isolated from rhizosphere showed plant growth promoting and antagonistic activity against phytopathogens. *Microbiological Research* **167**, 493–499. doi: 10.1016/j.micres.2012.05.002
- Kuramshina, Z.M., Khairullin, R.M. & Sattarova, L.R. 2015. The influence of endophytic bacteria strains *Bacillus subtilis* on mycorrhization of roots of cereals in the imitation of a soil drought. *Agrochemistry* **5**, 69–73 (in Russian).
- Kuramshina, Z.M., Smirnova, Y.V. & Khairullin, R.M. 2016. Increasing *Triticum aestivum* tolerance to cadmium stress through endophytic strains of *Bacillus subtilis*. *Russian Journal of Plant Physiology* **63**(5), 636–644 (in Russian).
- Muzzarelli Riccardo, A.A. 1977. *Chitin*. Pergamon Press, Oxford, 309 pp.
- Li, H., Zhao, J., Feng, H., Huang, L. & Kang, Z. 2013. Biological control of wheat stripe rust by an endophytic *Bacillus subtilis* strain E1R-j in greenhouse and field trials. *Crop Protection* **43**, 201–206. doi: 10.1016/j.cropro.2012.09.008
- Priyatkin, N.S., Arkhipov, M.V., Guskova, L.P., Potrakhov, N.N., Kropotov, G.I., Tzibizov, I.A. & Vinerov, I.A. 2018. Introsopic methods of seed quality evaluation: state of problem and prospects of realization. *Agrophysica* **2**, 29–39. doi: 10.25695/FGRP.2018.02.05. (in Russian)
- Reddy, M.V.B., Arul, J., Angers, P. & Couture, L. 1999. Chitosan treatment of wheat seeds induces resistance to *Fusarium graminearum* and improves seed quality. *Journal of agricultural and food chemistry* **47**, 1208–1216. doi: 10.1021/jf981225k
- Reynolds, M., Foulkes, M.J., Slafer, G.A., Berry, P., Parry, M.A.J., Snape, J.W. & Angus, W.J. 2009. Raising yield potential in wheat. *Journal of Experimental Botany* **60**(7), 1899–1918. <https://doi.org/10.1093/jxb/erp016>
- Roberts, W.K. & Selitrennikoff, C.P. 1988. Plant and Bacterial Chitinases Differ in Antifungal Activity. *Journal of General Microbiology* **134**, 169–176.
- Shcherbakova, E.N., Shcherbakov, A.V., Rots, P.Yu., Gonchar, L.N., Mulina, S.A., Yahina, L.M., Laktionov, Yu.V. & Chebotar, V.K. 2018. Inoculation technology for legumes based on alginate encapsulation. *Agronomy Research* **16**(5), 2156–2168. <https://doi.org/10.15159/AR.18.186>

- Sidorova, T.M., Asaturova, A.M. & Homyak, A.I. 2018. Biologically active metabolites of bacillus subtilis and their role in the control of phytopathogenic microorganisms. *Sel'skokhozyaistvennaya biologiya [Agricultural Biology]* **53**(1), 29–37 (in Russian) doi: 10.15389/agrobiology.2018.1.29eng
- Silva, V.N., Cicero, S.M. & Bennett, M. 2013. Associations between X-ray visualised internal tomato seed morphology and germination. *Seed Science and Technology* **41**(2), 225–234.
- Sivasakthi, S., Kanchana, D., Usharani, G. & Saranraj, P. 2013. Production of plant growth promoting substance by *Pseudomonas fluorescens* and *Bacillus subtilis* isolates from paddy rhizosphere soil of Cuddalore district, Tamil Nadu, India. *International Journal of Microbiological Research* **4**(3), 227–233. doi: 10.5829/idosi.ijmr.2013.4.3.75171
- Smirnova, J.V., Kuramshina, Z.M., Horohorina, E.E. & Khairullin, R.M. 2016. Influence of bacteria *Bacillus subtilis* antioxidant enzymes *Triticum aestivum* under the joint action of cadmium and lead. *Modern problems of science and education* **6**, 541. <http://www.science-education.ru/ru/article/view?id=25757>
- Stein, T. 2005. Bacillus subtilis Antibiotics: Structures, Synthesis and Specific Functions. *Molecular Microbiology* **56**, 845–857. <http://dx.doi.org/10.1111/j.1365-2958.2005.04587.x>
- Yakushkina, N.I. & Bakhtenko, E.Yu. 2005. *Plant physiology*. Moscow. Vlados, pp. 342–368 (in Russian).