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ORIGINAL PAPER

EFFECT OF BIOLOGICAL PREPARATIONS AND MINERAL NITROGEN FERTILISATION ON THE CONTENT OF PROTEIN AND MACROELEMENTS IN SPRING WHEAT GRAIN[1]

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Abstract

Researchers from the Siedlce University of Natural Sciences and Humanities carried out an investigation in 2017-2019, on a family farm located in Krzymosze (52°03′27″N, 22°33′74″E) near Siedlce, Poland, aiming at determining the effect of biofertilisers and nitrogen fertiliser regime on the protein content as well as macroelements in the grain of spring wheat produced in a sustainable agriculture system. The following two factors were examined: (I) biological preparations: control where no biological preparations were applied, *Azotobacter vinelandii*, L-*α* proline, *Azotobacter vinelandii* + L-α proline; (II) mineral nitrogen regime: unamended control, 60 kg N ha^{-1}, 90 kg N ha^{-1}, 120 kg N ha^{-1}. Spring wheat grain was sampled to determine the total protein content and macroelements (P, K, Ca and Mg). The results demonstrated that the biological nitrogen preparations *Azotobacter vinelandii* + L-*α* proline applied simultaneously with 90 kg N ha^{-1} contributed to the production of spring wheat grain with the highest concentration of protein and phosphorus, potassium, calcium and magnesium, all being very important in human nutrition. The use of the biological preparation *Azotobacter vinelandii* and L-*α* proline biostimulator with a fertilisation dose of mineral nitrogen of 90 kg N ha^{-1} ensures favorable chemical composition of spring wheat grain. Moreover, such form of fertilisation protects the soil environment, an issue of growing importance in modern agriculture. It should be recommended to apply bioproducts in combination with lower nitrogen mineral fertilisation doses in spring wheat cultivation.

Keywords: *Triticum aestivum* L., macroelements; nitrogen, biofertiliser, biostimulator.

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INTRODUCTION

Wheat is the basic cereal used for human consumption worldwide (Abou-el--Seoud, Abdel-Megeed 2012). In order to provide superior quality grain, it is recommended that the species be produced in the system of sustainable agriculture (Szulc 2013, Wilczewski et al. 2013), where an application of mineral nitrogen to produce spring wheat should be partially substituted by biological nitrogen through the application of eco-friendly nitrogen bio-products (Muhammad et al. 2016). Biofertilisers containing nitrifying bacteria of the genus *Azospirrilum* and *Azotobacter* positively affect plant growth and development, which translates to better cereal grain yield and chemical composition (Youssef and Eissa 2014, Kumar 2018). Also, they increase the soil availability of macroelements, as a result of which their uptake by plants increases. This is of particular importance in human nutrition as wheat grain is a basic source of nutrients, including protein and minerals (Amraei et al. 2015, Jarecki et al. 2019). There is paucity of works pertaining to this subject. The future will see an expansion of sustainable agriculture which will rely on biofertilisers whose application will be suplemented by mineral nitrogen fertiliser applied at lower doses, which will positively affect cereal grain chemical composition and protect the soil environment, the issue being of particular relevance at present. The research reported here is an attempt to fill this gap. It aimed at determining the impact of new biological products and mineral nitrogen fertiliser regime on the content of protein and macroelements in the grain of spring wheat grown in the system of sustainable agriculture. The experiment assumed that the biological preparations used and the doses of mineral nitrogen fertilisation would vary the content of total protein and macronutrients in spring wheat grain.

MATERIALS AND METHODS

A field experiment was conducted on a family-run farm located in Krzymosze (52°03′27″N, 22°33′74″E) near Siedlce, Poland, in 2017-2019. The experimental soil was a Stagnic Luvisol according to WRB FAO (World Reference Base for Soil Resources 2014) and the available macroelement content was as follows: P – 8.2 mg 100 g^{-1} soil, K – 18.7 mg 100 g^{-1} soil and Mg – 4.8 mg 100 g^{-1} soil. The soil reaction was neutral (pH 6,5) and the humus content was 1.88%. The content of mineral nitrogen was N-NH_4^+ – 4.97 mg kg^{-1} of soil and N-NO_3^- – 7.84 mg kg^{-1} of soil. Chemical analyses of the soil were performed at the Chemical and Agricultural Station in Warsaw. The size of a plot for harvest was 16 m^2.

