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# MULTIFUNCTIONAL BIOPREPARATIONS AND COMPLEXES BASED ON MICROORGANISMS AND CHITOSAN INCREASE DISEASES RESISTANCE, PRODUCTIVITY AND LEAF PHOTOSYNTHETIC PIGMENT CONTENTS IN SPRING SOFT WHEAT (*Triticum aestivum* L.)

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## Abstract

The application of useful microorganisms and biologically active molecules lies at the basis of the modern concept of agroecosystems phytosanitary optimization. The increase of the protective properties of preparative forms, which include phytopathogen antagonists and chitosan, is due to the ability of chitosan polysaccharide to induce systemic plant disease resistance. In addition, multifunctional compositions with multiple action mechanisms, effective against a wide range of phytopathogens, can positively effect on the functional state of plants, including their photosynthetic activity, quantitative and qualitative changes in the entire pigment system, which often reflect the nature of adaptive reactions under stress. However, studies of changes in the photosynthetic apparatus in relation to the disease resistance and plants productivity under the influence of such compositions are extremely few. It was shown for the first time that the multifunctional complexes Vitaplan, CL + Chitosan II and Vitaplan, SC + Chitosan II significantly increase wheat productivity and disease resistance, while the content of chlorophylls a and b in leaves also turned out to be the highest. The ratio of chlorophylls a + b and carotenoids content, which serves as one of the indicators of plant stress resistance, was maximal when using the Vitaplan, CL + Chitosan II complex. This study aimes to estimate the potential wheat productivity by morphometric indicators of plant development, susceptibility to root rot, brown and yellow rust, powdery mildew, Septoria leaf blotch, and the content of chlorophylls a, b, carotenoids in leaves when using multifunctional biopreparations and complexes combining the useful properties of microorganisms — antagonists of phytopathogens and chitosan as plant disease resistance activator. Seeds of the Leningradka 6 cultivar (k-64900, VIR collection) of soft spring wheat (*Triticum aestivum* L.) were treated before sowing with biopreparations based on *Bacillus subtilis* strains VKM B-2604D and *B. subtilis* VKM B-2605D Vitaplan, SP, Vitaplan, CL and the complexes Vitaplan, CL + Chitosan II, Vitaplan, SC + Chitosan II. In the field during the growing season, plants were sprayed with the same preparations vs. control (without treatment). In general, the used complexes turned out to be more effective than biopreparations by 16.2 %. The multifunctional compositions application significantly reduced wheat plants harm by diseases complex (by 17.9 % at $p < 0.05$). The highest values of potential productivity (0.94±0.02 g/plant) and chlorophyll a (1.32±0.02 mg/g) and b (2.15± 0.04 mg/g) content in the leaves were detected when using the multifunctional complex Vitaplan, CL + Chitosan II, which exceeded the control by 57.1 %, 16.7 % and 4.3 %, the other variants — by 19,7 %, 23,7 %, and 11,0

%. Differences in the content of chlorophyll a and chlorophyll b photosynthetic pigments in wheat flag leaves were revealed when using the multifunctional complex Vitaplan, CL + Chitosan II compared to biopreparations by 16.8 %, 3.7 % and 2.0 %, with Vitaplan, SC + Chitosan II — by 1.1 %, 17.7 %, and 27.0 %, respectively. The strongest correlation was found between the chlorophyll b content in the flag leaves and wheat productivity ($r = 0.69$, $p = 0.03$), the chlorophyll b content in the flag leaves and the grains number per spike ($r = 0.79$, $p = 0.006$), the grains weight per spike and the spike weight ($r = 0.69$, $p = 0.03$; $r = 0.72$, $p = 0.02$). Correlations between a decrease in the yellow rust development and an increase in the chlorophylls a and b content in leaves were found ($r = 0.66$, $p = 0.04$; $r = 0.87$; $p = 0.005$). The highest values of the chlorophyll a to chlorophyll b ratio in the leaves compared to control occurred when using Vitaplan, CL + Chitosan II and Vitaplan, SC + Chitosan II complexes. The ratio of the chlorophylls a and b to the carotenoid pigments, as an indicator of plant resistance to negative external factors, also reached maximum values with Vitaplan, CL + Chitosan II. According to the indicators sum, the most promising for use in wheat cultivation is the multifunctional complex Vitaplan, CL + Chitosan II which has a pronounced growth-stimulating and protective effect on plants upon preventive use.

Keywords: *Triticum aestivum*, soft wheat, multifunctional biological products, chlorophyll a, chlorophyll b, carotenoids, wheat productivity, wheat diseases, brown rust, yellow rust, septoriosis, powdery mildew, root rot

Modern technology for cultivating grain crops requires a set of measures to protect against diseases, in particular chemical treatment of seeds and spraying of crops with fungicides, which is environmentally unsafe and leads to the formation of stable populations of phytopathogens. Therefore, alternative, environmentally friendly means are needed [1].

*Bacillus subtilis* strains, due to the diversity and high variability of their biochemical properties and the synthesis of a spectrum of bioactive metabolites - cyclic lipopeptides, polypeptides, proteins and non-peptide compounds [2-4], are widely used in the fight against pathogens of agricultural crops. *B. subtilis* strains are known to produce three ribosomal antibiotics (TasA, subtilosin, and sublacin), four nonribosomal antibiotics (bacitracin, bacilisin, plipastatin, and surfactin), the novel phospholipid antibiotic bacilisocin, and the amino sugar antibiotic neotrehalosadiamine (NTD) [2]. Nonribosomal cyclic oligopeptides, such as surfactin, iturin group compounds and fengycin, containing a chain of fatty acids, exhibit high antifugal and antibacterial activity [5]. *B. subtilis* strains produce various hydrolytic enzymes that destroy the cell wall of phytopathogenic fungi [6]. A number of active compounds produced by these microorganisms have elicitor activity and trigger mechanisms of induced resistance [7, 8].

Numerous data have also been published regarding the ability of beneficial microorganisms of the rhizo- and phyllosphere to synthesize metabolites that affect the resistance and growth of plants and have signaling and hormonal functions. Auxins, gibberellins, cytokinins, abscisic (ABA), salicylic, jasmonic acids are natural growth regulators [9-11]. Many strains of bacteria belonging to the genera *Bacillus*, *Azospirillium*, *Pseudomonas* can synthesize auxins, which stimulates the development of the plant root system, as a result, the absorption of water and nutrients is activated. These processes collectively increase disease resistance and allow plants to more quickly pass through those developmental stages when plants are most susceptible to pathogens [12-14].

The most promising for protecting agricultural crops and increasing their productivity are compositions that combine the beneficial properties of microorganisms - antagonists of pathogens and activators of plant disease resistance, such as chitosan and its derivatives [15]. Chitosan and preparations based on it have found practical use as inducers that increase resistance to fungal, bacterial and viral diseases [16-19].

The biological activity of chitosan as an inducer of resistance is determined by its ability to activate protective reactions and induce nonspecific

cellular immunity, one of the lines of defense for the innate immunity of plants [19, 20]. Chitosan and chitin, present in the cell walls of many parasitic microorganisms and fungi, are the molecular patterns of phytopathogenic fungi (pathogen-associated molecular patterns, PAMPs), which are recognized by plant protein receptors (pattern-recognition receptors, PRR) [21] and serve as a signal for activation protective responses (pattern-triggered immunity, PTI), preventing the development of infection [22, 23]. Protective responses induced by chitosan include an increase in cytosolic $Ca^{2+}$ concentration, oxidative burst (formation of reactive oxygen species, ROS) [24-26], hypersensitive response (HR), synthesis of pathogenesis-related proteins (PR), with antimicrobial and lytic activity, induction of defense hormones (abscisic acid, jasmonates, salicylic acid), formation of phytoalexins [26-31]. In addition, defense reactions induced by chitosan in plants are characterized by increased synthesis of lignin [32] and callose [30], which leads to the strengthening of cell walls and the creation of physical barriers to the penetration and movement of pathogens in plants [18]. These reactions, aimed at suppressing the growth of the pathogen, lead to the formation of systemic resistance in plants and protect them from subsequent attack by a wide range of pathogens, and also increase plant resistance to unfavorable abiotic factors.

