Effect of Biopreparations Obtained from Carboxymethylated Plant Raw Material on the Wheat Growth, Crop Capacity, and Biochemical Parameters of Grain

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Abstract—The effect of biopreparations produced by carboxymethylation of plant raw materials on the growth and development of spring wheat has been studied. A presowing treatment of seeds with aqueous solutions of the tested preparations (150 g per 10 L per 1 ton of seeds) has provided some positive effect on the plant growth processes. Activation of the growth and development of wheat has been observed at the early developmental phases, from the germination phase (radicle appearance) to intense tillering phase. The studied preparations provide an increase in the yield of wheat by 30–43% (small-plot experiment), 15% (field experiment), and 21–32% (large-scale experiment). The yield increase in wheat caused by the use of growth regulators is not always accompanied by an increase in the protein and gluten content in grain. A decrease in the protein content in grain, which can be observed, for example, in the case of a nonfallow forecrop and a limited soil nitrogen, can be determined as a "growth dilution effect" caused by an increase in the yield volume per unit of area as well as by a productive tillering, which is of critical importance for the uniform grain ripening and grain quality.

Keywords: carboxymethylation, plant processing waste, yield, wheat, grain quality, gluten, protein, plant growth regulators

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INTRODUCTION

Almost all companies processing agricultural products face a serious problem of recycling of the resulting wastes. The Altai region is not an exception as it takes the leading position among Russian regions in both production and processing of agricultural crops. The region takes $\sim 30\%$ of the total production of cereals in Russia including up to 55% of the oatmeal, 50% of buckwheat, and $\sim 30\%$ of pearl and peeled barley production. Growing and processing of these cereals is accompanied with a large volume of cereal processing waste; for example, sunflower and buckwheat husk makes 14-16 and 20-22% of the total production volume, respectively.

The total volume of agricultural wastes in Russia may reach 630–650 million tons, and the total volume of wastes generated by the forestry and wood processing industry may reach 700 million tons [1]. The most part of generated organic wastes are burned or stored at landfills until their natural decomposition. Industrial companies cannot directly use such wastes (due to the specificity of each individual manufacturing pro-

cess), so the vast majority of wastes comes to landfills. Being generated in huge quantities, agricultural wastes represent a source of pollution and worsen the sanitary, epidemiological and aesthetic qualities of nature [2].

One of the possible ways to solve this problem is chemical modification (carboxymethylation) of organic crop wastes intended to obtain water-soluble polymeric products characterized by a complex of valuable properties including the ability to regulate the growth and development of plants. Based on some studies [3, 4], some innovative biopreparations were developed (technical specification 928900-005-02067818-2015), and the possibility of their use as plant growth regulators and phytomeliorants capable of increasing water stability of soil aggregates was confirmed [4–8]. The growth-regulating activity of such compounds is at least similar to that of widely used peat-humic "Flora-C" fertilizers and the chitosanand succinic acid-based plant growth activator (JOY) [9]. Lignin carboxymethylation in plant raw materials results in the formation of structures similar to the molecules of auxin-type growth regulators, whose typical feature is the presence of an aromatic ring or a group of rings and a side chain with an acid group [10].

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To date, use of growth regulators is considered as the environmentally friendly and cost-effective way to increase the yield of agricultural crops, which provides more complete implementation of a crop potential. Moreover, such preparations may have a positive effect not only on the yield, but also on grain quality, particularly, on the crude gluten content [11].

Thus, the study of the effect of plant growth regulators on the crop capacity and grain quality of spring wheat represents a relevant task.

The purpose of this study was evaluation of the effect of preparations obtained from carboxymethylated plant raw materials on the activity of growth processes, crop capacity, and biochemical grain quality parameters of spring wheat.

