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NUMBERS OF PHOSPHATE SOLUBILIZING MICROORGANISMS AND PHOSPHATASES ACTIVITIES IN THE RHIZOSPHERE SOIL OF ORGANICALLY AND CONVENTIONALLY GROWN WINTER WHEAT

Summary

In the years 2014-2016 four commercial winter wheat cultivars, released during 2008-2011, were grown in a field experiment at RZD Osiny according to organic and conventional farming systems. Each year in the first or second decade of May samples of rhizosphere soil were analyzed for numbers of colony forming units (CFU) of phosphate solubilizing microorganisms (PSM) and for acid and alkaline phosphatase activities. The synthesis of three-year results has shown that PSM numbers and activity of acid phosphatase in the rhizosphere soil of the tested cultivars were not significantly influenced by the farming systems used, nor by the cultivars. On the other hand, in all years the activity of alkaline phosphatase was significantly higher in the rhizosphere soil of all winter wheat cultivars grown according to the organic farming system than in soil under these cultivars grown in the conventional system.
***Key words**: phosphate solubilizing microorganisms, phosphatase, winter wheat, rhizosphere soil, organic farming, conventional farming*

LICZEBNOŚĆ MIKROORGANIZMÓW ROZPUSZCZAJĄCYCH FOSFORANY ORAZ AKTYWNOŚĆ FOSFATAZ W GLEBIE RYZOSFEROWEJ PSZENICY OZIMEJ UPRAWIANEJ W SYSTEMIE EKOLOGICZNYM I KONWENCJONALNYM

Streszczenie

Cztery komercyjne odmiany pszenicy ozimej, wpisane do rejestru w latach 2008-2011, uprawiano w sezonach wegetacyjnych 2014-2016 według zasad rolnictwa ekologicznego (organicznego) i konwencjonalnego na poletkach doświadczalnych w RZD Osiny. Każdego roku w maju pobierano spod badanych odmian próbki gleby ryzosferowej i oznaczano w nich liczebność (CFU – colony forming units) mikroorganizmów rozpuszczających fosforany (PSM) oraz aktywność fosfatazy kwaśnej i zasadowej. Synteza trzyletnich wyników wykazała, że liczebność PSM, jak i aktywność fosfatazy kwaśnej w glebie ryzosferowej badanych odmian nie były istotnie uzależnione zarówno od systemu uprawy, jak i od odmiany pszenicy ozimej. Jednak we wszystkich latach objętych badaniami aktywność fosfatazy alkalicznej była istotnie większa w glebie ryzosferowej wszystkich odmian pszenicy ozimej uprawianych w systemie ekologicznym (organicznym) niż tych samych odmian uprawianych w systemie konwencjonalnym.
***Słowa kluczowe**: mikroorganizmy rozpuszczające fosforany, fosfatazy, pszenica ozima, gleba ryzosferowa, uprawa ekologiczna, uprawa konwencjonalna*

1. Introduction

In conventional or integrated crop management systems requirements of agricultural crops with respect to phosphorus (P) are satisfied mainly through the application of mineral phosphate fertilizers, which also build-up soil reserves of this nutrient in cropped soils [1, 3, 5, 9]. Numerous soil fertilization trials have shown that up to 70-90% of soluble inorganic fertilizer phosphate added to soils may become immobilized soon after application as a result of its fixation and precipitation with highly reactive Al^{3+} and Fe^{3+} in acidic, and Ca^{2+} in neutral or calcareous soils [3, 5, 8, 9]. P complexes with these metals constitute usually the largest fraction of inorganic phosphate in soils. Soil P occurs also in various fractions of organic matter [5, 9, 10, 12] and organic forms of P constitute usually about 50% of the total phosphorus content in most soils, although they may range from as low as 5% to as high as 95% [5, 12].

Different groups of soil microorganisms possess the ability to liberate phosphates from organic and inorganic pools and thus they can enhance the availability of P to plants [1, 2, 7]. The liberation of organic phosphates by microorganisms is mediated mainly through the synthesis of enzymes such as phosphatases, while the principal mechanism for mineral phosphate solubilization is the production of organic acids, siderophores, protons, hydroxyl ions and CO_2 [1, 2, 5, 8]. These processes seem to be particularly important is the case of organic agriculture in which arable crops acquire essential nutrients, including phosphates, mainly from microbiologically transformed organic amendments (composts, green or animal manures) and natural rocks, like apatites [4, 6].