The mechanisms of the protective action of chitosan and its high efficiency in protecting various types of agricultural plants from the action of a wide range of phytopathogens are discussed in detail in numerous review articles [17, 30]. In addition to protecting against phytopathogens, chitosan accelerates plant growth, increases resistance to stress (frost, drought, excess moisture) and the productivity of grain and vegetable crops [33-36]. Treatment with polysaccharide increases the rate of photosynthesis, increases the number of shoots, leaf size, and plant height in wheat, corn, beans, tomatoes, and rice, which provides increased yield [37-42].

Among the factors determining high grain productivity of wheat, chlorophyll photosynthetic potential of crops, which characterizes the total amount of chlorophyll in leaves or whole plants per unit area of crops during the growing season or a certain period thereof. There is a strong positive relationship between chlorophyll photosynthetic potential during the reproductive period and wheat yield [43]. It has been established [44) that the increase in the yield of the modern winter wheat variety Favoritka compared to the Mironovskaya 808 variety bred in the 1960s is associated with an increase in the content and gross amount of chlorophyll, as well as an extension of the period of functioning of the photosynthetic apparatus of sowing during the reproductive period. The modern variety is characterized by more efficient use of absorbed light energy, which leads to an increase in photosynthetic productivity. The authors conclude that increasing photosynthetic efficiency is a promising strategy for increasing crop productivity (44). Optimizing the operation of the photosynthetic apparatus at different levels of its organization can increase grain productivity by 10-60% [45, 46].

In plants, chlorophylls are found only in pigment-protein complexes (PPCs), since in their free form, being strong photosensitizers, they can cause destruction of thylakoid membranes and chloroplast stroma due to the photodynamic effect. PPCs allow optimizing the operation of the photosynthetic apparatus. There are four main types of PBPCs: two of them are reaction centers of photosystem (PS)I and PSII, the other two are light-harvesting complexes (light-harvesting complexes) of PSI and PSII. In chloroplasts, the antenna complex contains a large number (200-400) of chlorophyll molecules and a relatively small amount of carotenoids non-covalently bound to protein.

Chlorophyll b serves as an auxiliary light-harvesting pigment, capturing

and transmitting light energy to the reaction centers of photosystems. It accounts for approximately 15-25% of the total chlorophyll content. Unlike chlorophyll a, which is part of the core complexes of photosystems, chlorophyll b is found only in the light-harvesting complexes of photosystems (LHC I and LHC II) and in the so-called small antenna of PSII. In LHC I, chlorophyll b makes up apprx. 22% of the total amount of chlorophylls, in LHC II apprx. 43%, in the pigment-protein complex of the small antenna 31-46% [47].

Carotenoids are auxiliary photosynthetic pigments and an essential component of the pigment systems of all photosynthetic organisms. In chloroplasts, carotenoids are found in the PPC and partly in the lipophilic phase of thylakoid membranes. Reaction centers, which are a complex of proteins, pigments and other cofactors and provide the reaction of converting light energy into chemical energy during photosynthesis, include only chlorophyll a and β-carotene, and light-harvesting antennas include chlorophylls a and b, carotenes and xanthophylls. Carotenoids, which are part of the light-harvesting antenna, expand the spectral range of photosynthetically active radiation (PAR). In addition to participating in the absorption of solar radiation energy and its migration from additional pigments to the main ones, carotenoids also perform a protective function (quenching triplet chlorophyll and singlet oxygen), protecting the photosynthetic apparatus from photodestruction.

Previously, the biosynthesis of photosynthetic pigments, in particular chlorophylls, was not considered a factor activating signaling pathways that lead to the initiation or delay of ontogenesis phases. However, recent work has demonstrated the important role of the accessory photosynthetic pigment chlorophyll b in the regulation of plant ontogeny [48]. In addition, data have been published that indicate that the absence of chlorophyll b negatively affects the change in periods of ontogenesis in barley. The chlorina mutants of both species differed from the plants of the parental lines in the later onset of floral transformation. In addition, in 30-40% of barley mutants, the growth and differentiation of the structural elements of the ear stopped [49]. Photosynthetic structures may be involved in the plant's adaptive response to stress [50]. In this case, a change in the content of pigments (chlorophyll a, chlorophyll b, the sum of chlorophylls a + b and carotenoids) is likely. Abiotic stress reduces the efficiency of photosynthesis primarily due to the negative impact on chlorophyll biosynthesis, the functioning of photosystems, electron transport mechanisms, and gas exchange parameters [51, 52].

It should be noted that some researchers either have not identified a direct relationship between the efficiency of photosynthesis and yield, or have established a negative correlation between these indicators in many plants, including grain crops [53].

Nevertheless, photosynthesis is the basis of primary bioproductivity both in natural ecosystems and in the formation of crop yields; therefore, under changing environmental conditions it is necessary to maintain and increase the photosynthetic productivity of plants [54-56].

Indirectly, resistance to abiotic stress can be assessed by quantitative changes in the pigment complex [57-60]. A number of repors reflect the influence of plant damage by pathogens on the composition of the pigment complex. In particular, when woody plants are damaged by parasitic fungi, the content of chlorophylls decreases and the content of carotenoids increases [61]. As a result, structural-functional and physiological-biochemical rearrangements occur, which ultimately leads to a decrease in photosynthetic activity [61]. However, similar studies linking the productivity and resistance of plants to biotic stress (damage by

phytopathogens) with the state of the photosynthetic complex are extremely few.

In the presented work, we showed for the first time that the multifunctional complexes Vitaplan, CL + Chitosan II and Vitaplan, SC + Chitosan II significantly increase the productivity and disease resistance of wheat, while the content of chlorophylls a and b in leaf blades also turned out to be the highest. The ratio of the content of chlorophylls a + b and carotenoids, which serves as one of the indicators of plant stress resistance, was maximum when using the Vitaplan, CL + Chitosan II complex.

The purpose of our study is to assess the potential productivity of wheat based on morphometric indicators of plant development, their susceptibility to the most harmful diseases (root rot, brown and yellow rust, powdery mildew, septoria) and the content of chlorophylls a, b, carotenoids in leaves when using multifunctional biological products and complexes, combining the beneficial properties of microorganisms that are antagonists of phytopathogens and the activator of plant disease resistance, chitosan.

*Materials and methods.* Experimental studies were carried out in an experimental field (Federal Research Center Vavilov All-Russian Institute of Plant Genetic Resources — VIR, St. Petersburg-Pushkin, 2016-2018) on spring soft wheat (*Triticum aestivum* L.) variety Leningradskaya 6 (k-64900, provided by the Department of Wheat Genetic Resources of the VIR). In the field experiment, wheat was sown on plots with an area of 1.0 $m^2$ in a row with row spacing of 15 cm and a distance in the row of 2 cm. For each sample, the registration plot consisted of 6 rows; 50 grains were placed in each row when sowing. The seeding rate was 300 seeds per 1 $m^2$. The seed placement depth is 5-6 cm. The field experiment was carried out in three repetitions. All activities were carried out in accordance with generally accepted VIR recommendations and methods.

The experimental design included no treatment (control); treatment with biological product Vitaplan, WP (wettable powder, standard), containing cells of the strains *Bacillus subtilis* VKM B-2604D and *B. subtilis* VKM B-2605D (viable cell titer 1011 CFU/g); Vitaplan, CL, the culture liquid of *B. subtilis* VKM V-2604D and *B. subtilis* VKM V-2605D (1:1) with a titer of viable *B. subtilis* cells of 1010 CFU/ml; multifunctional complex Vitaplan, CL + Chitosan II (the composition of the culture liquid CL in the multifunctional complex Vitaplan, CL + Chitosan II and in the preparation Vitaplan, CL is the same); multifunctional complex Vitaplan, SC (suspension concentrate, viable cell titer $5\times10^{10}$ CFU/ml) + Chitosan II. Chitosan II with a molecular weight of 50 KDa was obtained by the method of oxidative destruction [62] from chitosan with a molecular weight of 300 KDa and a degree of deacetylation of 85% (OOO Bioprogress, Russia). Chitosan was dissolved in succinic acid and, with constant stirring, added to the culture liquid to a final concentration of 0.1% [63].

