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ORIGINAL PAPER

Effect of plant growth-promoting bacteria and undersown crops on the total protein content of spring barley grain grown in organic agriculture

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Abstract

The use of innovative cereal cultivation technology based on the application of plant growth-promoting bacteria together with undersown legume is gaining particular importance, especially in organic cereal cultivation, which is expected to provide raw material with high protein content under changing climate conditions. To address this, a field study was conducted in 2019-2021 to determine the effects of plant growth-promoting rhizobacteria (PGPR) and phosphorus-releasing bacteria applied together with undersown crops on the nitrogen content, total protein and total protein yield of spring barley grown in organic agriculture. Two factors were investigated in the experiment. A: bacterial formulation: control (without bacterial formulation), plant growth-promoting rhizobacteria (PGPR) (*Bacillus subtilis, Bacillus amyloliquefaciens, Pseudomonas fluorenscens*), phosphorus-releasing bacteria (*Bacillus megaterium var. phosphaticum, Arthrobacter agilis*). B: undersown crops: control (no undersown crops), red clover, red clover + Italian ryegrass, Italian ryegrass. The nitrogen content was determined in spring barley grain, and subsequently total protein content and total protein yield were calculated. The results substantiate the conclusion that the development of an innovative technology of spring barley cultivation based on the application of PGPR bacteria together with undersown red clover or a mixture of red clover and Italian ryegrass will ensure obtaining grain with high total protein and total protein yield. Such grain meets high quality parameters, and is a valuable raw material for the production of groats and flakes that are functional foods from organic cultivation.

Keywords: *Hordeum vulgare* L., bacterial formulation, red clover, Italian ryegrass

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INTRODUCTION

Spring barley grain from organic agriculture is destined for consumption, and its nutritional value increases with increasing protein content (Alharbi et al. 2022). In recent years, the world, including Poland, has been experiencing climatic change due to periodic precipitation deficits and rising temperatures during the growing season of cereals, which negatively affects their yield and grain chemical composition (Hafez et al. 2021, Koryagin et al. 2022). Thus, it is necessary to introduce innovative technologies for grain cultivation to mitigate the detrimental effects of drought, which will ensure high grain yields with high nutrient content. This will also enable an uninterrupted supply of food, with good quality, increasing food security.

Global research in recent years has shown that the use of PGPR bacteria provides plant resistance to environmental stresses, including drought stress (Hafez et al. 2019). PGPR bacteria alter root morphologies, resulting in better absorption of nutrients, including nitrogen, and they increase soil water content (Glick 2014). Bacteria that stimulate plant growth and development work through various mechanisms. Many studies have demonstrated that PGPR bacteria can act as biofertilizers that directly promote plant growth by improving acquisition of certain nutrients through different processes, such as nitrogen fixation, mineral solubilization, phosphate and potassium absorption, siderophore production and iron sequestration (Hardoim et al. 2015). PGPR bacteria can also produce cytokinins, gibberellins or both to promote plant growth, thereby increasing the nutritional value of cereal grains (Kang et al. 2014). Also, microorganisms such as phosphorus-releasing bacteria tend to produce enzymes and solubilize insoluble phosphate from organic and inorganic phosphate sources (Ahmad et al. 2017, Iqbal et al. 2022). Phosphorus is a macronutrient essential for proper plant growth and development, but it is often the limiting nutrient in the soil, and its deficiency degrades the chemical composition of cereal grains. Therefore, the acquisition of P from the soil by plant roots is of great interest, including the use of an organic, alternative form of this element, i.e. form phosphorus-releasing bacteria, which stimulate plant growth and have a beneficial effect on the nutritional value of grain (Pan et al. 2019, Psakia et al. 2019, Zaballa et al. 2020). Undersown crops also protect the soil from water loss by increasing the compactness of the canopy (van Diggelen et al. 2021, Liu et al. 2022, Poudel et al. 2022). In addition, legume undersowns, thanks to nodule bacteria, have the ability to biologically reduce molecular nitrogen from the air (Płaza et al. 2020). They release its excess into the soil environment, which is then taken up by grain crops, resulting in an increase in the nitrogen and protein content in grain (Wittwer et al. 2017). In Poland, there is a lack of research on the combined use of bacterial formulations with undersown crops as a live mulch in cereal crops. Therefore, an attempt was made to develop an innovative technology for spring barley cultivation based on the use of PGPR bacteria together with undersown crops, which will ensure obtaining spring barley grain with high protein content for consumption use. The aim of the study was to determine the effect of PGPR bacteria and phosphorus-releasing bacteria applied together with undersown crops on the nitrogen content, total protein and total protein yield of spring barley grown in organic agriculture.

MATERIALS AND METHODS

The spring barley grain used for chemical analyses came from a field experiment conducted in 2019-2021, on an organic farm located in the village of Wyłazy (52°12′35″ N 22°11′05″ E) near Siedlce, Poland. The weather conditions during the field experiment are shown in Figure 1.

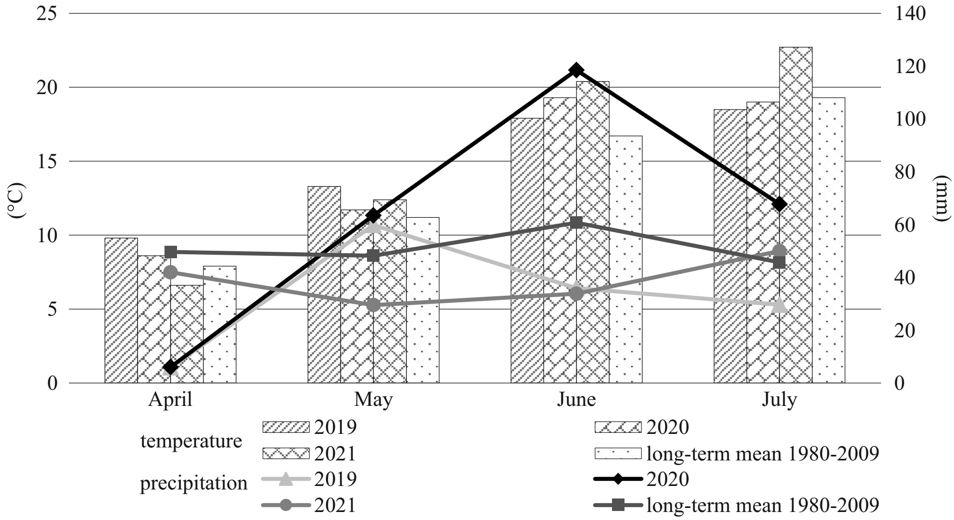

Fig. 1. Weather conditions during the growing season of spring barley, according to the Zawady Meteorological Station