The field experiment was designed in a split-block arrangement with three replicates. The following two factors were examined: (I) the fist factor presented in Table 1; (II) the second factor was a mineral nitrogen regime:

Table 1

Biological preparations studied in the experiment

Biological preparations	Control obiect	Biological preparation 1 *(Azotobacter vinelandii)* 1 dm^3 ha^{-1}	Biological preparation 2 (L-α proline) 2 g ha^{-1}	Biological preparation 1 *(Azotobacter vinelandii)* 1 dm^3 ha^{-1} + Biological preparation 2 (L-α proline) 2 g ha^{-1}

unamended control, 60 kg N ha^{-1} (preplant), 90 kg N ha^{-1} (60 kg N ha^{-1} preplant + 30 kg N ha^{-1} at the shooting stage), 120 kg N ha^{-1} (60 kg N ha^{-1} preplant + 30 kg N ha^{-1} at the shooting stage + 30 kg N ha^{-1} – foliar application of 8% urea solution at the stage of initial ear formation).

Maize was the crop chosen to proceed spring wheat cv. Mandaryna. Phosphorus and potassium fertiliser doses, established based on soil availability, were 30.8 and 99.6 kg ha^{-1} of P and K, respectively. The mineral nitrogen regime was applied as described for factor II above. Spring wheat was sown in early April and the sowing density was 500 grains per 1 m^2. Mineral nitrogen fertilisation before sowing and in the shooting stage (BBCH 30) was applied in the form of 34% ammonium nitrate. Additionally, foliar fertilisation was carried out, with 46% urea in the form of an 8% solution, in the heading stage (BBCH 50). During the plant growing season, two treatments were carried out: the first with the herbicide Gold 450 SC (2,4 D ester + fluroxypyr) at a dose of 1.25 dm^3 ha^{-1}, fungicide (the first treatment Bumper Super 490 EC (prochloraz, propiconazole) at a dose of 1 dm^3 ha^{-1}; the second one with Falcon 460 EC (spiroxamine, tebuconazole, triadimenol) and the growth regulator Cerone 480 SL (etephon) in a dose of 480 SL 0.75 dm^3 ha^{-1}. After harvesting the preceding crop, pre-winter plowing was performed and spring soil tillage was carried out before sowing the wheat.

The biological products were applied once at the stage of spring wheat tillering. Biological preparation 1 (Azofix) contains the *Azotobacter vinelandii* MVY-010 (1x10 CFU/l) and micro-elements Mn, Fe, Cu, Mo, Zn, Co and B-group vitamins: B1, B3, B6 (max. 0.02%), and it is a biological product intended for increasing the nitrogen content in soil. It contains the non-symbiotic, free-living soil bacterium *Azotobacter vinelandii*, which effectively assimilates the atmospheric nitrogen and extracts bioactive substances that improve the development of plants, as well as polysaccharide alginates having influence on the formation of water-resistant units in soil. Biological preparation 2 (Maxprolin) contains L-α proline (purity 99.5%), a biostimulator increasing the natural resistance of plants to stress. Both biological preparations should be dissolved in water and sprayed with 250 dm^3 ha^{-1} of water. The distributor of these biological preparations in Poland is PHU Biotel Sp. zoo. Dzikowice 87, 67-300 Szprotawa.

During the spring wheat harvest, which took place in early August, grain was sampled to perform chemical analyses. The total protein content in grain was determined by the Kjeldahl method, and the following elements were determined: P (spectrophotometric method), K (flame photometry method), Mg (flame atomic absorbtion spectroscopy FAAS). Then, the plant material was mineralized in acids in a microwave digestor Milesone Ethos Plus. The content of micronutrients was determined in the mineralizate by emission spectrometry with excitation in inductively coupled plasma and an optical detector (ICP-OES), using an emission spectrometer Perkin Elmer Optima 8300. Each assay was performed in triplicate.