EXPERIMENTAL

Carboxymethylation of plant wastes. The plant raw materials used in this study included various plant wastes, such as pine sawdust (NaCMW preparation), oat lodicules (NaCMO), buckwheat husk (NaCMB), sunflower husk (NaCMS), maize ear shanks (NaCMM), and fallen poplar leaves (NaCML). The process of plant waste carboxymethylation was carried out using a RVPE-0.2 industrial pilot plant (UVS Ltd., Obninsk, Russia) [12].

Chemical composition studies. Carboxymethylated products of plant wastes were analyzed for the content of carboxymethyl groups (CMG) [13], carboxymethylated lignin (Komarov's method) [14], and sodium salt of carboxymethyl cellulose (NaCMC) [15] as well as for their water solubility [13].

A small-plot experiment was arranged at the educational-experimental agricultural station of the Altai State Agricultural University. Prior sowing, spring wheat seeds were treated with 1.5% water solutions of the tested biopreparations at a dosage of 150 g per 10 L per 1 ton of seeds). The experimental layout included four replicates arranged systematically with shifts; the plot area was 1 m².

A field experiment was arranged at the same station. For each variant, the field area was 1.0 hectare. In this experiment, the effect of 1.5% aqueous solutions of NaCMW and NaCMB was assessed. In the last case, wheat was sown after the fallow or rape.

A large-scale experiment to determine the effect of the studied biopreparations on the growth and development of wheat was arranged at the Kiprinskoe JSC (Shelabolikha district), Vektor LLC (Kalmanka district), and Brusentsevo village (Ust-Pristan district). The NaCMW preparation (1.5% aqueous solution) was used to treat wheat seeds immediately prior to sowing. A Baryer Kolor preparation (a.i. tebuconazole) belonging to the triazole group and possessing preventive and curative systemic fungicidal action was used as a reference preparation. The experiment area was 10 hectares.

The content of protein and gluten as well as the grain-unit were determined by a near-infrared spectroscopy using a FOSS NIRSystems 4500 reflection spectrophotometer [16].

RESULTS

Carboxymethylation of plant raw materials resulted in the obtaining of preparations, whose chemical composition is shown in Table 1. The process of carboxymethylation of different types of plant raw materials and agricultural waste materials provided products with the water solubility of 46–78%. The content of carboxymethyl groups varied within a wide range (13.3–35.8%) depending on the initial raw material. The carboxymethylation process involved all major structural components of plant raw materials. Fallen leaves were characterized by the easiest carboxymethylation process, whereas buckwheat and sunflower husk as well as corn processing wastes were hardly carboxymethylated. Obviously, this fact is determined by the structural organization of plant cells in these objects and by the availability of hydroxyl groups of the main structural components of cells for chemical modification.

Under conditions of a small-plot experiment, all studied preparations showed a positive effect on the crop capacity of wheat (Table 2). The yield increase of spring wheat was significant and varied from 35.6 to $50.8 \text{ g/m}^2 (30-43\%)$.

In general, all studied preparations showed a quite high efficiency. A more thorough investigation of the effect of such preparations on the wheat yield and grain quality was carried out with the NaCMW and NaCMB preparations. The reasons for the choice of these preparations were the following. NaCMW was obtained using a pine timber. Pine is a perennial plant widely used in the industry that generates a large amount of waste materials, which are not used in a qualified way. At the same time, pine timber is easily carboxymethylated with the formation of products characterized by a good water solubility (46%) and a high CMG content (29.3%). NaCMB was obtained from buckwheat husk and represented an example of processing of wastes obtained from annual plants; it was characterized by a poor carboxymethylation level, and the resulting product contained low CMG amount (17.6%).