The field experiment was conducted on Stagnic Luvisol soil (IUSS Working Group WRB 2022). The content of available mineral nutrients in the soil before the establishment of the experiment is presented in Table 1.

Table 1

The content of available mineral nutrients in the soil

Content of available mineral nutrients (mg kg^{-1} soil)			pH in KCL	Organic carbon, % p.s.m.
P	K	Mg		
86	123	43	6.0	1.06

The experiment was set up in triplicate. Two factors were researched: A – application of bacterial formulations, B – undersown crops. The detailed treatments used in growing spring barley and the experimental scheme are shown in Figure 2.

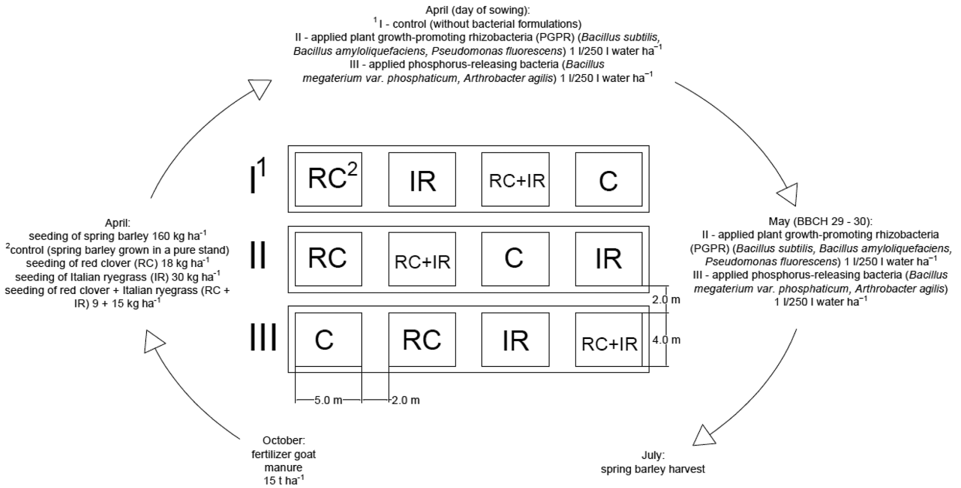

Fig. 2. The schematic presentation of the field experiment

The bacterial species used for inoculation came from the collection of the Department of Soil Science and Microbiology of the Poznań University of Life Sciences. The density of bacterial cells in the bacterial formulations was determined using direct microscopy in a Thoma cell counting chamber, revealing 10^8 cells in 1 mL of culture. Spring barley grains were harvested from an area of $1m^2$ in each experimental plot. After spring barley was harvested, the grain was transported to a laboratory for grain yield and quality evaluation. Grain yield and chemical analyses were performed at an average grain moisture content of 13.6%. The nitrogen content of spring barley grain was determined using the Kjeldahl method (Krełowska-Kułas 1993). Total protein content was calculated by multiplying the nitrogen content by a factor of 6.25, and total protein yield was determined by multiplying the total protein content by grain yield. Data for each studied characteristic were analyzed with ANOVA. The significance of sources of variability was tested using the Fisher-Snedecor F-test ($F \leq 0.05$), and the differences between the compared averages were verified using the Tukey's HSD test ($p \leq 0.05$). The strength of the relationships between spring barley grain yield, total protein content and total protein yield was assessed by calculating the Pearson's correlation coefficients. All the calculations were performed in Statistica, version 13.3.

RESULTS AND DISCUSSION

Statistical analysis demonstrated a significant effect of the experimental factors studied and their interaction on the total protein content of spring barley grain (Table 2).

Table 2

Total protein content in spring barley grain depending on bacterial formulations and undersown crops means across 2019-2021 (g kg^{-1} d.m.)

Bacterial formulations[#] (A)	Undersown crops (B)				Means
	control	red clover	red clover + Italian ryegrass	Italian ryegrass	
I	106.0 ± 13.1	108.2 ± 14.6	109.1 ± 11.8	105.1 ± 13.2	107.1 ± 13.6
II	107.5 ± 13.1	111.8 ± 12.9	111.5 ± 12.7	106.3 ± 13.3	109.3 ± 13.2
III	106.7 ± 11.5	110.4 ± 12.8	109.9 ± 12.6	106.2 ± 13.3	108.3 ± 12.8
Means	106.7 ± 12.7	110.1 ± 13.5	110.2 ± 12.7	105.9 ± 13.3	
$HSD_{0.05}$: A – 0.4; B – 0.4; A × B – 0.8					

[#] I – control (without bacterial formulations), II – PGPR bacteria (*Bacillus subtilis*, *Bacillus amyloliquefaciens*, *Pseudomonas fluorescens*), III – phosphorus-releasing bacteria (*Bacillus megaterium* var. *phosphaticum*, *Arthrobacter agilis*), ± – standard deviation