Each of the characteristics studied was analysed by means of ANOVA for a split-block arrangement. Comparison of means for significant sources of variation was achieved by the Tukey's test at the significance level of $P \leq 0.05$. All the calculations were performed in Statistica®, version 12.0, and MS Excel.

RESULTS

Total protein content in spring wheat grain

Total protein content in spring wheat grain was significantly affected by the experimental factors and their interaction (Table 2). The highest total protein content was recorded in the grain of spring wheat treated with *Azotobacter vinelandii* + L-*α* proline. It decreased significantly in wheat from the plots treated with either *Azotobacter vinelandii* or L-*α* proline. However, the spring wheat grain content of total protein following an application of these products was higher compared with the untreated control values.

Table 2

Total protein content in spring wheat grain (means across 2017-2019), g kg^{-1} d.m.

Biological preparations (A)	Mineral fertilisation with nitrogen (B) (kg N ha^{-1})				Means
	control	60	90	120	
Control	12.58	13.08	14.07	15.08	13.70
Azotobacter vinelandii	13.13	13.69	16.51	17.82	15.29
L-*α* proline	12.96	13.46	14.75	17.13	14.58
Azotobacter vinelandii+L-*α* proline	14.88	15.51	18.01	18.13	16.63
Means	13.39	13.94	15.84	17.04	-
ANOVA	*P* – value				$HSD_{0.05}$
Biological preparations (A)	< 0.001				0.82
Mineral fertilisation with nitrogen (B)	< 0.001				0.83
Interaction: AxB	< 0.001				0.94

Also, mineral fertilisation with nitrogen significantly changed the total protein content in spring wheat grain. Increasing mineral nitrogen fertiliser doses contributed to an increase in the spring wheat grain content of total protein, the highest concentration being recorded after spring wheat had been fertilised with the highest nitrogen dose, i.e. 120 kg N ha^{-1}. An interaction was identified: the highest total protein content was determined in the grain of spring wheat amended with *Azotobacter vinelandii* + L-*α* proline and fertilised with 90 kg N ha^{-1} + foliar application, as well as *Azotobacter vinelandii* accompanied by nitrogen applied at 90 kg N ha^{-1} + foliar fertilisation. In contrast, the lowest concentration of total protein was determined in the control unit, following an application of *Azotobacter vinelandii* or L-α proline unaccompanied by mineral nitrogen fertiliser, and in the control unit where the nitrogen had been 60 kg N ha^{-1}.

Phosphorus content in spring wheat grain

Statistical analysis revealed a significant effect of the experimental factors and their interaction on the phosphorus content in spring wheat grain (Table 3). The highest phosphorus concentration was determined in the grain

Table 3

Phosphorus content in spring wheat grain (means across 2017-2019), g kg^{-1} d.m.

Biological preparations (A)	Mineral fertilisation with nitrogen (B) (kg N ha^{-1})				Means
	control	60	90	120	
Control	2.123	2.299	2.367	2.347	2.284
Azotobacter vinelandii	2.498	2.587	2.712	2.693	2.623
L-*α* proline	2.406	2.496	2.593	2.584	2.520
Azotobacter vinelandii+L-*α* proline	2.637	2.834	3.112	2.987	2.893
Means	2.416	2.554	2.696	2.653	-
ANOVA	*P* – value				$HSD_{0.05}$
Biological preparations (A)	< 0.001				0.218
Mineral fertilisation with nitrogen (B)	< 0.001				0.219
Interaction: AxB	< 0.001				0.226

of spring wheat treated with the mixture *Azotobacter vinelandii* + L-*α* proline. When applied separately, these products resulted in a significant decline in the spring wheat grain content of phosphorus, the differences between these units being insignificant. However, the phosphorus content in the grain of spring wheat treated with these biological products was higher compared with the untreated control. Mineral fertilisation with nitrogen significantly affected the phosphorus content in spring wheat grain, too. Increasing mineral nitrogen doses contributed to an increase in the spring wheat grain content of phosphorus when lower than 90 kg N ha^{-1}. The highest dose of mineral fertilisation with nitrogen was followed by a decline in the concentration of phosphorus in spring wheat grain. An interaction was confirmed indicating that the highest phosphorus content was accumulated in the grain of spring wheat fertilised with *Azotobacter vinelandii* + L-α proline accompanied by mineral nitrogen applied at a dose of either 90 kg N ha^{-1} or 90 kg N ha^{-1} + foliar application. In contrast, the lowest concentration of phosphorus was recorded in the grain harvested in the control unit, where neither bioproducts nor nitrogen were applied, or when bioproducts were accompanied by 60 kg N ha$^{-1,}$ or 90 kg N ha^{-1} + foliar application.