In field tests, the seeds of wheat variety Leningradka 6 were treated before sowing and vegetative plants were sprayed with the same preparations 4 times during the tillering, booting, heading and flowering phases.

Ninteen parameters were used to analyze the elements of wheat productivity that characterize the morphological characteristics of plants and the structure of the crop in the heading, flowering and ripening phases. During the heading-flowering phases, the following set of indicators was taken into account: productive and general bushiness (pcs.), area of flag and pre-flag leaves ($cm^2$), plant height (cm), spike length (cm), number of spikelets per ear (pcs.), weight ear (g). In addition, the number and length of roots (main embryonic root, embryonic and coleoptile roots) extending from the epicotyl were determined, and the number

and length of nodal roots were accounted. The masses of the roots and vegetative parts of wheat plants were determined (the mass of the vegetative parts was calculated from the total mass of the stem and leaves without taking into account the mass of roots and ears).

The field germination of samples [64] and the stage of plant ontogeny were determined by the J.C. Zadoks's scale [65]. In the ripening stage (stage of full ripeness), the structure of the wheat harvest was studied with regard to the number of spikelets per ear (pcs.), spike length (cm), weight of the spike with grain (g), the number of grains per spike (pcs.), weight of grains with ear (g), 1000-grain weight (g).

The potential (biological) productivity of a single plant $P_p$ was calculated based on the productive tillering capacity $T_p$ and the weight of grains per ear of one plant $W_g$ (g/plant): $P_p = W_g \cdot T_p$.

Wheat leaf area was calculated by the well-known formula [66], using linear measurement data - length ($l$, cm) and maximum width of the leaf at the base ($d$, cm): $S = {}^2/_3\, ld$.

To evaluate field germination, the number of wheat plants in the germination phase was counted for each registration plot, based on the sowing rate of 300 seeds per 1 $m^2$.

The sample size for each experimental variant with 3-fold repetition of plot placement was 60 plants.

The degree of damage to wheat plants ($Pd$, %) by helminthosporium root rot, the causative agent is *Cochliobolus sativus* (S. Ito & Kurib.) Drechsler ex Dastur, according to experimental options was assessed in laboratory conditions in the tillering and heading-flowering phases in accordance with the generally accepted methodology [67] according to the formula:

$$P_d = \frac{\sum(ab)100\,\%}{AK},$$

where $a$ is the number of plants with the same signs of damage; $b$ is the corresponding damage score; $A$ is the number of plants in a set (healthy and sick); $K$ is the highest score of the accounting scale (maximum score of damage is 3).

The intensity of damage to wheat by pathogens of leaf diseases was determined using generally accepted accounting scales, as well as additional indicators of pathogenesis [68]. The intensity or degree of development of rust is determined as a percentage according to classical scales [63]: brown rust (*Puccinia recondita* Rob. ex Desm f. sp. *tritici* by Rusakov or Peterson, yellow rust (*Puccinia striiformes* Westend) by Manners, septoria (*Septoria tritici* Roberge in Desmaz.) by James, powdery mildew (*Blumeria graminis* (DC.) Speer f. sp. *tritici* March) by Peterson. In addition, indicators that further characterize the pathogenesis were determined: the number of pustules (total per leaf), the number of stripes with pustules, length stripes with pustules, the area of the pustule and their number in the stripe. Pathogenesis indicators were examined using a stereoscopic microscope MBS-9 (LLC PTP ASMA-Pribor, Russia) and a trinocular microscope Micromed 1 (var. 3 LED) (OOO Observational Devices, Russia).

The size of infectious structures on leaves during pathogenesis (spots, pustules, etc.) was determined using object and ocular micrometers. The values of the pustule area were calculated assuming their elliptical shape: $S = m \cdot \pi ab$ where $a$ and $b$ are the length of the semi-axes of the ellipse (in the lines of the ocular micrometer), $m$ is the microscope scale factor.

The content of chlorophylls a and b in wheat flag leaves was determined spectrophotometrically as described [69] using a SPEKOL-11 spectrophotometer (Carl Zeiss AG, Germany). To determine the content of photosynthetic pigments,

we used samples from the middle part of the leaf blade, from which alcoholic extracts were prepared [69].

The concentration of pigments in the extracts (mg/dm$^3$) was calculated using the following formulas:

$$C_p = 9.784 \cdot D_{662} - 0.99 \cdot D_{644};$$
$$C_b = 21.426 \cdot D_{644} - 4.650 \cdot D_{662};$$
$$C_a + C_b = 5.134 \cdot D_{662} + 20.436 \cdot D_{644};$$
$$C_{car} = 4.695 \cdot D_{440.5} - 0.268 \cdot (C_a + C_b),$$

where $C_p$ is the concentration of pigments, $C_a$ is the concentration of chlorophyll a, mg/dm$^3$; $C_b$ is chlorophyll b concentration, mg/dm$^3$; $C_{car}$ is concentration of carotenoids, mg/dm$^3$; $D_{662}$ is optical density at $\lambda = 662$ nm, units; $D_{644}$ is optical density at $\lambda = 644$ nm; $D_{440.5}$ is optical density at v 440.5 nm.

The concentration of pigments in flag leaves (mg/100 g) was calculated as $X = 100CVV_2 \cdot (mV_1)^{-1} \cdot 1000$, where $C$ is the pigment concentration, mg/dm$^3$; $V$ is the initial extract volume, cm$^3$; $V_1$ is the volume of the initial extract taken for dilution, cm$^3$; $V_2$ is volume of diluted extract, cm$^3$; m is the mass of the sample, g.

Statistical analysis of the results was carried out in the programs IBM SPSS Statistics 21, Statistica 6.0 (StatSoft, Inc., USA), Microsoft Excel 2016. The calculations used methods of analysis of variance and the Scheffй multiple comparison test, methods of parametric statistics (calculation of means $M$ and their standard errors ±SEM, 95% confidence intervals and Student's t-test), nonparametric method of Spearman's rank correlations, linear and nonlinear regression analysis based on the least squares algorithm.

*Results.* In the field experiment scheme, we did not use the variant with Chitosan II with a molecular weight of 50 KDa separately, since our earlier field experiments showed that its use caused a significant increase only in the mass of the vegetative part of plants [63]. The complex Vitaplan, CL + Chitosan II caused a significant increase in the largest number of wheat productivity indicators compared to the control (63). In the same variant of the experiment, the maximum significant increase in yield (82.6%) and maximum effectiveness against helminthosporium root rot were recorded (reduction in the development of root rot by 80-100% compared to the control) (63).

**1. Grain productivity of spring soft wheat (*Triticum aestivum* L.) variety Leningradka 6 treated with biologicals and multifunctional complexes based on Bacillus subtilis strains and chitosan** ($n = 17$, $N = 3$, $M$±SEM; experimental field of the Federal Research Center Vavilov All-Russian Institute of Plant Genetic Resources, St. Petersburg-Pushkin, 2018)

| Treatments | Productivity, g per plant | Student's *t*-test | Confidence interval at a 5% level of significance | To control, % |
|---|---|---|---|---|
| Conrol (water) | 0.60±0.02 | | 0.56–0.64 | |
| Vitaplan, CL | 0.81±0.03 | 1.17 | 0.75–0.87 | 35.3 |
| Vitaplan, CL + Chitozan II | 0.94±0.02 | 2.40 | 0.90–0.98 | 57.1 |
| Vitaplan, WP | 0.80±0.05 | 0.90 | 0.70–0.90 | 33.7 |
| Vitaplan, SC + Chitozan II | 0.93±0.03 | 1.97 | 0.88–0.99 | 55.7 |

N o t e. For a description of the drugs, see the Materials and methods section.