The highest total protein content was recorded in spring barley grain after the application of PGPR bacteria (*Bacillus subtilis, Bacillus amyloliquefaciens, Pseudomonas fluorenscens*), significantly lower after the application of phosphorus-releasing bacteria (*Bacillus megaterium var. phosphaticum, Arthrobacter agilis*), and the lowest on the control, without the bacteria application. Studies by Basu et al. (2021), Elkelish et al. (2021) and Sedri et al. (2022) demonstrated positive effects of PGPR bacteria on both quantitative and qualitative traits of crop plants, including total protein content of cereal grains. Also in a study by El-Sawah et al. (2021) the application of PGPR bacteria (*Bradyrhizobium* sp. and *Bacillus subtilis*) led to improvements in shoot length, root length, number of branches, plant dry weight, leaf area index (LAI), chlorophyll content, nutrient uptake, including nitrogen and seed and protein yield compared to control plants. Also, studies by Gupta et al. (2013), Kudoyarova et al. (2017), Amna et al. (2019), Sood et al. (2020) and Mirskaya et al. (2022) demonstrated that PGPR bacteria stimulate plant growth and development through various mechanisms. They directly affect plant growth through production of phytohormones (Guta et al. 2013, Kudoyarova et al. 2017), ACC deaminase activity, nitrogen-fixing activity, and solubilization of potassium, phosphorus and zinc (Gupta et al. 2013, Amna et al. 2019, Sood et al. 2020, Rashid et al. 2021). As many as 80% of the bacteria inhabiting the root zone of plants synthesize auxins, which stimulate root cell proliferation and increase the host plant's uptake of minerals and nutrients from the soil, including nitrogen, which translates into an increase in the total protein content of cereal grains (Mirskaya et al. 2022). A study by Zaballa et al. (2020) demonstrated that the phosphorus-releasing bacteria *Entobacter ludwigii* stimulates the growth of *Hordeum valgare*, which indirectly increases total protein content in grain. In our study, the use of undersown crops also significantly differentiated the total protein content of spring barley grain. The highest content of this component was recorded in the grain of spring barley grown when undersown with a mixture of red clover and Italian ryegrass and red clover, significantly lower on the control, without undersown crops, and the lowest when undersown with Italian ryegrass. This is because grasses compete with cereals for nutrients, including nitrogen, resulting in lower concentrations of nitrogen in cereal grain, even compared to cereals grown in pure sowing (Liu et al. 2022, Poudel et al. 2022). On the other hand, legume undersowns, especially those grown in mixtures with grasses, yield more stably and show the ability to biologically reduce molecular nitrogen from the air due to symbiosis with nodule bacteria of the genus Rhizobium (Płaza et al. 2020). They release excess nitrogen into the soil environment, from where cereals uptake it, resulting in an increase of the total protein content of cereal grains (Toukabri et al. 2021). Also, a study by Pellegrini et al. (2021) showed an increase in the protein content of wheat grain grown with living mulch of Persian clover compared to wheat grown in pure sowing. The experiment here also demonstrated an interaction implicating that after the application of PGPR bacteria, the highest total protein content was recorded in spring barley grain grown when undersown with red clover and a mixture of red clover and Italian ryegrass, significantly lower in the control variant, and the lowest when undersown with Italian ryegrass. After the application of phosphorus-releasing bacteria, the highest concentration of total protein was recorded in spring barley grain undersown with red clover and a mixture of red clover and Italian ryegrass and significantly lower in the control and undersown of Italian ryegrass. On the other hand, on plots without bacterial application, the highest total protein content was recorded in the grain of spring barley undersown with a mixture of red clover and Italian ryegrass, significantly lower when undersown red clover, followed by the control, and the lowest when undersown with Italian ryegrass.

Statistical analysis demonstrated a significant effect of the growing season conditions and their interaction with bacterial formulations on the total protein content of spring barley grain (Table 3).

The highest total protein content was recorded in the grain of spring barley grown in the favourable 2020, significantly lower in the dry 2019 and the lowest in 2021. It should be noted that the use of bacterial formulations mitigates the effects of drought and increases the nitrogen content of spring barley grain. Also, studies by Hafez et al. (2021) and Koryagin et al. (2022) showed that PGPR bacteria effectively overcome the negative effects

Table 3

The total protein content in spring barley grain in relation to bacterial formulations and growing season conditions (g kg^{-1} d.m.)

Bacterial formulations[#] (A)	Years (Y)		
	2019	2020	2021
I	104.8 ± 2.3	124.4 ± 1.7	92.2 ± 1.5
II	107.2 ± 3.3	126.0 ± 1.9	94.7 ± 2.3
III	105.6 ± 2.5	125.4 ± 1.6	93.9 ± 2.0
Means	105.9 ± 2.9	125.3 ± 1.9	93.6 ± 2.2
$HSD_{0.05}$: Y – 0.36, Y × A – 0.70			