Potassium content in spring wheat grain

The potassium content in spring wheat grain was significantly affected by the experimental factors and their interaction (Table 4). The highest concentration of potassium was recorded in the grain of spring wheat fertilised with *Azotobacter vinelandii* + L-α proline. However, when applied separately, the products contributed to a significant decline in the spring wheat grain content of potassium. Despite this, the content of this element was still higher than in the untreated control unit. Mineral fertilisation with nitrogen had a significant impact on the potassium content in spring wheat grain. In the control unit, without mineral nitrogen fertilisation or where a dose of 60 kg N ha^{-1} had been applied, the spring wheat grain content of potassium was the lowest. A dose of either 90 kg N ha^{-1} or 90 kg N ha^{-1} + foliar application was followed by a significant increase in potassium content in spring wheat grain, the contents in these units remaining at a similar level. An interaction was found indicating that the highest potassium content was accumulated in the grain of spring wheat treated with *Azotobacter vinelandii* + L-α proline and fertiliserd with the following mineral nitrogen rates 60 kg N ha^{-1}, 90 kg N ha^{-1} or 90 kg N ha^{-1} + foliar application. In contrast, the lowest concentration of potassium was determined in the grain

Table 4

Potassium content in spring wheat grain (means across 2017-2019), g kg^{-1} d.m.

Biological preparations (A)	Mineral fertilisation with nitrogen (B) (kg N ha^{-1})				Means
	control	60	90	120	
Control	3.018	3.122	3.287	3.226	3.163
Azotobacter vinelandii	3.373	3.586	3.825	3.797	3.645
L-α proline	3.246	3.427	3.687	3.621	3.495
Azotobacter vinelandii+L-α proline	3.683	3.894	4.103	3.984	3.916
Means	3.330	3.507	3.726	3.657	-
ANOVA	*P* – value				$HSD_{0.05}$
Biological preparations (A)	< 0.001				0.237
Mineral fertilisation with nitrogen (B)	< 0.001				0.235
Interaction: AxB	< 0.001				0.244

of spring wheat grown in the untreated control unit where no mineral nitrogen fertiliser had been applied, or for the mineral nitrogendoses of either 60 kg N ha^{-1} or 90 kg N ha^{-1} + foliar application.

Calcium content in spring wheat grain

Statistical analysis demonstrated a significant influence of the experimental factors and their interaction on the calcium content in spring wheat grain (Table 5).The highest calcium concentration was recorded in the grain

Table 5

Calcium content in spring wheat grain (means across 2017-2019), g kg^{-1} d.m.

Biological preparations (A)	Mineral fertilisation with nitrogen (B) (kg N ha^{-1})				Means
	control	60	90	120	
Control	2.218	2.294	2.318	2.305	2.284
Azotobacter vinelandii	2.761	2.996	3.207	3.120	3.021
L-α proline	2.554	2.773	3.005	2.924	2.814
Azotobacter vinelandii+L-α proline	2.897	3.200	3.536	3.461	3.274
Means	2.608	2.816	3.017	2.953	-
ANOVA	*P* – value				$HSD_{0.05}$
Biological preparations (A)	< 0.001				0.187
Mineral fertilisation with nitrogen (B)	< 0.001				0.188
Interaction: AxB	< 0.001				0.196