The results obtained in this work showed a significant positive effect of the studied multifunctional compositions on the phytosanitary state of the agrocenosis of spring soft wheat and its productivity indicators. In 2018, the potential yield of wheat in the Vitaplan, CL + Chitosan II variant exceeded the control by 57.1% (Table 1), and for 2016-2018 on average by 64.1% (Fig. 1, A).

We descrived changes in some morphometric indicators associated with the grain productivity of bread wheat when using biologicals and multifunctional complexes (Table 2-4).

For Vitaplan, CL + Chitosan II, there was a reduction in the period of wheat ripening by ontogenesis phases (by 10.2%), a significant ($p \leq 0.05$) increase in plant height (by 24.3%), and root length (by 11.3%). 0%), root mass (by 50.6%), flag leaf area (by 30.9%), number of spikelets per ear (by 6.1%).

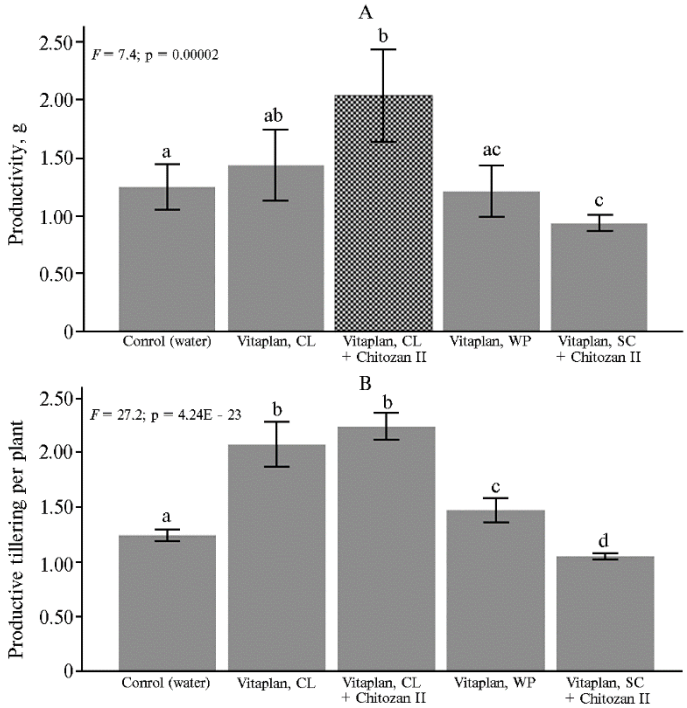


**Fig. 1. Potential grain productivity** (A) **and productive tillering** (B) **in spring soft wheat (*Triticum aestivum* L.) variety Leningradka 6 treated with biological products and polyfunctional complexes based on *Bacillus subtilis* strains and chitosan** ($n$ = 51, $N$ = 3, $M$±SEM; experimental field of the Federal Research Center Vavilov All-Russian Institute of Plant Genetic Resources, St. Petersburg-Pushkin, 2016-2018). For a description of the drugs, see the Materiasl and methods section. The same letters indicate values that do not differ from the control and from each other by the Scheffe's test at $p < 0.05$; $F$ is Fisher's test.

A strong correlation was identified between potential productivity and productive tillering (Spearman non-parametric correlation coefficient $r$ = 0.76; p = 0.029E–9). In our tests, the highest productive tillering was observed when using the multifunctional complex Vitaplan, CL + Chitosan II (see Fig. 1, B).

On average, over the observation period (2016-2018), this figure increased by 80.1% compared to the control.

In 2018, a significant ($p \leq 0.05$) increase in the field germination of wheat was noted for the multifunctional complexes Vitaplan, CL + Chitosan II and Vitaplan, SC + Chitosan II, as well as the biological product Vitaplan, CL (Fig. 2). The maximum increase in field germination was recorded when using the multifunctional complex: Vitaplan, CL + Chitosan II (in 2018 by 20.8%, for the period 2016-2018 by 19.6% compared to the control, $p \leq 0.05$). Perhaps this is due to the most pronounced reduction in the development of helminthosporium rot on wheat compared to other experimental options (in 2018 by 13.7%, in 2016-2018 by 24.8% compared to the control; p ≤ 0, 05).

The potential productivity of wheat was, to an average extent, positively correlated with root mass ($r$ = 0.46; p = 0.005E-8) (Fig. 3, A) and flag leaf area ($r$ = 0.40; p = 0.006E-11 (see Fig. 3, B) The maximum values of root mass were recorded for the multifunctional complex Vitaplan, CL + Chitosan II.

**2. Morphometric parameters of productivity (roots) in spring soft wheat (*Triticum aestivum* L.) variety Leningradka 6 treated with biologicals and multifunctional complexes based on *Bacillus subtilis* strains and chitosan** ($n$ = 51, $N$ = 3, $M$±SEM; experimental field of the Federal Research Center Vavilov All-Russian Institute of Plant Genetic Resources, St. Petersburg-Pushkin, 2016-2018)

| Treatments | Parameter | Cource of development | | Plant height | | Root number | | Root length | | Nodal root mumber | | Nodal root length | | Root weight | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | score | to control, % | cm | to control, % | total | to control, % | mm | to control, % | total | to control, % | mm | to control, % | g | to control, % |
| Conrol | *M* | 51.0 | | 48.4 | | 4.9 | | 73.2 | | 9.6 | | 58.0 | | 0.5 | |
| (water) | ±SEM | 1.5 | | 2.7 | | 0.2 | | 2.6 | | 0.6 | | 2.2 | | 0.0 | |
| Vitaplan, CL | *M* | 52.4 | 2.7 | 51.4 | 6.1 | 5.5 | 14.1 | 85.6 | 17.0 | 9.4 | –2.0 | 59.3 | 2.3 | 0.6 | 11.5 |
| | ±SEM | 1.9 | | 3.2 | | 0.3 | | 2.9 | | 0.6 | | 2.0 | | 0.1 | |
| Vitaplan, CL | *M* | 56.2 | 10.2* | 60.2 | 24.3* | 5.3 | 9.5 | 81.2 | 11.0* | 10.1 | 5.9 | 58.6 | 1.1 | 0.7 | 50.6* |
| + Chitozan II | ±SEM | 1.3 | | 3.2 | | 0.3 | | 2.3 | | 0.7 | | 2.4 | | 0.1 | |
| Vitaplan, WP | *M* | 52.4 | 2.8 | 53.8 | 11.0 | 4.9 | 1.1 | 74.2 | 1.3 | 10.2 | 6.6 | 63.7 | 9.9 | 0.7 | 35.6 |
| | ±SEM | 1.9 | | 3.7 | | 0.3 | | 3.2 | | 0.6 | | 2.7 | | 0.2 | |
| Vitaplan, SC | *M* | 48.9 | –4.1 | 37.6 | –22.3 | 4.8 | –0.8 | 67.3 | –8.1 | 8.9 | -7.3 | 48.0 | –17.2* | 0.3 | –32.1* |
| + Chitozan II | ±SEM | 2.0 | | 3.2 | | 0.2 | | 3.5 | | 0.4 | | 1.9 | | 0.0 | |

N o t e. For a description of the drugs, see the Materiasl and methods section.

* Differences from control are statistically significant according to Student's $t$-test at $p < 0.05$.