[#] see Table 2

of drought in plants. Most of these bacteria have the ability to support plant growth and development under natural conditions by fixing nitrogen, producing phytohormones, and improving nutrient availability in many drought-prone plants (Mumtaz et al. 2019). Thus, the use of PGPR bacteria in the organic farming of spring barley is an innovative technology that should be recommended in modern agriculture facing increasingly frequent droughts. Research by other authors shows that weather conditions during the growing season of cereals, especially during flowering, grain filling and ripening, affect the efficiency of protein accumulation in grains (Jolánkai et al. 2018, Wan et al. 2020). Research by Garcia Del Moral et al. (2007) revealed high protein content in wheat grains grown in warm and dry climates. The effect of lower total protein content in years with high precipitation is most often attributed to the dilution effect (Yang et al. 2018). This is not consistent with the results obtained in the authors' own research, in which higher total protein content was characterized by barley grains grown in the year with the highest precipitation. However, in the analyzed years of the research, the distribution of precipitation was very uneven. The higher sum of precipitation in year 2020 was mainly due to the high precipitation recorded in June. The protein content in cereals is conditioned mainly by the uptake of nitrogen by vegetative organs up to the flowering stage and the translocation of nitrogen reserves to grains at the filling stage (Arisnabarreta, Miralles 2008). Research by Hu et al. (2021) revealed 20% less nitrogen uptake by wheat in a year with lower precipitation. The authors also noted that precipitation deficiencies in April and May, the period before flowering, were crucial. Hu et al. (2021) also observed that precipitation deficiency in the post-flowering period could interfere with nitrogen translocation to grain. Also, Michaletti et al. (2018) suggest that drought stress during the period during flowering and grain filling reduces nitrogen utilization, leading to lower grain protein content. In some field studies, several researchers (Hu et al. 2018, Kondić-Špika et al. 2019) obtained higher total protein contents in cereal grains in seasons with higher water availability with the distribution of precipitation during the growing season similar to that in the study reported in this article. It can be concluded that the effect of weather conditions on the total protein content of grain is not totally determined by the sum of precipitation during the entire growing season versus its distribution during the stages of plant development. Thus, to fully assess the impact of weather conditions, it is necessary to consider the sum of precipitation and temperature at different stages of plant growth. In our research, an interaction was demonstrated showing that in the dry year 2019, the highest total protein content was recorded after the application of PGPR bacteria, significantly lower after the application of phosphorus--releasing bacteria, while the lowest was on control sites without bacterial formulation. On the other hand, in 2020 and 2021, the lowest total protein content was recorded on the plots where no bacterial formulations were applied, while significantly higher content was revealed after the application of PGPR bacteria and phosphorus-releasing bacteria. The interaction of growing season conditions with undersown crops was demonstrated (Figure 3).

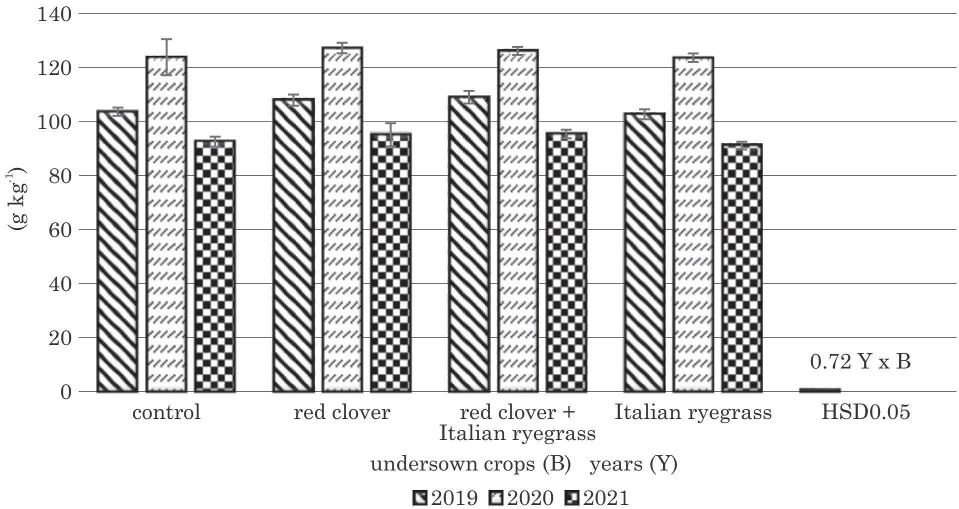

Fig. 3. The total protein content in spring barley grain in relation to undersown crops and growing season conditions (g kg^{-1} d.m.)

In the favourable year 2020, the highest total protein content was recorded in the grain of spring barley grown when undersown with red clover, significantly lower with a undersown of a mixture of red clover and Italian ryegrass and the lowest on the control, without being undersown and with an undersown of Italian ryegrass. In 2021, with less total precipitation, the highest total protein content was recorded in spring barley grain grown when undersown with red clover and a mixture of red clover and Italian ryegrass, lower on the control, without undersown crops, and lowest with undersown of Italian ryegrass. On the other hand, in dry 2019, the highest total protein content was recorded in the grain of spring barley grown with undersown of mixed red clover with Italian ryegrass, significantly lower with undersown of red clover, then with undersown of Italian ryegrass, and the lowest on the control. It should be explained that in dry years, undersown legumes are relatively unreliable in yield, legume-grass mixtures prove to thrive better, providing more nitrogen to the soil, which is taken up by cereals, resulting in an increase in the total protein content in grain.

Total protein yield from spring barley grain was significantly differentiated by the research factors of the experiment and their interactions (Table 4).

Table 4

The total protein yield from spring barley grain in relation to bacterial formulations and undersown crops, means across 2019-2021 (kg ha^{-1})

Bacterial formulations[#] (A)	Undersown crops (B)				Means
	control	red clover	red clover + Italian ryegrass	Italian ryegrass	
I	296.8 ± 60.0	418.1 ± 69.7	379.4 ± 88.3	283.9 ± 108.2	344.5 ± 100.6
II	388.2 ± 101.0	530.6 ± 115.4	530.4 ± 99.1	391.5 ± 114.5	460.2 ± 128.6
III	326.7 ± 69.4	478.0 ± 71.6	435.0 ± 109.2	339.6 ± 84.1	394.8 ± 106.2
Means	337.6 ± 87.3	475.6 ± 99.4	448.8 ± 116.9	338.3 ± 112.1	
$HSD_{0.05}$: A – 19.3, B – 22.3, A × B – 36.2					