According to the State Standard 9353-2016, spring wheat grain quality indicators, which determine the grain class and the purchasing price, include the typical composition, state, odor, color, gluten content and quality, falling number, glassiness, presence and type of a foreign material, and the presence of germinated grains. The grade of a grain and, therefore, its cost depends mainly on its protein and gluten content. The content of crude protein should be within the range of 11–17%. If the protein content rises above 17–19% or

	Properties of carboxymethylation products, %						
Initial raw material (preparation)	Carboxy- methylated cellulose	Carboxy- methylated lignin	Content of carboxy- methyl groups (CMG)	CMG in carboxy- methylated cellulose	water solubility		
Buckwheat husk (NaCMB)	19.8 ± 0.2	17.3 ± 0.5	17.6 ± 0.5	12.2 ± 0.3	63.8 ± 0.7		
Fallen poplar leaves (NaCML)	14.0 ± 0.2	13.5 ± 0.4	35.8 ± 0.5	23.5 ± 0.2	78.3 ± 0.6		
Oat lodicules (NaCMO)	28.7 ± 0.4	12.4 ± 0.3	13.3 ± 0.3	21.2 ± 0.3	75.2 ± 0.8		
Sunflower husk (NaCMS)	21.5 ± 0.7	17.1 ± 0.4	19.0 ± 0.4	14.2 ± 0.4	59.6 ± 1.3		
Scotch pine (NaCMW)	32.4 ± 0.5	16.5 ± 0.2	29.3 ± 0.3	21.3 ± 0.1	67.6 ± 0.9		
Maize ear shanks (NaCMM)	23.4 ± 0.6	13.2 ± 0.4	18.0 ± 0.5	15.1 ± 0.4	48.9 ± 0.6		

Table 1. Chemical composition of carboxymethylation products obtained from different plant raw materials

Table 2. Effect of preparations obtained from carboxymethylated plant raw materials on the crop yield of soft wheat (var. Omskaya 36) under conditions of a small-plot trial

Parameter	Preparation						LSD ₀₅ ,	
Parameter =	Control	NaCMO	NaCMS	NaCMW	NaCMB	NaCMM	NaCML	g/m^2
Yield, g/m ²	117.2	152.8	151.6	158.8	165.2	165.2	168.0	21.4

falls below 11%, the bread quality significantly deteriorates. Gluten is a highly hydrated gel consisting of proteins (mainly alkali-soluble glutenins and alcoholsoluble gliadins) as well as carbohydrates, lipids, and minerals. Gluten determines the baking quality of flour obtained from wheat grain. According to the State Standard 9353-90, the highest-class grain should contain 36% of gluten, while the grain of the first, second, third, and fourth classes should contain 32, 28, 23, and 18% of gluten, respectively. The grainunit represents the mass of one liter of grain. This index indirectly characterizes the degree of grain swelling and grain ripening as well as its nutritional value. For wheat this indicator varies between 700-840 g/L; grain characterized by a high grain-unit value provides more flour and less bran.

The field study of the NaCMW effect on the growth and development of spring wheat (var. Omskaya 36) showed a positive effect on both crop yield and grain quality. Application of NaCMW resulted in the yield increase by 15.1%, a thousand-kernel weight increase by 1.1 g, protein content

increase by 0.9%, and gluten content increase by 1.4% (Table 3).

Under conditions of a large-scale experiment at the Kiprinskoe JSC, NaCMW also provided a positive effect on the crop yield (Tables 4–6). A presowing treatment of wheat seeds with the NaCMW preparation resulted in a significant yield increase by 0.34 t/ha (21%) as well as an improvement of the grain quality in relation to the protein content (+1.5%), gluten content (+4.1%), and grain-unit (+6.4 g). The observed positive effect of the preparation on the wheat yield and grain quality exceeded that of a Baryer Kolor seed dresser representing a wide-range systemic fungicide for cereals (Table 4).

Another experiment arranged at the Brusentsevo village (Ust-Pristan district) showed such pre-sowing treatment resulted in the yield increase by 0.42 tons (24%), protein content increase by 0.6%, gluten content increase by 3.9%, and the grain-unit increase by 6.5 g (Table 5).