**3. Morphometric parameters of productivity (abovt-ground part) in spring soft wheat (*Triticum aestivum* L.) variety Leningradka 6 treated with biologicals and multifunctional complexes based on *Bacillus subtilis* strains and chitosan** ($n$ = 51, $N$ = 3, $M$±SEM; experimental field of the Federal Research Center Vavilov All-Russian Institute of Plant Genetic Resources, St. Petersburg-Pushkin, 2016-2018)

| Treatments | Parameter | Flag leaf area | | Pre-flag leaf area | | Vegetative part weight | | Ear leangth | | Spikelet number per ear | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | $cm^2$ | to control, % | $cm^2$ | to control, % | g | to control, % | mm | to control, % | total | to control, % |
| Conrol (water) | *M* | 3.8 | | 4.3 | | 2.2 | | 63.2 | | 13.1 | |
| | ±SEM | 0.3 | | 0.3 | | 0.2 | | 1.6 | | 0.2 | |
| Vitaplan, CL | *M* | 4.8 | 27.1 | 4.7 | 9.1 | 2.4 | 8.1 | 57.7 | –8.7 | 13.2 | 1.2 |
| | ±SEM | 0.5 | | 0.4 | | 0.2 | | 1.7 | | 0.3 | |
| Vitaplan, CL + Chitozan II | *M* | 5.0 | 30.9* | 4.5 | 5.1 | 2.5 | 14.1 | 64.6 | 2.1 | 13.8* | 6.1* |
| | ±SEM | 0.3 | | 0.3 | | 0.2 | | 2.4 | | 0.2 | |
| Vitaplan, WP | *M* | 5.4 | 42.6* | 4.7 | 9.8 | 3.0 | 33.6* | 60.2 | –4.9 | 13.8 | 6.0 |
| | ±SEM | 0.6 | | 0.5 | | 0.3 | | 2.3 | | 0.3 | |
| Vitaplan, SC + Chitozan II | *M* | 2.3 | –40.6* | 2.5 | –40.8* | 2.0 | -8.9 | 62.3 | –1.4 | 11.5 | –11.6 |
| | ±SEM | 0.1 | | 0.2 | | 0.2 | | 2.9 | | 0.2 | |

N o t e. For a description of the drugs, see the Materiasl and methods section.
* Differences from control are statistically significant according to Student's $t$-test at $p < 0.05$.

**4. Morphometric parameters of productivity (ear structure) in spring soft wheat (*Triticum aestivum* L.) variety Leningradka 6 treated with biologicals and multifunctional complexes based on *Bacillus subtilis* strains and chitosan** ($n$ = 60, $N$ = 3, $M$±SEM; experimental field of the Federal Research Center Vavilov All-Russian Institute of Plant Genetic Resources, St. Petersburg-Pushkin, 2016-2018)

| Treatments | Parameter | Ear weight | | Grain number per ear | | Grain weight per ear | | 1000-grain weight | | Ear weigh wigh grain | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | g | to control, % | total | to control, % | g | to control, % | g | to control, % | g | to control, % |
| Conrol (water) | *M* | 0,6 | | 28,9 | | 0,9 | | 30,7 | | 1,1 | |
| | ±SEM | 0,0 | | 0,7 | | 0,0 | | 0,9 | | 0,0 | |
| Vitaplan, CL | *M* | 0,6 | –2,6 | 30,1 | 4,3 | 0,9 | 5,8 | 31,1 | 1,3 | 1,3 | 12,5 |
| | ±SEM | 0,0 | | 1,0 | | 0,0 | | 1,1 | | 0,1 | |
| Vitaplan, CL + Chitozan II | *M* | 0,6 | –5,1 | 28,3 | –1,8 | 0,8 | –6,1 | 28,6 | –6,8 | 1,1 | -3,1 |
| | ±SEM | 0,0 | | 0,8 | | 0,0 | | 1,0 | | 0,0 | |
| Vitaplan, WP | *M* | 0,8 | 27,3 | 31,3 | 8,6 | 1,0 | 13,7* | 31,0 | 1,0 | 1,3 | 19,5* |
| | ±SEM | 0,1 | | 1,0 | | 0,0 | | 1,2 | | 0,0 | |
| Vitaplan, SC + Chitozan II | *M* | 0,5 | –22,7* | 28,4 | –1,6 | 0,9 | 4,6 | 33,4 | 8,8* | 1,2 | 7,9 |
| | ±SEM | 0,0 | | 0,7 | | 0,0 | | 0,4 | | 0,0 | |

N o t e. For a description of the drugs, see the Materiasl and methods section.
* Differences from control are statistically significant according to Student's $t$-test at $p < 0.05$.

**5. Chlorophylls a and b content in flag leaves of spring soft wheat (*Triticum aestivum* L.) variety Leningradka 6 treated with biologicals and multifunctional complexes based on *Bacillus subtilis* strains and chitosan** ($n$ = 17, $N$ = 3, $M$±SEM; experimental field of the Federal Research Center Vavilov All-Russian Institute of Plant Genetic Resources, St. Petersburg-Pushkin, 2018)

| Treatments | 1 | 2 | 3 | | 4 | 5 | 2 | 3 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Conrol (water) | 1.13±0.07 | | 0.99 | 1.26 | | 2.06±0.20 | | 1.67 | 2.45 | | 0.55 |
| Vitaplan, CL | 1.13±0.10 | 0.06 | 0.94 | 1.33 | 0.69 | 2.07±0.12 | 0.05 | 1.84 | 2.30 | 0.58 | 0.55 |
| Vitaplan, CL + Chitozan II | 1.32±0.02 | 2.55* | 1.27 | 1.36 | 16.72 | 2.15±0.04 | 0.43 | 2.07 | 2.23 | 4.30 | 0.61 |
| Vitaplan, WP | 1.05±0.20 | –0.36 | 0.67 | 1.44 | –6.62 | 1.99±0.40 | –0.15 | 1.20 | 2.78 | –3.24 | 0.53 |
| Vitaplan, SC + Chitozan II | 0.96±0.17 | –0.90 | 0.64 | 1.29 | –14.38 | 1.63±0.27 | –1.26 | 1.10 | 2.16 | –20.70 | 0.59 |

N o t e. 1 — chlorophyll a, mg/g; 2 — Student's $t$-test; 3 — confidence interval at a 5% level of significance; 4 — change in chlorophyll a content vs. control, %; 5 — chlorophyll b, mg/g мг/г; 6 — change in chlorophyll b content vs. control, %; 7 — chlorophyll a/chlorophyll b; 8 — change in chlorophyll a/chlorophyll b vs. control, %. For a description of the drugs, see the Materiasl and methods section.

* Differences from control are statistically significant according to Student's $t$-test at $p < 0.05$.

**6. Chlorophylls a + b to carotenoids concentration in flag leaves of spring soft wheat (*Triticum aestivum* L.) variety Leningradka 6 treated with biologicals and multifunctional complexes based on *Bacillus subtilis* strains and chitosan** ($n$ = 17, $N$ = 3, $M$±SEM; experimental field of the Federal Research Center Vavilov All-Russian Institute of Plant Genetic Resources, St. Petersburg-Pushkin, 2018)

| Treatments | Chlorophylls a + b to carotenoids | Student's $t$-test | Confidence interval at a 5% level of significance | Change in chlorophyll a + b/carotenoids vs. control, % |
|---|---|---|---|---|
| Conrol (water) | 7.60±2.30 | | 3.09–12.11 | |
| Vitaplan, CL | 10.80±4.20 | 0.67 | 2.57–19.03 | 42.09 |
| Vitaplan, CL + Chitozan II | 11.80±2.23 | 1.31 | 7.42–16.17 | 55.22 |
| Vitaplan, WP | 6.92±1.62 | –0.24 | 3.74–10.10 | –8.92 |
| Vitaplan, SC + Chitozan II | 8.26±0.87 | 0.27 | 6.56–9.97 | 8.73 |

N o t e. For a description of the drugs, see the Materiasl and methods section.