[#] see Table 2

The highest yield of total protein was obtained from spring barley grain after the application of PGPR bacteria (*Bacillus subtilis, Bacillus amyloliquefaciens, Pseudomonas fluorenscens*), significantly lower after the application of phosphorus-releasing bacteria (*Bacillus megaterium var. phosphaticum, Arthrobacter agilis*), and the lowest on the control, without the application of bacteria. Biofertilizers are an innovative technology used in cereal cultivation, especially in organic farming. They provide improvements in yield quality and quantity (Chittora et al. 2020). Also, in a study by El-Sawah et al. (2021), the use of PGPR bacteria (*Bradyrhizobium* sp. and *Bacillus subtilis*) led to an increase in seed and protein yield compared to control plants. In our study, undersown crops also significantly differentiated the yield of total protein from spring barley grain. The highest total protein yield was obtained from spring barley grain grown when undersown with red clover, significantly lower with undersown mixture of red clover and Italian ryegrass, and the lowest with undersown Italian ryegrass and on a control plot without any undersown crop. It should be noted that undersown legumes and their mixtures with grasses are also a source of nitrogen for cereals, especially in organic farming. The best-known effect of legumes on the soil is its enrichment in nitrogen bound by nodule bacteria living in symbiosis with legumes (Shendy 2015, Płaza et al. 2020). The nitrogen is then taken up by grain, which increases the protein content and yield of the grain (Toukabri et al. 2021). In our study, we also found an interaction implicating that after the application of PGPR bacteria the highest yield of total protein was obtained from spring barley grain undersown with red clover and a mixture of red clover and Italian ryegrass, and the lowest – from spring barley with undersown Italian ryegrass and on the control plot. On the other hand, after the application of phosphorus-releasing bacteria, the highest yield of total protein was obtained from spring barley undersown with red clover, significantly lower with undersown mixture of red clover and Italian ryegrass, and the lowest with undersown Italian ryegrass and in the control.

Statistical analysis showed a significant effect of growing season conditions and their interaction with bacterial formulations on the yield of total protein obtained from spring barley grain (Table 5).

Table 5

The total protein yield from spring barley grain in relation to bacterial formulations and growing season conditions (kg ha^{-1})

Bacterial formulations[1] (A)	Years (Y)		
	2019	2020	2021
I	254.0 ± 59.1	411.4 ± 113.5	370.3 ± 26.8
II	318.4 ± 65.9	572.7 ± 60.0	489.5 ± 90.1
III	283.4 ± 67.3	471.3 ± 67.8	429.9 ± 72.6
Means	285.3 ± 69.4	485.1 ± 107.0	429.9 ± 84.1
$HSD_{0.05}$: Y – 17.4, Y × A – 33.4			

[#] see Table 2

The highest yield of total protein was obtained in the favourable 2020, significantly lower in 2021 with less precipitation and the lowest in the dry 2019. Total protein yield is the product of grain yield and total protein content. Therefore, total protein yield was lowest in the dry year 2019, caused by low grain yield. The use of PGPR bacteria increases plant tolerance to drought, accelerates nutrient uptake and increases soil moisture (Hafez et al. 2019). Also, research by Basu et al. (2021) and Sedri et al. (2022) demonstrated the beneficial effect of PGPR bacteria on grain protein yield, even under drought conditions. PGPR bacteria can improve plants' drought tolerance by stimulating the production of drought-tolerant substances, such as amino acids, 1-aminocyclopropane-1- carboxylate deaminase, volatile organic compounds, sugars (that prevent degenerative processes), bacterial exopolysaccharides and phytohormones, like auxin or indole-3-acetic acid, cytokinin (CK), abscisic acid, ethylene, salicylic acid. These stress relievers can prevent the buildup of reactive oxygen species (ROS) through the production of antioxidant enzymes (Chieb, Gachomo 2023). In the experiment in question, an interaction was demonstrated, showing that in all years of study, the highest yield of total protein from spring barley grain was recorded after the application of PGPR bacteria in spring barley, and significantly lower after the application of phosphorus-releasing bacteria. This is due to the fact that PGPR bacteria stimulate plant growth and development and increase nutrient uptake by grains, resulting in an increase in total protein yield.

In our study, we also demonstrated the interaction of weather conditions with undersown crops on the total protein yield obtained from spring barley grain (Figure 4).

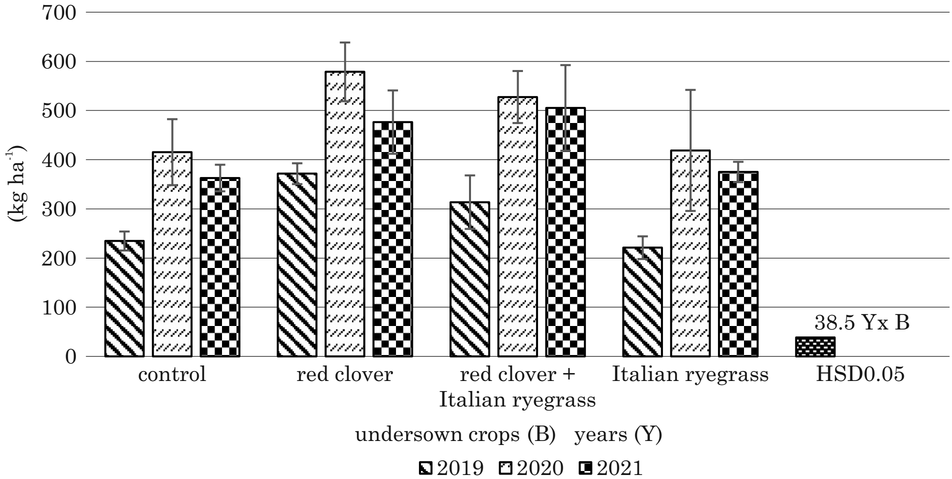

Fig. 4. The total protein yield from spring barley grain in relation to undersown crops and growing season conditions (kg ha^{-1})

In the favourable 2020, as well as in the dry 2019 years, the highest total protein yield was obtained from spring barley grain grown when undersown with red clover, significantly lower with undersown mixture of red clover and Italian ryegrass, and the lowest with undersown Italian ryegrass and in the control. In 2021, with less precipitation, the highest total protein yield was obtained from spring barley grain grown together with undersown red clover and a mixture of red clover and Italian ryegrass, and the lowest – with undersown Italian ryegrass and in the control plots.

In order to evaluate the effect of total protein content and grain yield of spring barley on the total protein yield, the Pearson's correlation coefficient analysis was performed (Table 6).

