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Increasing Wheat Productivity and Disease Resistance through Combined Use of Polymer Hydrogel and Protein Hydrolysates with Varied Composition and Molecular Weight

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Abstract: This study explores the effectiveness of protein hydrolysates derived from by-products of farm animals' processing. In previous research, we utilized protein hydrolysates with amino acids rich in glycine and with peptides of molecular weight ranging from 200 to 1000 Da. Subsequently, we modified the production process, resulting in a new hydrolysate from by-products of meat, chicken, and fish processing, with a molecular weight of 700,000 Da. During the period 2021–2022, we conducted a comparative analysis of protein hydrolysates of diverse nature, both independently and as components of multifunctional compositions with acrylic hydrogel. This analysis focused on their impact on productivity metrics and plant disease severity. Our experimental approach involved pre-soaking wheat seeds in the new protein hydrolysate before sowing, while introducing acrylic hydrogel into the soil at rates of 6 g/m² or 60 kg/ha. This regimen led to a remarkable increase in wheat yield (73.3% higher than the control group). The improvement was attributed to a boost in field germination of wheat seeds by 21.9%, an elevation in plant height by 17.0%, an increment in the number of spikelets per spike by 7.8%, an enlargement of preflag leaf area by 36.0%, and a reduction in the incidence of critical diseases such as brown rust (21.7%), yellow rust (28.3%), and root rot (5.6%). Our findings underscore the influence of hydrolysate molecular weight and the composition of acrylic hydrogel on their efficacy. Furthermore, these factors contribute to the economic viability of their application in wheat cultivation.

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1. Introduction

Currently, the development of effective technologies for crops production based on principles of resource conservation and the implementing requirements for adaptive landscape and precision agriculture is continuing. Physical characteristics of soils can be improved by using polymer hydrogels that bind and preserve water inside it, retain nutrients, and reduce soil erosion [1]. Utilising biohydrolysates (protein hydrolysates, PH; biostimulants, PBs) allows to significantly increase crop productivity and grain quality [2], as well, reducing negative effects of abiotic and biotic stresses via using

biohydrolysates (protein hydrolysates, PH; biostimulants, PBs) obtained from raw materials of animal or vegetable origin [3] and consisting of a mixture of peptides and amino acids [4].

There is evidence of biostimulants impacts on morphological, physiological and biochemical changes in plants, increasing yield and crop quality [5], plant stress resistance [6,7], on the relationship among photosynthetic, enzymatic activity and accumulating phenolic compounds [8,9]. However, in order to fully achieve the potential of protein hydrolysates, further research is needed, it is required to study the mechanisms that ensure their beneficial effects on plants and determine the optimal amino acid composition and methods for applying in various crop cultivation technologies [4]. So far, the possibilities for using polymer compositions in crops cultivation, including their combined use with biohydrolysates of various nature, have not been fully disclosed.

According to the above, purpose of this work is the biological substantiation of combined use of polymer hydrogel with protein hydrolysates of various compositions and molecular weights in common wheat.

2. MATERIALS AND METHODS

The plant material for the study was the cultivar Leningradskaya 6, k-64900 given by the Department of Wheat Genetic Resources of the Federal Research Center «N. I. Vavilov All-Russian Institute of Plant Genetic Resources» (VIR). The objects of the study were a moisture-absorbing polymer hydrogel composition based on potassium acrylate and methylenebisacrylamide, obtained using a unique technology at the Centre for Chemical Engineering of ITMO University, and protein hydrolysates of various compositions developed at the Faculty of Biotechnologies of ITMO University from products of processing of slaughtered animals. They include polypeptides of different molecular weights and amino acids in various combinations that impact productivity and adaptive potential of crops to cultivation conditions.

Previously, in our studies, a protein hydrolysate from reticular dermis layer of bovine beef (R) was used. A detailed description of the technology for obtaining protein hydrolysates from animal and vegetable by-products, methods for studying the molecular weight distribution of fractions and their amino acid composition are presented in the publication of the journal *Agronomy Research* [10]. The characteristics and technological scheme for synthesizing a composite supermoisture absorbent (hydrogel) and protein hydrolyzate [11] are given in the works [12–14].

In this study, a hydrolysate obtained from hog skin trimmings (RM) was used. Its distinctive feature in relation to previously used (R) is not only the type of raw material, but also the molecular weight, the composition of amino acids. Taking into account the multifactorial variability of the composition of processed animal raw materials, the content of glycine was re-analysed, which in our case plays a decisive role in regulating plants growth and development. Protein hydrolysate RM, which was treated with wheat plants in 2021-2022, had a molecular weight of 700,000 Da, with a glycine content of 17% more relative to R.