**7. Brown rust, powdery mildew and septoria damage to flag leaves of spring soft wheat (*Triticum aestivum* L.) variety Leningradka 6 treated with biologicals and multifunctional complexes based on *Bacillus subtilis* strains and chitosan** ($n = 60$, $N = 3$, $M \pm$SEM; experimental field of the Federal Research Center Vavilov All-Russian Institute of Plant Genetic Resources, St. Petersburg-Pushkin, 2016-2018)

| Treatments | Parameter | Brown rust | | | | | | Powdery mildew | | | | | | Septoria | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | damage | | number of pustules | | pustule area | | damage | | spot number | | spot area | | | |
| | | % | to control, % | total | to control, % | mm$^2$ | to control, % | % | to control, % | total | to control, % | mm$^2$ | to control, % | % | to control, % |
| Conrol (water) | *M* | 12.5 | | 81.6 | | 0.08934 | | 5.8 | | 7.6 | | 3.6 | | 17.5 | |
| | ±SEM | 3.8 | | 24.6 | | 0.00880 | | 1.9 | | 2.3 | | 1.0 | | 2.5 | |
| Vitaplan, CL | *M* | 7.6 | –4.9 | 133.2 | 63.2 | 0.04766 | –46.7* | 1.8 | -4.0 | 2.8 | –63.3 | 1.3 | –63.0 | 7.5 | –10.0 |
| | ±SEM | 1.8 | | 49.0 | | 0.00524 | | 0.8 | | 1.4 | | 0.4 | | 2.5 | |
| Vitaplan, CL + | *M* | 4.8 | –7.7 | 42.2 | –48.3 | 0.07286 | –18.5 | 3.8 | –2.0 | 5.5 | –27.9 | 2.5 | –29.2 | 2.0 | –15.5 |
| Chitozan II | ±SEM | 1.3 | | 22.6 | | 0.01149 | | 1.4 | | 1.7 | | 0.4 | | 1.0 | |
| Vitaplan, WP | *M* | 10.2 | -2.4 | 52.2 | –36.0 | 0.10333 | 15.7 | 2.9 | –2.9 | 3.1 | –59.0 | 4.3 | 21.7 | 0.0 | –17.5* |
| | ±SEM | 5.0 | | 33.2 | | 0.02187 | | 1.1 | | 1.0 | | 1.3 | | 0.0 | |
| Vitaplan, SC + | *M* | 4.1 | –8.5* | 15.1 | –81.5* | 0.05016 | –43.9* | 1.0 | –4.8* | 1.0 | –86.9* | 5.5 | 53.9 | 0.0 | –17.5* |
| Chitozan II | ±SEM | 1.2 | | 5.0 | | 0.00688 | | 0.2 | | 0.1 | | | | 0.0 | |

N o t e. For a description of the drugs, see the Materiasl and methods section.
* Differences from control are statistically significant according to Student's *t*-test at $p < 0.05$.

**8. Powdery mildew and septoria damage to pre-flag leaves and root rot of spring soft wheat (*Triticum aestivum* L.) variety Leningradka 6 treated with biologicals and multifunctional complexes based on *Bacillus subtilis* strains and chitosan** ($n$ = 60, $N$ = 3, $M$±SEM; experimental field of the Federal Research Center Vavilov All-Russian Institute of Plant Genetic Resources, St. Petersburg-Pushkin, 2016-2018)

| Treatments | Parameter | Powdery mildew | | | | | | Septoria | | Root rot | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | damage | | spot number | | spot area | | | | | |
| | | % | to control, % | total | to control, % | mm$^2$ | to control, % | % | to control, % | % | to control, %% |
| Conrol (water) | *M* | 22.0 | | 23.1 | | 3.3 | | 31.5 | | 39.7 | |
| | ±SEM | 5.0 | | 4.7 | | 0.5 | | 8.4 | | 2.9 | |
| Vitaplan, CL | *M* | 24.2 | 2.2 | 22.3 | –3.3 | 2.9 | –11.8 | 21.9 | –9.6 | 19.7 | –20.0* |
| | ±SEM | 5.8 | | 3.8 | | 0.4 | | 7.8 | | 5.5 | |
| Vitaplan, CL + Chitozan II | *M* | 18.6 | –3.4 | 20.6 | –10.7 | 2.8 | –14.4 | 8.8 | –22.8* | 14.9 | –24.8* |
| | ±SEM | 4.3 | | 3.9 | | 0.3 | | 2.7 | | 6.5 | |
| Vitaplan, WP | *M* | 30.5 | 8.5 | 43.7 | 88.9 | 4.8 | 45.4* | 6.3 | –25.2* | 33.8 | –5.9 |
| | ±SEM | 5.9 | | 13.4 | | 0.5 | | 3.1 | | 6.2 | |
| Vitaplan, SC + Chitozan II | *M* | 0.0 | –22.0* | 0.0 | –100.0* | 0.0 | –100.0* | 15.9 | –15.6 | 28.3 | –11.3* |
| | ±SEM | 0.0 | | 0.0 | | 0.0 | | 8.0 | | 4.7 | |

N o t e. For a description of the drugs, see the Materiasl and methods section.
* Differences from control are statistically significant according to Student's $t$-test at $p < 0.05$.

**9. Yellow rust damage to flag leaves of spring soft wheat (*Triticum aestivum* L.) variety Leningradka 6 treated with biologicals and multifunctional complexes based on *Bacillus subtilis* strains and chitosan** ($n$ = 60, $N$ = 3, $M$±SEM; experimental field of the Federal Research Center Vavilov All-Russian Institute of Plant Genetic Resources, St. Petersburg-Pushkin, 2018)

| Treatments | Parameter | Damage | | Strip number | | Strip length | | Pustules | | | | Pustule area | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | number per strip | | sum of pustules | | | |
| | | % | to control, % | total | to control, % | mm | to control, % | total | to control, % | total | to control, % | $mm^2$ | to control, % |
| Control (water) | *M* | 9.5 | | 2.2 | | 40.9 | | 102.7 | | 221.7 | | 0.02312 | |
| | ±SEM | 3.3 | | 0.5 | | 7.6 | | 26.4 | | 97.8 | | 0.00391 | |
| Vitaplan, CL | *M* | 5.7 | –3.8 | 2.6 | 18.2 | 31.9 | –22.0 | 66.0 | –35.7 | 168.8 | –23.9 | 0.02329 | 0.7 |
| | ±SEM | 1.4 | | 0.5 | | 2.6 | | 6.0 | | 45.4 | | 0.00183 | |
| Vitaplan, CL + Chitozan II | *M* | 2.8 | –6.7 | 1.4 | –36.4 | 28.5 | –30.3 | 58.1 | –43.4 | 106.6 | –51.9 | 0.01327 | –42.6* |
| | ±SEM | 1.8 | | 0.3 | | 3.4 | | 6.8 | | 28.5 | | 0.00143 | |
| Vitaplan, WP | *wp* | 7.7 | –1.8 | 3.3 | 50.0 | 18.2 | –55.5* | 41.9 | –59.2* | 148.3 | –33.1 | 0.01653 | –28.5 |
| | ±SEM | 4.1 | | 1.1 | | 1.7 | | 6.4 | | 77.6 | | 0.00341 | |
| Vitaplan, SC + Chitozan II | *M* | 2.3 | –7.2 | 1.3 | –40.9 | 26.3 | –35.7 | 62.3 | –39.3 | 83.0 | –62.6 | 0.01022 | –55.8* |
| | ±SEM | 1.3 | | 0.3 | | 8.0 | | 32.8 | | 38.6 | | 0.00086 | |

N o t e. For a description of the drugs, see the Materiasl and methods section.
* Differences from control are statistically significant according to Student's $t$-test at $p < 0.05$.