The aim of this work was to find out if organically and conventionally grown winter wheat cultivars differ with respect to numbers of phosphate solubilizing microorganisms (PSM) and activities of phosphatases (acid and alkaline) in their rhizosphere soil.

2. Material and Methods

The study was based on a long-term field experiment established in 1994 on an Haplic Luvisol (loamy sand) in Osiny Experimental Station (51°28'N, 22°30'E) belonging to the Institute of Soil Science and Plant Cultivation, Pulawy, Poland. Some basic properties of this soil are as follows: C org. – 0.82%; N tot. – 0.1%; pH(KCl) – 5.9; P_2O_5, K_2O and Mg (mg/kg) – 86, 102 and 91, respectively. Main characteristics of weather conditions at this site are presented in Table 1. In this experiment agricultural crops are grown according to three different crop management systems (organic, integrated and conventional). For the purpose of this study fields under the organic and conventional systems were used. The organic system consists of five fields (about 1 ha each) on which the following crops are rotated: potato – spring wheat (under-sown with clover-grass mixture) – clover-grass mixture – winter wheat – oats-field peas mixture. No N mineral fertilizers are used in the organic system, but phosphorus (150 kg/ha) of powdered phosphate rock was applied once per rotation. With respect to K fertilization the winter wheat field was treated with 150 kg/ha of potassium sulfate before sowing. Organic fertilization in this system included 30 Mg ha^{-1} of compost plowed in before potato planting. No synthetic pesticides were applied to control pests and spring harrowing was used to reduce weed infestation in winter wheat plots.

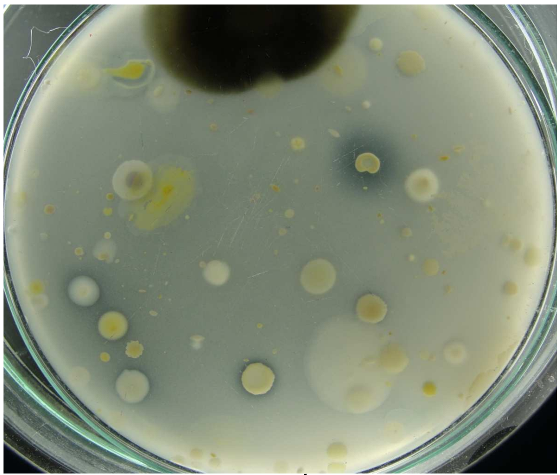
The conventional system consists of three fields (1 ha each) on which winter rape - winter wheat - spring wheat are rotated and managed according to an intensive crop production technology used in Poland. In this farming system the average annual rates of mineral fertilizers were 140 + 60 + 80 kg ha^{-1} of NPK. After harvest, shredded winter wheat straw and rape stems were plowed in every second cropping year. To control weeds and pests, appropriate synthetic pesticides are used according to general rules. To prevent soil acidification in both systems, lime was applied once per each rotation. In 2014, 2015 and 2016 four commercial winter wheat cultivars (Table 2), released during 2008-2011, were grown within winter wheat fields of both cropping systems. In the absence of winter wheat cultivars bread for the purpose of organic agriculture, conventionally bread cultivars were chosen for their high yielding potential and good tolerance to foliar fungal diseases. Each cultivar was planted (4.5 million grains/ha) in 4 replicated and randomized micro-plots (35 m^2).

Samples of the rhizosphere soil were collected in the first or second decade of May 2014, 2015 and 2016 by digging out about 15 plants with soil adhering to their roots. In the laboratory only soil closely adhering to the roots was collected. Moist, sieved (2 mm) soil samples were refrigerated (4^{o}C). Within one week soil samples were analyzed for: acid (AcP) and alkaline (AlP) phosphatases, using *p*-nitrophenyl phosphate (PNP) as the substrate according to Tabatabai & Bremner method [11] and for numbers of colony forming units (CFU) of phosphate solubilizing microorganisms (PSM) using the soil dilution-plate method on Pikowskya agar medium containing $(Ca)_3PO_4$ [7]. On this medium the ability of microorganisms to dissolve tri-calcium phosphate is indicated by clear zones around their colonies (Figure 1).