Previously, the experiment technique was applying hydrogel and protein hydrolysate into soil before sowing (presowing introduction), both individually and in various combinations [13,14]. In the presented results for 2021-2022, experimental methodology was altered. The polymer hydrogel was applied to soil in a dry form before sowing wheat, and wheat seeds were pre-soaked in solutions of protein hydrolysates at a concentration of 0.195 g/l.

Experiment scheme in 2021-2022 was as following options, placed systematically in triplicate: Without applying treatments into soil (control)–K; Hydrogel (in terms of ability for binding 200 ml of moisture in the soil), 3 g per 1 m² or 30 kg / ha–0.5G; Hydrogel (in terms of ability for binding 400 ml of moisture in the soil), 6 g per 1 m² or 60 kg/ha–1G; Hydrogel, 3 g per 1 m² or 30 kg/ha + seeds soaked in protein hydrolyzate–0.5G:1R; Hydrogel, 6 g per 1 m² or 60 kg/ha + seeds soaked in protein hydrolyzate, BSR–1G:2R;

Hydrogel, 3 g per 1 m² or 30 kg/ha + seeds soaked in protein hydrolyzate–0.5G:1RM; Hydrogel, 6 g per 1 m² or 60 kg/ha + seeds soaked in protein hydrolyzate–1G:2RM.

Wheat productivity was studied in developing phases of germ shoot, heading-flowering and ripening according to a set of indicators [13,14]. The severity of wheat damage by diseases was determined by using both the generally accepted criterion–disease development, and additional ones. In particular, damage to flag leaf by leaf rust (*Puccinia recondita* Rob. ex Desm. f. sp. *tritici* Eriks.) was characterized by total number of pustules per leaf and pustules area; yellow rust (*Puccinia striiformis* West.)–number of bands with pustules, length of band with pustules, number of pustules in band, total number of pustules per leaf, pustules area; powdery mildew (*Blumeria graminis* Speer.)–by number and area of plaque spots. The characteristics of pathogenesis indicators were given according to measurements results of wheat leaves in laboratory using MBS-9 binocular and the Micromed-6 trinocular. The area of infectious structures of micromycetes was determined by the ellipse formula [13,14]. Under laboratory conditions, Damage degree to plants by Helminthosporium root rot *Bipolaris sorokiana* (Sacc.) Shoem was assessed in the phases of wheat tillering (finished tillering stage) and heading-flowering in accordance with the generally accepted method [15]. To calculate the biological effectiveness (BE) in terms of reducing wheat affection by pathogens, the Abbott formula was used [16].

When assessing the effect of stimulating treatments on wheat sowing, the significance of differences between the average values of phytometric and phytopathological indicators in experimental variants in comparison with control was determined based on Student's test. The relationship between individual indicators of pathogenesis and productivity elements was studied by the method of non-parametric correlation analysis, based on the calculation of Spearman correlation coefficient.

3. RESULTS AND DISCUSSION

The highest potential wheat yield for the period 2021-2022 ($Y_p = 4.41 \pm 0.47$ t/ha) was detected in the variant 1G:2RM with combined use of a polymer hydrogel and a new protein growth hydrolysate RM (Figure 1). In this variant wheat yield significantly increased by 73.3% compared with control ($Y_p = 2.54 \pm 0.29$ t/ha). A significant rise in potential yield of wheat (by 60.9%) was also registered in the experimental variant 0.5G:1R ($Y_p = 4.09 \pm 0.52$ t/ha).

When studying the dynamics of potential wheat yield, it was found that in 2021, with 1G:2RM treatment, wheat yield increased by 43.6 % and amounted to $Y_p = 4.65 \pm 0.43$ t/ha, and in 2022–by 119.9% ($Y_p = 4.65 \pm 0.43$ t/ha) compared to control ($Y_p = 2.54 \pm 0.29$ t/ha).

A significant enhancement in wheat yield in 1G:2RM compared with the control can be explained by statistically significantly higher values of following indicators: field germination of wheat–by 21.9%, plant height–by 17.0%, spikelets number per spike–by 7.8%, area of pre-flag leaf–by 36.0%. At the same time, the 'weight of 1000 grains of wheat' indicator in the 1G:2R treatment varied greatly over the study years: in 2021 it increased by 13.7%, and in 2022 it decreased by 11.4%. A significant impact on wheat yield with this variant of experiment and had a significant reduction in plant damage by brown rust–by 21.7% and yellow rust–by 28.3%, as well as root rot–by 5.6%. A significant decrease in spots area of powdery mildew on the flag and pre-flag leaves of wheat was noticed–by 77.8% compared with control.