Table 6

Correlation coefficients between total protein content and grain yield of spring barley and total protein yield

Parameter	Grain yield	Total protein content
total protein yield	0.8524**	0.3929**
** $p<0.01$		

The correlation analysis demonstrated a highly significant (p<0.01) effect of spring barley grain yield on the total protein yield obtained. On the other hand, a highly significant (p<0.01) but lower correlation value was also obtained between grain total protein content and spring barley total protein yield. The correlation analysis demonstrated that in the presented study, spring barley grain yield has a higher influence on the obtained total protein yield compared to grain total protein content.

CONCLUSIONS

1. The highest total protein content and the highest total protein yield were recorded from spring barley grain after application of PGPR bacteria.

2. Spring barley grain undersown with red clover and a mixture of red clover and Italian ryegrass had the highest total protein content and total protein yield.

3. The conditions of the growing season significantly differentiated the total protein content and total protein yield of spring barley grain.

4. The innovative technology of spring barley cultivation based on the use of PGPR bacteria together with undersown red clover or a mixture of red clover and Italian ryegrass ensures the production of grain with high total protein content, which meets high quality parameters, and is a valuable raw material for the production of groats and flakes that are functional foods from organic farming.

Author contributions

R.G. – methodology, project administration, software, writing – original draft preparation, writing – review & editing, investigation, supervision, visualization, A.P – writing – original draft preparation, writing – review & editing, investigation, funding acquisition, supervision, visualization, A.N. – formal analysis, project administration, resources, K.G. – formal analysis, resources, R.R. – software, visualization. All authors have read and agreed to the published version of the manuscript.

Conflicts of interest

The authors ensure that they have neither professional nor financial connections related to the manuscript sent to the Editorial Board

REFERENCES

Ahmad, M., Zahir, Z.A., Jamil, M., Nazli, F. and Iqbal, Z. (2017) 'Field application of ACC-deaminase biotechnology for improving chickpea productivity in Bahawalpur', *Soil & Environment*, 36(2), 93-102, available: https://doi.org/10.25252/SE/17/51189

Alharbi, K., Rashwan, E., Mohamed, H.H., Awadalla, A., Omara, A.E.-D., Hafez, E.M. and Alshaal, T. (2022) 'Application of silica nanoparticles in combination with two bacterial strains improves the growth, antioxidant capacity and production of barley irrigated with saline water in salt-affected soil', *Plants,* 11(15), 2026, available: https://doi.org/10.3390/plants11152026

Amna, Ud Din, B., Sarfraz, S., Xia, Y., Kamran, M.A., Javed, M.T., Sultan, T., Munis, M.F.H. and Chaudhary, H.J. (2019) 'Mechanistic elucidation of germination potential and growth of wheat inoculated with exopolysaccharide and ACC-deaminase producing *Bacillus strains* under induced salinity stress', *Ecotoxicology and Environmental Safety*, 183, 109466, available: https://doi.org/10.1016/j.ecoenv.2019.109466

Andrews, J.S., Sanders, Z.P., Cabrera, M.L., Saha, U.K. and Hill, N.S. (2018) 'Nitrogen dynamics in living mulch and annual cover crop corn production systems', *Agronomy Journal*, 110(4), 1309-1317, available: https://doi.org/10.2134/agronj2017.10.0609

Arisnabarreta, S., Miralles, D.J. (2008) 'Radiation effects on potential number of grains per spike and biomass partitioning in two- and six-rowed near isogenic barley lines', *Field Crops Research*, 107, 203-210, available: https://doi.org/10.1016/j.fcr.2008.01.005

Basu, A., Prasad, P., Das, S.N., Kalam, S., Sayyed, R.Z., Reddy, M.S. and El Enshasy, H. (2021) 'Plant growth promoting rhizobacteria (PGPR) as green bioinoculants: recent developments, constraints, and prospects', *Sustainability*, 13(3), 1140, available: https://doi.org/10.3390/su13031140

Chieb, M. and Gachomo, E.W. (2023) 'The role of plant growth promoting rhizobacteria in plant drought stress responses', *BMC Plant Biology,* 23, 407, available: https://doi.org/10.1186/s12870-023-04403-8

Chittora, D., Meena, M., Barupal, T., Swapnil, P. and Sharma, K. (2020) 'Cyanobacteria as a source of biofertilizers for sustainable agriculture', *Biochemistry and Biophysics Reports*, 22, 100737, available: https://doi.org/10.1016/j.bbrep.2020.100737

Garcia Del Moral, L.F., Rharrabti, Y., Martos, V. and Royo, C. (2007) 'Environmentally induced changes in amino acid composition in the grain of durum wheat grown under different water and temperature regimes in a Mediterranean environment', *Journal of Agricultural and Food Chemistry*, 3, 8144-8151, available: https://doi.org/10.1021/jf063094q

Elkelish, A., El-Mogy, M.M., Niedbała, G., Piekutowska, M., Atia, M.A.M., Hamada, M.M.A., Shahin, M., Mukherjee, S., El-Yazied, A.A., Shebl, M., Jahan, M.S., Osman, A., Abd El-Gawad, H.G., Ashour, H., Farag, R., Selim, S. and Ibrahim, M.F.F. (2021) 'Roles of exogenous *α*-lipoic acid and cysteine in mitigation of drought stress and restoration of grain quality in wheat', *Plants*, 10(11), 2318, available: https://doi.org/10.3390/plants10112318

El-Sawah, A.M., El-Keblawy, A., Ali, D.F.I., Ibrahim, H.M., El-Sheikh, M.A., Sharma, A., Alhaj Hamoud, Y., Shaghaleh, H., Brestic, M., Skalicky, M., Xiong, Y. and Sheteiwy., M.S. (2021) 'Arbuscular mycorrhizal fungi and plant growth-promoting rhizobacteria enhance soil key enzymes, plant growth, seed yield, and qualitative attributes of guar', *Agriculture,* 11(3), 194, available: https://doi.org/10.3390/agriculture11030194