of spring wheat fertilised with *Azotobacter vinelandii* + L-α proline. When applied separately, the bioproducts contributed to a significant decline in the spring wheat grain content of calcium. Still, the calcium content in the grain of spring wheat treated with one or the other product was significantly higher than in the untreated control. Mineral fertilisation with nitrogen significantly affected the calcium content in spring wheat grain. An increase in a nitrogen fertiliser dose, particularly to the level of 90 kg N ha^{-1} or 90 kg N ha^{-1} + foliar application, was followed by a significant rise in the spring wheat grain content of calcium. An interaction was confirmed indicating that the superior concentration of calcium was determined in the grain of spring grain fertilised with *Azotobacter vinelandii* + L-α proline in combination with the mineral nitrogen dose of 90 kg N ha^{-1} or 90 kg N ha^{-1} + foliar application. By contrast, the lowest calcium content was determined in the grain of spring wheat grown in the untreated control units at all the levels of mineral nitrogen regime.

Magnesium content in spring wheat grain

The magnesium content in spring wheat grain was significantly affected by the experimental factors and their interaction (Table 6). The highest con-

Table 6

Magnesium content in spring wheat grain (means across 2017-2019), g kg^{-1} d.m.

Biological preparations (A)	Mineral fertilisation with nitrogen (B) (kg N ha^{-1})				Means
	control	60	90	120	
Control	0.903	0.945	0.997	0.969	0.954
Azotobacter vinelandii	0.978	1.232	1.356	1.318	1.221
L-α proline	0.927	0.996	1.218	1.127	1.067
Azotobacter vinelandii+L-α proline	1.112	1.347	1.612	1.521	1.398
Means	0.980	1.130	1.296	1.234	-
ANOVA	P – value				$HSD_{0.05}$
Biological preparations (A)	< 0.001				0.118
Mineral fertilisation with nitrogen (B)	< 0.001				0.120
Interaction: AxB	< 0.001				0.126

centration of magnesium was recorded in the grain of spring wheat treated with *Azotobacter vinelandii* + L-α proline. Both the products, when applied separately, resulted in a significant decline in the spring wheat grain content of magnesium, the values still being higher compared with control grain. Mineral fertilisation with nitrogen contributed to a significant increase in the magnesium content in spring wheat grain, particularly after the dose of 90 kg N ha^{-1} or 90 kg N ha^{-1} + foliar application had been used. An interaction was confirmed indicating that the highest magnesium was recorded in the grain of spring wheat treated with *Azotobacter vinelandii* + L-α proline when combined with the mineral nitrogen fertiliser dose of 90 kg N ha^{-1} or 90 kg N ha^{-1} + foliar application. The lowest concentration of magnesium was determined in spring wheat grain harvested in the untreated control unit at all levels of mineral nitrogen fertiliser regime or following an application of only *Azotobacter vinelandii* or L-α proline.

DISCUSSION

Cereal grain has been human food for thousands of years (Awika 2011). Wheat grains are a source of nutrients and play an important role in the diet of many countries, so it is crucial to produce high quality raw material (Dewettinck et al. 2008, Poutanen 2012). Wheat grain is the main raw material for the production of bakery products rich in protein and minerals essential in human nutrition (Muhammad et al. 2016). The research reported here demonstrated that the protein content in the grain of spring wheat grown in Poland is affected by fertilisation with bioproducts. It is worth stressing that simultaneous application of *Azotobacter vinelandii* + L-α proline had a superior influence on the protein content in spring wheat grain. Also, Kizilkaya (2008), Kumar (2018) and Kumar and Sarkar (2019) reported that application of biological fertiliser containing bacteria from the genus *Azospirrilum* and *Azotobacter* increases the soil availability of nitrogen, which translates into a higher nitrogen concentration in cereal grain compared with separate application of these bacteria. It can be explained by the fact that the two nitrogen bioproducts, when applied into the soil, enrich it with more bacteria which reduce elemental nitrogen from the air and make it available for the crop plant continuously throughout the whole growing season. Unlike high doses of mineral nitrogen fertilisation, this ensures the element is transformed into protein nitrogen. Also, in the present work, the L-α proline-based bioproduct positively affected nitrogen uptake by wheat plants, which in turn increased the grain content of protein. Similarly to findings by Sułek and Cacak-Pietrzyk (2008), Wilczewski et al. (2013) and Szmigiel et al. (2014), in the experiment discussed here, increasing nitrogen doses contributed to an increase in the cereal grain content of protein. However, application of high doses of mineral fertilisation with nitrogen leads to soil environment pollution (Wang et al. 2008, Munees, Mulugeta 2014). Thus, other solutions should be considered. In the developing system of sustainable agriculture, it is recommended to apply lower doses of mineral fertilisation with nitrogen, and supplement it with bioproducts, as this ensures production of high quality grain and protects the soil environment (Yousset et al. 2014), which was confirmed in the present study. A superior protein content in spring wheat grain was recorded following the application of two nitrogen bioproducts *Azotobacter vinelandii* + L-α proline when combined with mineral fertilisation with nitrogen at a dose of 90 kg N ha^{-1}.