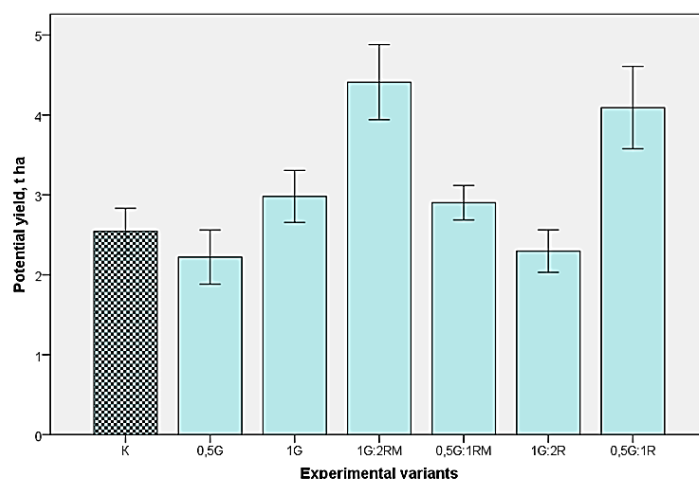


Figure 1. Potential yield of common wheat when using polymer hydrogel and protein growth hydrolysates of various molecular weights 2021-2022.

When using the indicator “biological yield per plant”, calculated without taking into account data on sowing density for the period 2021-2022, its maximum increase by 80.8% ($Yr.p = 3.77 \pm 0.54$ g/plant) compared with control ($Yr.p = 2.09 \pm 0.27$ g/plant) was registered in the experimental treatment 0.5G:1R. The maximum growth of the indicator (by 43.6%) was revealed in 2021 in variant of the 1G:2RM experiment, and in 2022–1G (by 68.5%). In the 0.5G:1R experiment, the increase in wheat yield was 18.2% (2021) and 62.2% (2022).

It should be noticed that on average for the period 2021-2022 in experimental variant 0.5G:1R, a statistically significant increase in the maximum number of phytometric characteristics was observed in comparison with other variants of the study: plant phase–by 2.3%, length of primary roots–by 22.9%, spike length–by 60.6 %, spike weight–by 46.9%, spikelets number per spike–by 16.4%, grains number per spike–by 28.6%, grains weight per spike–by 54.0%, weight of 1000 grains–by 23.5%. At the same time, an increase in wheat susceptibility to brown rust was noticed–by 13.0% and the same damage to plants by root rot as in the control ($Rg = 22\%$).

The maximum increase in grains number per spikelet compared with control was registered in experimental variant with the combined use of protein growth hydrolysate and hydrogel: 0.5G:1R–by 9.5%. In the experimental variants when using only hydrogel: 0.5G and 1G, the index values decreased by 8.9% and 19.6%, respectively.

In most study variants, there was an absence or a statistically significant decrease in the reproductive parts of plants compared with control: 0.5G–by 32.4%, 0.5G: 1RM–by 34.5%, 1G: 2R–by 35.4%. A similar trend can be shown in relation to the general tillering of wheat. Only in the 1G:2RM experimental variant in 2021, a significant increase in overall tillering by 77.6% was revealed.

The values of empty spikelets number per spike in the experimental variants either did not statistically significantly differ from control, or significantly exceeded it. In the experimental variants with utilising polymer hydrogel 0.5G and 1G, the index values exceeded the control by 98.3% and 161.6%, respectively. However, when hydrogel was used together with protein hydrolysate of growth in the 0.5G:1R variant, a significant decrease in empty spikelets number per spike by 69.6% was noted.

In the variant 0.5G:1R the largest number of indicators (50%), which differed statistically significantly in the values of relative change to control were revealed. The remaining variants of experiment can be ranked in descending order of the specified criterion as follows: 1G:2RM (16.7%)–1G:2R (12.5%)–1G and 0.5G (8.3%)–0.5G: 1RM (0%).

The combined use of hydrogel and RM growth protein hydrolysate in the 1G:2RM and 0.5G:1RM variants of the study, compared with the control, caused a significant decrease in severity of brown rust Rb by 15.4%, pustules number Np.b by 59.9% and 59, 4%,

respectively. In addition, a significant decrease in plant susceptibility to leaf rust was noted in the variant where using only hydrogel 1G: Rb-by 17.0%, Np.b-by 83.6%. The average value of pustule area of leaf rust Sp.b significantly decreased by 66.9% in the experimental variant 1G:2RM.