Glick, B.R. (2014) 'Bacteria with ACC deaminase can promote plant growth and help to feed the world', *Microbiological Research*, 169(1), 30-39, available: https://doi.org/10.1016/j.micres.2013.09.009

Gupta, G., Panwar, J. and Jha, P.N. (2013) 'Natural occurrence of Pseudomonas aeruginosa, a dominant cultivable diazotrophic endophytic bacterium colonizing *Pennisetum glaucum* (L.) R. Br.', *Applied Soil Ecology*, 64, 252-261, available: https://doi.org/10.1016/j.apsoil.2012.12.016

Hafez, E., Omara, A.E.D. and Ahmed, A. (2019) 'The coupling effects of plant growth promoting Rhizobacteria and salicylic acid on physiological modifications, yield traits, and productivity of wheat under water deficient conditions', *Agronomy*, 9(9), 524, available: https://doi.org/10.3390/agronomy9090524

Hafez, E.M., Osman, H.S., Gowayed, S.M., Okasha, S.A., Omara, A.E.D., Sami, R., Abd El-Monem, A.M. and Abd El-Razek, U.A. (2021) 'Minimizing the adversely impacts of water deficit and soil salinity on maize growth and productivity in response to the application of plant growth-promoting Rhizobacteria and silica nanoparticles', *Agronomy*, 11(4), 676, available: https://doi.org/10.3390/agronomy11040676

Hardoim, P.R., van Overbeek, L.S., Berg, G., Pirttilä, A.M., Compant, S., Campisano, A., Döring, M. and Sessitsch, A. (2015) 'The hidden world within plants: ecological and evolutionary considerations for defining functioning of microbial endophytes', *Microbiology and Molecular Biology Reviews*, 79(3), 293-320, available: https://doi.org/10.1128/mmbr.00050-14

Hu, Y., Barmeier, G. and Schmidhalter, U. (2021) 'Genetic variation in grain yield and quality traits of Spring malting barley. *Agronomy*, 11(6), 1177, available: https://doi.org/10.3390/agronomy11061177

IUSS Working Group WRB (2022) 'World Reference Base for Soil Resources. International soil classification system for naming soils and creating legends for soil maps. 4th edition', *International Union of Soil Sciences (IUSS)*, Vienna, Austria

Jolánkai, M., Kassai, M.K., Tarnawa, Á., Pósa, B. and Birkás, M. (2018) 'Impact of precipitation and temperature on the grain and protein yield of wheat (*Triticum aestivum* L.) varieties', *Journal of the Hungarian Meteorological Service*, 122(1), 31-40, available: https://doi.org/10.28974/idojaras.2018.1.3

Iqbal, Z., Bushra, Hussain, A., Dar, A., Ahmad, M., Wang, X., Brtnicky, M. and Mustafa, A. (2022) 'Combined use of novel endophytic and rhizobacterial strains upregulates antioxidant enzyme systems and mineral accumulation in wheat', *Agronomy*, 12(3), 551, available: https://doi.org/10.3390/agronomy12030551

Kang, S.M., Khan, A.L., You, Y.H., Kim, J.G., Kamran, M. and Lee, I.J. (2014) 'Gibberellin production by newly isolated strain *Leifsonia soli* SE134 and its potential to promote plant growth', *Journal of Microbiology and Biotechnology*, 24(1), 106-112, available: http://dx.doi.org/10.4014/jmb.1304.04015

Kondić-Špika, A., Mladenov, N., Grahovac, N., Zorić, M., Mikić, S., Trkulja, D., Marjanović--Jeromela, A., Miladinović, D. and Hristov, N. (2019) 'Biometric analyses of yield, oil and protein contents of wheat (*Triticum aestivum* L.) genotypes in different environments. *Agronomy*, 9(6), 270, available: https://doi.org/10.3390/agronomy9060270

Koryagin, Y.V., Kulikova, E.G., Koryagina, N.V. and Trishina, V.A. (2022) 'Application of microbiological fertilizers in barley cultivation technology', *IOP Conference Series: Earth and Environmental Science*, 953, available: https://doi.org/10.1088/1755-1315/953/1/012005

Krełowska-Kułas, M. (1993) 'Examination of foodstuff quality', PWE, Warszawa, p. 1-588 (in Polish)

Kudoyarova, G.R., Vysotskaya, L.B., Arkhipova, T.N., Kuzmina, L.Y., Galimsyanova, N.F., Sidorova, L.V., Gabbasova, I.M., Melentiev, A.I. and Veselov, S.Y. (2017) 'Effect of auxin producing and phosphate solubilizing bacteria on mobility of soil phosphorus, growth rate, and P acquisition by wheat plants', *Acta Physiologiae Plantarum*, 39, 253, available: https://doi.org/10.1007/s11738-017-2556-9

Liu, X., Wang, S., Zhuang, Q., Jin, X., Bian, Z., Zhou, M., Meng, Z., Han, C., Guo, X., Jin, W. and Zhang, Y. (2022) 'A Review on carbon source and sink in arable land ecosystems', *Land*, 11(4), 580, available: https://doi.org/10.3390/land11040580

Michaletti, A., Naghavi, M.R., Toorchi, M., Zolla, L. and Rinalducci, S. (2019). Metabolomics and proteomics reveal drought-stress responses of leaf tissues from spring-wheat. *Scientific Reports*, 8, 5710, available: https://doi.org/10.1038/s41598-018-24012-y

Mirskaya, G.V., Khomyakov, Y.V., Rushina, N.A., Vertebny, V.E., Chizhevskaya, E.P., Chebotar, V.K., Chesnokov, Y.V. and Pishchik, V.N. (2022) 'Plant Development of Early -Maturing Spring Wheat (*Triticum aestivum* L.) under Inoculation with *Bacillus* sp. V2026', *Plants*, 11(14), 1817, available: https://doi.org/10.3390/plants11141817