In the case of a field experiment arranged at the Vektor LLC (Kalmanka district), the reliable yield increase made 0.6 t/ha (+32%). However, a slight

Table 3. Effect of the NaCMW preparation on the crop capacity and grain quality of soft spring wheat (var. Omskaya 36) under field trial conditions

Variant	1000 kernel weight, g	Protein content, %	Gluten content, %	Yield, t/ha
Control	32.2	11.0	19.3	1.46
NaCMW	33.1	11.9	20.7	1.72
LSD ₀₅				0.19

Variant Protein content, % Gluten content, % Grain-unit, g/L Yield, t/ha 32.54 Control 16.02 790.8 1.61 **NaCMW** 1.95 17.49 36.63 796.4 Barver Kolor 17.21 35.15 786.7 1.79 LSD_{05} 0.17

Table 4. Effect of the NaCMW preparation on the crop capacity and biochemical indices of grain of soft spring wheat under conditions of a large-scale trial at the Kiprinskoe JSC

Table 5. Effect of the NaCMW preparation on the crop capacity and biochemical indices of grain of soft spring wheat under conditions of a large-scale trial at the Brusentsevo village (Ust-Pristan district)

Variant	Protein content, %	Gluten content, %	Grain-unit, g/L	Yield, t/ha
Control	14.32	31.39	867.8	1.72
NaCMW	14.99	35.25	877.3	2.14
LSD ₀₅				0.22

decrease in the grain quality was reported in relation to the protein and gluten content (-1.7 and -4.8%, respectively; Table 6).

In all plant organisms, biochemical processes of the matter accumulation and transformation occur under certain combinations of physical factors (light, heat, moisture, etc.). A complete absence or limited accessibility of one of these factors (either dominant, or secondary), causes changes in all physiological and biochemical processes of a plant. The yield size and the protein content in grain depend mainly on the nitrogen supply. In our case, decrease in the protein and gluten content in grain along with a significant yield increase may result from the "growth dilution effect," due to which, in the presence of a limiting factor (nitrogen content in soil), the content of nitrogencontaining components in plants decreases as the yield increases. As a result, the biosynthesis of carbohydrates or fats increases, while the protein synthesis decreases. In other words, accumulated nitrogen may be "diluted" in a larger organic mass of plants resulting in a relative decrease in the protein content in grain.

Phenological observations of the growth and development of the studied crop showed that carboxymethylated pine sawdust promoted more intensive development of the root system that represents one of manifestations of the effect of auxin-type growth regulators. Later this effect was manifested in a better develop-

ment of vegetative and generative organs of plants, increased yield, and improved grain quality (see Supplementary materials, Figs. 1, 2).

According to the earlier published data, activation of growth processes caused by auxin-type growth regulators occurs because one side of the stem or root accumulates more auxins than the other one that leads to uneven cell elongation and formation of a bend. Indolylacetic acid (IAA) synthesized in the apexes (shoot and root tips) represents ~80–95% of the total volume of plant auxins. Cell growth stimulation by elongation is based on the auxin-dependent cell elongation associated with an increased cell wall extensibility. A cell wall loosening may occur due to the auxin-induced activation of membrane H⁺-ATPases. These enzymes translocate H⁺ ions out of the cytosol causing acidification of a narrow space between the plasmalemma and the cell wall (so-called "acidic growth"). An acidic environment causes activation of cell wall-associated expansins, which break hydrogen bonds between cellulose microfibrils, allowing a cell to expand due to a turgor pressure. The direction of a cell growth depends on the orientation of cellulose fibrils, which is also controlled by auxins via a still-unknown way [17, 18].

An active ingredient of the studied preparations was carboxymethylated lignin, which is considered to work in accordance with the above-described process [10, 19].