In the 1G:2RM experiment, a statistically significant decrease in the development and area of yellow rust pustules by 28.3% and 38.5%, respectively, was revealed. In this variant of the experiment, a decrease in the number of bands with pustules by 69.9% and total number of pustules by 66.8% was recorded.

Significant decrease in the intensity of development (by 13.3%) and the number of plaque spots (by 34.8%) was determined in the variant of 0.5G:1RM. In 1G:2RM variant, no significant changes in disease development and number of spots with plaque were detected compared to control, however, a significant decrease in spots area with plaque was recorded (by 77.9%). There was a tendency to increase disease development by 71.3% and 10.4%, number of spots with plaque by 332.2% and 126.1% in the 1G and 0.5G:1R variants, respectively. In addition, in the variant of the experiment 1G, severe damage to flag leaves by the larvae of the chickweed was recorded (Rlp = 50%, lesions number Nlp = 33, the length of damage Llp = 27.0±9.1 mm) in contrast to the control (Rlp = 0%).

Severe damage to wheat by root rot was determined in the 1G and 0.5G:1R variants of the study: Rg = 23.3% and Rg = 22.2%, respectively. A significantly lower development of the disease Rg = 3.7% was recorded in experimental variant 0.5G:1RM (BE = 83.3%).

It was found that with an increase in spots area with a powdery mildew coating on the flag and pre-flag leaves, spike length increased ($r = 0.75$; $P = 0.03$). The grains number per spike and spikelets number per spike decreased with an increase in wheat damage by brown rust ($r = -0.52$; $P = 0.04$), with an increase in pustules number ($r = -0.9$; $P = 0.04$) and pustules area ($r = -0.8$; $P = 0.04$) of micromycetes on the flag leaf.

4. CONCLUSIONS

The highest increase in wheat yield compared with control was registered in the experimental variants with the combined use of polymer hydrogel with protein growth hydrolysate (1G:2RM) and with protein growth hydrolysate (0.5G:1R), respectively. The yield increase in the 1G:2RM experiment compared to 1G was 1.4 t/ha (0.1 g/plant), and in the 0.5G:1R experiment compared to 0.5G it was 1.9 t/ha (1.4 g/plant).

The increase in wheat yield in the 1G:2RM treatment was mainly due to a decrease in diseases intensity in wheat: brown rust-by 21.7%, yellow rust-by 28.3%, root rot-by 5.6%. A significant decrease in the area of powdery mildew spots on the flag and pre-flag leaves of wheat was noted-by 77.8% compared with control. In addition, the increase in wheat yield in the indicated variant of our study was caused by an increase in the following indicators: field germination-by 21.9%, plant height-by 17.0%, spikelets number per spike-by 7.8%, the area of pre-flag leaf-by 36.0%. It has been established that the hydrolysate from pork skin trimmings with a molecular weight of 700,000 Da contributes to a significant reduction in plant diseases.

A significant increase in wheat yield compared to control in the 0.5G:1R variant was mainly due to an increase in the largest number of wheat productivity indicators compared to control: plant phase-by 2.3%, length of primary roots-by 22.9%, spike length-by 60.6%, spike weight-by 46.9%, spikelets number per spike- by 16.4%, grains number per spike- by 28.6%, grains weight per spike-by 54.0% , the weight of 1000 grains-by 23.5%. At the same time, the development of root rot and yellow rust on wheat did not differ from the control, the intensity of plant damage by leaf rust and powdery mildew increased by 13.0% and 10.4%, respectively.

The highest biological efficiency (BEg = 83.3% and BEM = 81.5%) was found in relation to the reduction of development of root rot and powdery mildew with the combined use of protein growth hydrolysate and hydrogel in the experimental variant 0.5G:1RM. The wheat yield in this variant did not increase significantly compared to control-the change was 14.1%. The maximum reduction in the number of rust pustules was recorded

when using hydrogel in the 1G treatment (Beg = 83.6%), which caused a slight increase in yield by 17.2%. A significant reduction in wheat susceptibility to yellow rust (disease development–BEf = 68.5%; stripes number–BEpl.zh = 69.9%; pustules number BEp.zh = 69.9%) and powdery mildew (decrease in spots area with a bloom of BEpl m = 77.8%) was recorded in the variant 1G:2RM. It has been suggested that further study on proportion and composition effects of introduced acrylic hydrogel on developing effective technologies for cultivating grain crops, is required.

Thus, molecular weight of hydrolysates, as well as the proportion and composition of acrylic hydrogel, determine not only their properties, but also affect the quality indicators of plant products processed by them. This allows to regulate and influence positively the agrobiological processes of wheat production.

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