Mumtaz, M.Z., Barry, K.M., Baker, A.L., Nichols, D.S., Ahmad, M., Zahir, Z.A. and Britz, M.L. (2019) 'Production of lactic and acetic acids by *Bacillus* sp. ZM20 and *Bacillus cereus* following exposure to zinc oxide: A possible mechanism for Zn solubilization', *Rhizosphere*, 12, 100170, available: https://doi.org/10.1016/j.rhisph.2019.100170

Pan, Q., Shikano, I., Hoover, K., Liu, T-X. and Felton, G.W. (2019) '*Enterobacter ludwigii*, isolated from the gut microbiota of Helicoverpa zea, promotes tomato plant growth and yield without compromising anti-herbivore defenses', *Arthropod-Plant Interactions*, 13, 271-278, available: https://doi.org/10.1007/s11829-018-9634-9

Pellegrini, F., Carlesi, S., Nardi, G. and Barberi, P. (2021) 'Wheat-clover temporary intercropping under Mediterranean conditions affects wheat biomass, plant nitrogen dynamics and grain quality', *European Journal of Agronomy*, 130, 126347, available: https://doi.org/10.1016/j.eja.2021.126347

Poudel, P., Ødegaard, J., Mo, S.J., Andresen, R.K., Tandberg, H.A., Cottis, T., Solberg, H., Bysveen, K., Dulal, P.R., Mousavi, H. and Solberg, S.Ø. (2022) 'Italian ryegrass, Perennial ryegrass, and Meadow fescue as undersown cover crops in spring wheat and barley: Results from a mixed methods study in Norway', *Sustainability*, 14(20), 13055, available: https://doi.org/10.3390/su142013055

Psakia, O., Mainaa, S., Vlysidisb, A., Papanikolaoua, S., Machado de Castro, A., Freired, D.M.G., Dheskalie, E., Kookose, I. and Koutinasa, A. (2019) 'Optimisation of 2,3-butanediol production by *Enterobacter ludwigii* using sugarcane molasses', *Biochemical Engineering Journal*, 152, 107370, available: https://doi.org/10.1016/j.bej.2019.107370

Płaza, A., Gąsiorowska, B. and Rzążewska, E. (2020) 'Wsiewki roślin bobowatych i ich mieszanek z trawami źródłem azotu biologicznego dla ziemniaka jadalnego', Wydawnictwo UPH Siedlce: Siedlce, Poland, p. 62.

Rashid, U., Yasmin, H., Hassan, M.N., Naz, R., Nosheen, A., Sajjad, M., Ilyas, N., Keyani, R., Jabeen, Z., Mumtaz, S., Alyemeni, M.N. and Ahmad, P. (2021) 'Drought-tolerant Bacillus megaterium isolated from semi-arid conditions induces systemic tolerance of wheat under drought conditions', *Plant Cell Reports*, 41, 549-569, available: https://doi.org/10.1007/s00299-020-02640-x

Sedri, M.H., Niedbała, G., Roohi, E., Niazian, M., Szulc, P., Rahmani, H.A. and Feiziasl, V. (2022) 'Comparative analysis of plant growth-promoting rhizobacteria (PGPR) and chemical fertilizers on quantitative and qualitative characteristics of rainfed wheat', *Agronomy* 12(7), 1524, available: https://doi.org/10.3390/agronomy12071524

Shendy, M.Z. (2015) 'Evaluation of four new barley cultivars productivity intercropped with berseem clover at different seeding rate in new lands in Egipt', *The Bulletin, Faculty of Agriculture-Cairo University*, 66, 29-39.

Sood, G., Kaushal, R. and Sharma, M. (2020) 'Significance of inoculation with Bacillus subtilis to alleviate drought stress in wheat (*Triticum aestivum* L.)', *Vegetos*, 33, 782-792, available: https://doi.org/10.1007/s42535-020-00149-y

Toukabri, W., Ferchichi, N., Hlel, D., Jadlaoui, M., Kheriji, O., Zribi, F., Taamalli, W., Mhamdi, R. and Trabelsi, D. (2021) 'Improvements of durum wheat main crop in weed control, productivity and grain quality through the inclusion of fenugreek and clover as companion plants: Effect of N fertilization regime', *Agronomy*, 11(1), 78, available: https://doi.org/10.3390/agronomy11010078

Wan, Y., Wang, Y., Shi, Z., Rentsch, D., Ward, J.L., Hassall, K. and Hawkesford, M.J. (2021) 'Wheat amino acid transporters highly expressed in grain cells regulate amino acid accumulation in grain', *PLoS ONE*, 16, e024676, available: https://doi.org/10.1371/journal.pone.0246763

Wittwer, R., Dorn, B., Jossi, W. and van der Heijden, M.G.A. (2017) 'Cover crops support ecological intensification of arable cropping systems', *Scientific Reports* 7, 41911, available: https://doi.org/10.1038/srep41911 van Diggelen, R., Bobbink, R., Frouz, J., Harris, J. and Verbruggen, E. (2021) 'Converting agricultural lands into heathlands: The relevance of soil processes', In: Soils Landscape Restor; Stanturf, J.A., Callaham, M.A., Eds.; Academic Press: Cambridge, MA, USA, pp. 357-372, available: https://doi.org/10.1016/B978-0-12-813193-0.00013-8

Yang, R., Liang, X., Torrion, J.A., Walsh, O.S., O'Brien, K. and Liu, Q. (2018) 'The influence of water and nitrogen availability on the expression of end-use quality parameters of spring wheat. *Agronomy*, 8(11), 257, available: https://doi.org/10.3390/agronomy8110257

Zaballa, J.I., Golluscio, R. and Ribaudo, C. M. (2020) 'Effect of the phosphorus-solubilizing bacterium *Enterobacter ludwigii* on barley growth promotion', *American Scientific Research Journal for Engineering, Technology, and Sciences,* 63(1), 144-157.