Table 6. Effect of the NaCMW preparation on the crop capacity and biochemical indices of grain of spring wheat under conditions of a large-scale trial at the Vektor LLC

Variant	Protein content, %	Gluten content, %	Grain-unit, g/L	Yield, t/ha
Control	14.1	27.9	783.5	1.92
NaCMW	12.4	23.1	777.0	2.51
LSD ₀₅				0.28

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Variant	Productive tillering	1000-kernel weight, g	Protein content, %	Gluten content, %	Grain-unit, g/L	Yield, t/ha
Control	1.1	34.7	13.5	29.0	836.9	1.91
NaCMB	1.2	40.1	13.8	31.1	836.3	2.15
LSD ₀₅						0.19

Table 7. Effect of the NaCMB preparation on the yield and grain quality of fallow-preceded wheat (var. Torridon)

Table 8. Effect of the NaCMB preparation on the yield and grain quality of rape-preceded wheat (var. Torridon)

Variant	Productive tillering	1000-kernel weight, g	Protein content, %	Gluten content, %	Grain-unit, g/L	Yield, t/ha
Control	1.1	29.7	10.8	22.0	817.0	1.44
NaCMB	1.2	32.8	9.7	19.4	819.0	1.61
LSD ₀₅						0.13

A correct choice of preceding crops is one of the main conditions for obtaining high yield of wheat with a high grain quality. Preceding crops affect the soil moisture and nutrient content, seedling vigor and completeness, crop overwintering, phytosanitary condition of crops, and plant productivity [20].

The effect of NaCMB aqueous solution was studied with allowance for the preceding crop; wheat was sown after the fallow (variant 1) or rape (variant 2). According to the performed field trials, NaCMB provided the auxin-type enhancement of the growth and development of plants during the first growth phases (seed germination) that agreed with our earlier studies. Growth processes were activated starting from seed germination (radicle appearance) to more intense tillering and then to the full ripeness phase (see Supplementary materials, Figs. 3, 4).

In the case of fallow-preceding wheat, the yield increase caused by the presowing treatment of seeds with NaCMB was 12.6%. The treatment also improved productive tillering as well as increased the 1000-kernel weight and the gluten content by 5.4 g and 2.1%, respectively (Table 7).

In the case of rape-preceding wheat, the observed effect of NaCMB on the yield structure indices (1000-kernel weight and productive tillering) and crop capacity was similar to above-described (Table 8). At the same time, a small decrease in the gluten and protein content in grain was observed that can be explained by the growth dilution effect. The yield increase reached 10.6%. In this case, the soil was nitrogen-deficient, since the production of 100 kg of rape seeds with the corresponding amount of straw requires 6.2 kg of nitrogen taken from the soil [17].

Thus, large-scale trials of growth-regulating biopreparations obtained from carboxymethylated plant raw materials arranged under conditions of the educational-experimental agricultural station of the Altai State Agricultural University and farmer companies confirmed efficiency of their use with the corresponding yield increase up to 32%.

CONCLUSIONS

- (1) Carboxymethylation of different types of plant raw materials (timber processing or agricultural waste materials) results in a formation of products characterized by a water solubility range of 46–78% and the CMG content range of 13.3–35.8%. Carboxymethylation process involves all the main structural components of plant raw materials.
- (2) A presowing treatment of spring wheat seeds with biopreparations obtained from carboxymethylated plant processing wastes at a dosage of 150 g per 10 L per 1 ton of seeds positively influenced on the growth processes of the crop. The treatment resulted in activation of the growth and development of wheat plants at the early development phases, from seed germination (radicle appearance) to the more intensive tillering. The studied preparations provided an increased yield under conditions of small-plot (30–43%), field (11–15%), and large-scale (21–32%) trials.
- (3) Increase in the yield caused by the effect of growth regulators is not always accompanied with the increased protein and gluten content in grain. Negative changes in the protein content (for example, in the case of fallow-preceded wheat and limited nitrogen content in soil) can be considered as a "growth dilution effect" caused by an increased yield volume per unit of area as well as by productive tillering, which provides a key influence on the uniformity of ripening and grain quality. In other words, accumulated nitrogen may "be diluted" in a large organic mass of plants resulting in a relative drop of the protein content in grain.

COMPLIANCE WITH ETHICAL STANDARDS

The authors declare that they have no conflict of interest.

This article does not contain any studies with animals performed by any of the authors.

SUPPLEMENTARY INFORMATION

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