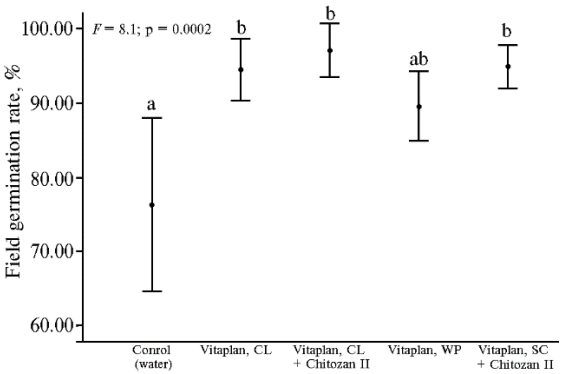


**Fig. 2. Field germination of spring soft wheat (*Triticum aestivum* L.) variety Leningradka 6 treated with biologicals and multifunctional complexes based on *Bacillus subtilis* strains and chitosan** ($n$ = 1050, $N$ = 3, $M \pm t_{05}$SEM; experimental field of the Federal Research Center Vavilov All-Russian Institute of Plant Genetic Resources, St. Petersburg-Pushkin, 2016-2018). For a description of the drugs, see the Materiasl and methods section. Identical letters indicate values that do not differ from the control and from each other according to the Scheffe's test at p < 0.05; $F$ is Fisher's test.

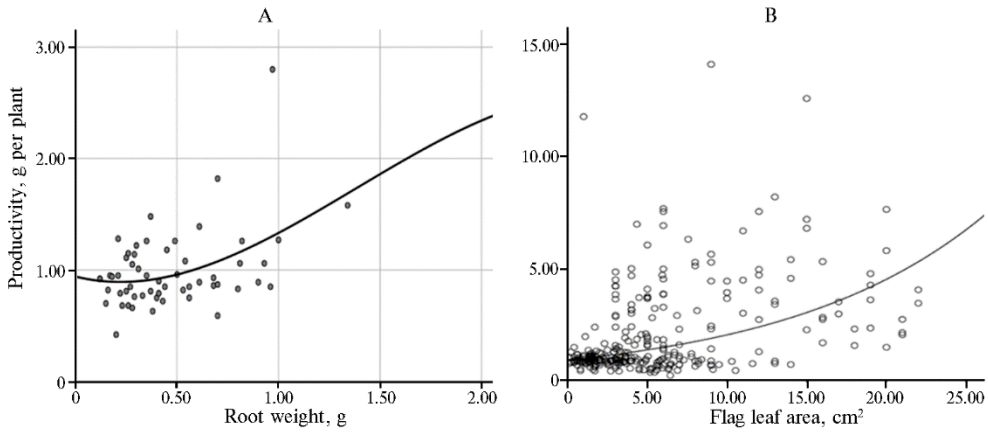


**Fig. 3. Regression dependence of potential productivity to root mass** (A, $W_r$) **and flag leaf area** (B, $S_{flag\ leaf}$) **of spring soft wheat (*Triticum aestivum* L.) variety Leningradka 6 withou trearments with biologicals and multifunctional complexes based on *Bacillus subtilis* strains and chitosan (control)** ($n$ = 17, $N$ = 3, $M$±SEM; experimental field of the Federal Research Center Vavilov All-Russian Institute of Plant Genetic Resources, St. Petersburg-Pushkin, 2018). For a description of the drugs, see the Materiasl and methods section. The resulting regression equations:

A — $P_p = 0.94 - 0.45W_r + 1.11W_r^2 - 0.27\ W_r^3$ ($r$ = 0.46; p = 0.005E–8);

B — $P_p = 0.807S_{flag\ leaf} - 0.160S_{flag\ leaf}^2 + 0.0085S_{flag\ leaf}^3$ ($r$ = 0.82; p = 1.1661E–45).

In 2018, with the Vitaplan complex, CL + Chitosan II, maximum chlorophyll a in flag leaves in the flowering stage occurred, 1.32±0.02 mg/g (16.7% more than in the control, p ≤ 0.05) (Table 5). The content of chlorophyll b changed slightly, but was 4.3% higher compared to the control (see Table 5). For Vitaplan, CL + Chitosan II, there was the greatest change in the ratio between the content of chlorophylls a, b and carotenoids (by 55.2%) vs. control (Table 6). Some authors believe that the ratio of the content of chlorophylls and carotenoids may be one of the indicators of resistance to external unfavorable factors and reflect the ecological plasticity of plants [70].

Nonparametric correlation analysis revealed that as the content of chlorophyll b (Chl b) in the flag leaves increased, there was an increase in the number of grains per ear (Spearman correlation coefficient $r$ = 0.79; p = 0.006), in the weight of grains per ear ($r$ = 0.69; p = 0.03); in ear weight with grains (r = 0.72; p = 0.02) and in overall potential yield (r = 0.69; p = 0.03). The dependence of the change in the mass of the ear with grains on the content of chlorophyll b in the flag leaves of wheat can be described by the regression equation: $M_e = 0.26$Chl b + 0.81; $R^2 = 0.45$ (Fig. 4).

The main diseases during the period of phytosanitary monitoring of wheat crops (2016-2018) were helminthosporium root rot caused by the fungus *Bipolaris*

*sorokiniana* (Sacc.) Shoemaker, brown rust (*Puccinia triticina* Eriks.), yellow rust (*P. striiformes* Westend); powdery mildew (*Blumeria graminis* (DC.) Speer.), septoria caused by *Stagonospora nodorum* (Berk.) Castellani & E.G. Germano and *Zymoseptoria tritici* (Desm.) Quaedvlieg & Crous.

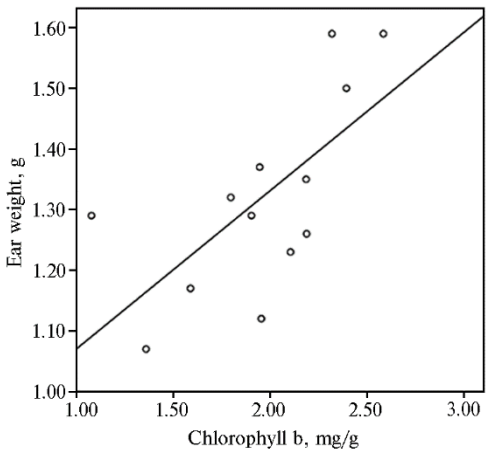


**Fig. 4. Dependence of changes in the weight of the ear with grain vs. the content of chlorophyll b in flag leaves of spring soft wheat (*Triticum aestivum* L.) variety Leningradka 6 withou trearments with biologicals and multifunctional complexes based on *Bacillus subtilis* strains and chitosan** (control) ($n$ = 13, $N$ = 3; experimental field of the Federal Research Center Vavilov All-Russian Institute of Plant Genetic Resources, St. Petersburg-Pushkin, 2018).

Multifunctional complexes significantly influenced the development of wheat diseases. According to the data obtained (Tables 7, 8), during the observation, the complex Vitaplan, CL and Chitosan II significantly ($p \leq 0.05$) reduced the root rot (by24.8%) and septoria infection on pre-flag leaves (by 22.8 %) compared to the control. The multifunctional complex Vitaplan, SC + Chitosan II significantly ($p \leq 0.05$) reduced the incidence of root rot in wheat (by 11.3%). The development of leaf rust decreased by 8.5%, the number of uredopustules by 81.5%, and the area of uredopustule by 43.9% ($p \leq 0.05$). When affected by powdery mildew, the number of spots on flag leaves decreased by 86.9%. We did not identify any symptoms of powdery mildew development on pre-flag leaves.

In 2018, for multifunctional complexes Vitaplan, CL + Chitosan II and Vitaplan, SC + Chitosan II, we noted that the intensity of development of yellow rust on wheat (R), the number of pustules ($N$) and the area of pustules ($S$) were minimal, R = 2.8±1.7%, N = 106.6±28.5, S = 0.013±0.001 mm$^2$ and R = 2.3±1.3%, N = 83.0±38.5, S = 0.010±0.0009 mm2, respectively) vs. control, R = 9.5±3.3%, $N$ = 221.7±97.8, $S$ = 0.023±0.004 mm$^2$ (Table 9). The most pronounced effect was on the area of the pustule, which decreased compared to the control.