Spring wheat grain is also the main source of essential minerals in human nutrition (Amraei et al. 2015, Chennappa et al. 2017). In the present work, the biological products were found to increase the macroelement content in spring wheat grain compared with untreated control. Also, Amroeci et al. (2015), Jākobsone et al. (2015), Kumar (2018), Chennappa et al. (2019) as well as Kumar and Sarkar (2019) reported that the application of bioproducts increased minerals in cereal grain, particularly when simultaneous bacterial inoculant applications were made. Also, in the work reported here, simultaneous application of biological preparation 1 containing the bacteria *Azotobacter vinelandii* and biological preparation 2 containing the amino acid L-α proline had the most positive effect on the spring wheat grain content of macroelements, which resulted in the best quality raw material. This is due to the fact that the application of a greater quantity of various bacteria, or L-alpha proline contained in biological preparation 2, does not directly provide the plant with nutrients. Their role is to enhance the microbiological process of increasing nutrient availability, the nutrients being more easily and consistently taken up by plants during the growing season, which ensures their high concentrations in plants. As a result, soil fertility increases and plant growth improves owing to the increased number and enhanced biological activity of beneficial microorganisms in soil. Such a function is not attributed to mineral nitrogen fertilisers. In the experiment discussed here, increasing doses of mineral fertilisation with nitrogen to the level of 90 kg N ha^{-1} resulted in an increase in phosphorus, potassium, calcium and magnesium in spring wheat grain. However, WILCZEWSKI et al. (2013) demonstrated that increasing doses of mineral fertilisation with nitrogen to the level of as much as 160 kg N ha^{-1} contributed to an increase in microelements in spring wheat grain when applied against the background of various preceding crops. In turn, GONDEK and GONDEK (2010), BUCZEK et al. (2011) and JARECKI et al. (2019) confirmed an insignificant increase in the cereal grain content of microelements following the application of high doses of mineral fertilisation with nitrogen. Thus, there is still insufficient research evidence concerning mineral content in cereal grain following a simultaneous application of nitrogen supplied with bioproducts and mineral fertilisers in sustainable agriculture. It seems that this agricultural system should be encouraged, as indicated by findings of the present work, where it was demonstrated that the simultaneous application of *Azotobacter vinelandii* + L-α proline and mineral fertilisation with nitrogen at a dose of 90 kg N ha^{-1} ensured production of spring wheat grain containing high quality protein and macroelements, which are important in human nutrition. What is more, this form of fertilisation protects the soil environment, an issue of growing importance in modern agriculture. In summary, simultaneous application of nitrogen bioproducts and lower doses of mineral fertilisation with nitrogen should be recommended when cultivating spring wheat in sustainable agriculture.

CONCLUSIONS

1. The highest content of total protein was recorded in spring wheat grain after application of *Azotobacter vinelandii* + L-α proline and with mineral nitrogen fertilisation at doses of 120 kg N ha^{-1} and 90 kg N ha^{-1}.

2. Fertilisation of spring wheat with the nitrogen dose of 90 kg N ha^{-1} and the use of *Azotobacter vinelandii* + L-α proline ensured the highest concentration of macronutrients in the grain.

3. The use of *Azotobacter vinelandii* and L-α proline at a dose of mineral nitrogen fertilisation of 90 kg N ha^{-1} ensures favorable chemical composition of spring wheat grain.

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