Plants are subject to the negative effects of stress factors of various natures throughout the growing season, which leads to a decrease in productivity due to inhibition of growth and photosynthesis. Many researchers note a significant decrease in the photosynthetic activity of plants when attacked by phytopathogenic fungi, which is associated with a decrease in the assimilation surface due to the death of leaf tissue or the growth of mycelium, with the destruction of chloroplasts, a decrease in chlorophyll content, and a violation of the outflow of photosynthetic products due to damage to the phloem [71, 72].

The results of our research showed that the plant treatment with multifunctional complexes significantly reduced the incidence of diseases in wheat, which had a positive effect on the chlorophyll content in the leaves. The content of chlorophylls a and b in wheat leaves increased with a decrease in the degree of yellow rust development as a percentage on the Manners scale (for chlorophyll a $r$ = –0.66; p = 0.04; for chlorophyll b $r$ = –0.87; p = 0.005), a decrease in the number of stripes ($r$ = –0.79; p = 0.02 and $r$ = –0.63; p = 0.04, respectively) and the number of yellow rust pustules (r = –0.73; p = 0.04 and $r$ = –0.97; p = 0.00007). The equations that describe the dependence of the content of chlorophylls in flag leaves on the intensity of yellow rust development are for Chl a R = –37.03 + 52.72Chl a, $R^2$ = 0.60, for Chl b R = 144.52 – 108.23Chl b + 20.53Chl b$^2$, $R^2$ = 0.81.

Thus, as a result of the studies, a statistically significant increase in the content of photosynthetic pigments (chlorophyll a in flag leaves of wheat) and a slight increase in

chlorophyll b were revealed when using biological products. When using the multifunctional complex Vitaplan, CL + Chitosan II, the highest potential productivity and the highest content of chlorophylls a and b in the leaves occurred. A correlation was revealed between an increase in the content of chlorophylls a and b in leaves and a decrease in the intensity of development of yellow rust. Based on the Spearman criterion, the strongest correlations between the chlorophyll b content and the weight of the ear, the weight of grains per ear and the number of grains in the ear are shown.

The observed positive effect of biological products and multifunctional complexes may be associated with the ability of beneficial microorganisms to synthesize complex bioactive complexes, including antibiotics of various chemical classes, enzymes, metabolites with signaling and hormonal functions, phytohormones, which can have a significant effect influence on photosynthetic function, growth and productivity of plants [9-11]. Thus, the stimulating effect of synthetic auxin (indolyl-3-butyric acid, IBA) and cytokinin (6-benzylaminopurine, BAP) on the accumulation of plant biomass, net productivity of photosynthesis, and the functioning of the photosynthetic apparatus of corn has been established [73]. It has been shown that gibberellin enhances the processes of photosynthetic phosphorylation, while the chlorophyll content decreases. Thus, the intensity of chlorophyll use per quantity increases under the influence of gibberellin; in addition, the assimilation number increases [9]. Many bacteria of the genera Bacillus, Azospirillium, and Pseudomonas, as already noted, synthesize auxins that stimulate the development of the root system. Together, these processes increase plant disease resistance [74]. Many strains of bacteria of the genus Bacillus can synthesize gibberellin [75]. Bacteria of the genera *Bacillus*, *Rhizobium*, *Arthtrobacter*, *Azotobacter*, Azospi*ri*llium, and *Pseudomonas* are capable of producing cytokinins. When inoculated with cytokinin-producing strains of *B. subtilis*, the content of chlorophyll and cytokinins in plants increased, which subsequently caused an increase in the biomass of the root system and vegetative part of the plant. Strains of *Bacillus*, *Brevibacterium*, *Azospirillum*, *Pseudomonas*, *Lysinibacillus* were found to be able to synthesize abscisic acid and influence its content in plants, which caused optimization of endogenous hormonal balance [76-79].

*B. subtilis* strains, which form the basis of Vitaplan, produce a variety of antimicrobial metabolites, the lipopeptides and polypeptides [80], which largely determines the fungicidal effect of the biological product against particularly dangerous phytopathogenic fungi. The biological activity of chitosan is determined by its ability to induce biochemical (signaling) pathways leading to the activation of defense reactions and increasing plant resistance to diseases [33-36]. It was reported that chitosan stimulates growth and development, increases the yield of many agricultural plants, e.g., corn [81-83], legumes [84], wheat [85], and rice [86]. It has been convincingly shown that chitosan treatment increases the rate of photosynthesis and chlorophyll content in rice and soybean plants [87, 88], corn [38], cowpea [89], beans [39], tomato [40], and wheat [37]. It is possible that chitosan can be a carbon source for the formation of antioxidants [90]. Although the exact mechanisms of chitosan's influence on photosynthesis have not been established, some studies have shown that in maize, chitosan and its derivatives improved photosynthesis and chlorophyll fluorescence, increasing stomatal activity, transpiration rate, and PSII activity [38]. There are reports that chitosan increases the endogenous level of cytokinins that stimulate chlorophyll synthesis [83). Some researchers associate the increase in yield when using chitosan with the effect of stimulating physiological processes and the subsequent active movement of photoassimilates into the tissues that consume them. This effect was noted in corn [38], beans [39], soybeans [87], cowpea [89], tomato [40], rice [41], and cucumber [91].

Thus, all mechanisms of action of beneficial microorganisms in combination with the biological activity of chitosan, described in the above scientific publications, are capable of optimizing the physiological state of plants by increasing the rate of photosynthesis, stimulating growth and development, which leads to increased stress and disease resistance - vigor and, as a consequence, crop yields.

The results of the studies suggest that the effect of the multifunctional complexes Vitaplan, CL + Chitosan II and Vitaplan, SC + Chitosan II is due to the mechanisms discussed above. It is reflected in a high protection against fungal infection, enhanced growth processes, stimulation of the reproductive properties of plants, increased content of photosynthetic pigments and, ultimately, in increasing the potential productivity of wheat.

In the compositions we have developed, the combination of an inhibitory effect on phytopathogenic microorganisms and a stimulating effect on plants provides higher efficiency and reliable protection compared to biological products. In our opinion, complex mechanisms for increasing disease resistance and ensuring stable plant productivity include adaptive reactions that involve the photosynthetic apparatus and the entire system of photosynthetic pigments. It can be assumed that this mechanism is universal, but the effectiveness of such compositions may depend on the biological characteristics of microorganism strains and the properties of inducers, as well as on the resistance of cultivated plant varieties.

So, it has been shown that the multifunctional complexes Vitaplan, CL + Chitosan II and Vitaplan, SC + Chitosan II optimize the physiological state of wheat plants, significantly increase its productivity and disease resistance (the incidence of plants with a complex of diseases decreased by 17.9%, $p < 0.05$). Plant treatment with the Vitaplan complex, CL + Chitosan II leads to the highest potential wheat productivity (0.94 ± 0.02 g/plant). The Vitaplan complex, SC + Chitosan II provides the least damage to plants by the disease complex. In addition, in these variants the highest content of chlorophylls a and b in the leaves occures. For the Vitaplan, CL + Chitosan II, it was 1.32±0.02 mg/g for chlorophyll a and 2.15±0.04 mg/g for chlorophyll b. For the complex Vitaplan, CL + Chitosan II, the ratio chlorophylls a, b and carotenoid pigments, which serves as one of the indicators of plant stress resistance, was also maximum. The strongest correlation was found between the content of chlorophyll b in flag leaves and wheat productivity ($r = 0.69$, $p = 0.03$), the content of chlorophyll b in flag leaves and the number of grains per ear ($r = 0.79$, $p = 006$), grain weight per ear and ear weight ($r = 0.69$, $p = 0.03$; $r = 0.72$, $p = 0.02$). A decrease in the development of yellow rust correlated цшер an increase in the content of chlorophylls a and b in the leaves ($r = -0.66$, $p = 0.04$; $r = -0.87$, $p = 0.005$). Our study shows that the multifunctional compositions based on the selected strains of bacterial antagonists of plant pathogens and inducers of disease resistance significantly reduces the incidence of a complex of diseases in wheat plants and has a positive effect on the content of photosynthetic pigments (chlorophylls a, b and carotenoids) and plant productivity.

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