The data were subject to the two-way analysis of variance (ANOVA) using the FR-ANALWAR software based on Microsoft Excel, with significance of differences assessed by Tukey test at $p < 0.05$.

3. Results and Discussion

Results of the synthesis for the years 2014, 2015 and 2016 are presented in Table 2 and they indicate that PSM numbers were not significantly influenced by the farming systems used, nor by the cultivars. Moreover, interactions between these factors and with years were also insignificant. However the maximum numbers of these bacteria were found in the rhizosphere soil of all cultivars in in 2014 and this effect was connected probably with the highest rainfall in May of that year (Table 1), which resulted in the highest moisture content of rhizosphere soil (Table 3). Only in 2016 the rhizosphere soil of all cultivars grown on conventional plots had markedly lower numbers of PSM than these cultivars grown on organic plots (Table 2).



Source: own elaboration / Źródło: opracowanie własne

Fig. 1. Colonies of phosphate solubilizing microorganisms (with clear zones) on agar medium containing tri-calcium phosphate

Rys. 1. Kolonie mikroorganizmów rozpuszczających fosforan (ze strefami przejaśnienia) na pożywce agarowej zawierającej trójfosforan wapnia

Similarly to PSM numbers the tested experimental factors and their interaction had no significant effect on the activity of acid phosphatase (AcP), but interactions between these factors and the years were significant (Table 2). In the years 2014 and 2016 activities of this enzyme in the rhizosphere soil of conventionally grown winter wheat cultivars were markedly lower than in the rhizosphere soil of organically grown cultivars. In 2015 soil on the conventional winter wheat field on which the studied cultivars were grown was in general substantially more acidic than soil under the organic farming (Table 3) and this lower soil pH was probably the main factor causing that the activity of AcP in the rhizosphere soil of conventionally grown cultivars was higher than that of organically grown cultivars (Table 2). These results indicate that not only farming system, but also soil properties, particularly soil pH, is an important factor influencing the activity of acid phosphatase. The dependence of the activity of soil phosphatases on soil pH is well documented [5, 8, 11]. For example, Lemanowicz and Koper [5] have shown that soil fertilization with increasing N rates caused a significant soil acidification which beneficially affected the activity of AcP in this soil.

Results presented in Table 2 show that the activity of alkaline phosphatase (AlP) was significantly affected by the farming system but not by the studied cultivars. Interactions between these factors had also significant effects. In all years included in this study the activity of this enzyme was significantly higher in the rhizosphere soil of winter wheat cultivars grown according to the organic farming system than in soil under these cultivars grown in the conventional system (Table 2) and this beneficial effect of organic farming on AlP occurred irrespective of soil pH (Table 3) and the cultivars. In our previous reports on the long-term experiment in Osiny, on which the present study was based, it was shown that soil in the organic system contained significantly more organic matter (OM) and microbial biomass C (MBC) than soil in the conventional system [4, 6]. It was also shown that in the soil environment AlP is produced by soil microorganisms, not by plant roots [2, 12]. Close positive relationships between OM and MBC contents in soils and AlP activities are well documented [2, 5, 11, 12] Thus, more active AlP in the organic soil found in our work was most probably related to higher contents of soil organic matter and microbial biomass in this soil as compared to that in the conventional system. Results of this study suggest that the activity of AlP could be used as a reliable biological indicator of organic farming beneficial effect on soil quality.

Table 1. Monthly mean air temperature and total precipitation (2013-2016), and long-term averages of these parameters (1988-2017) at Osiny Experimental Station

Tab. 1. Średnie miesięczne temperatury i opadów (2013-2016) oraz średnie wieloletnie (1988-2017) w RZD Osiny

Month	Temperature (^oC)				Precipitation (mm)			
	2013/ 2014	2014/ 2015	2015/ 2016	1988/2017	2013/ 2014	2014/ 2015	2015/ 2016	1988/2017
IX	11,8	14,7	15,3	13,5	58	16	126	58
X	10,2	10,0	7,0	8,3	8	19	30	41
XI	5,6	4,7	5,2	3,5	55	30	47	39
XII	2,0	0,6	4,0	-0,4	14	55	25	31
I	-2,2	0,6	-3,4	-1,7	46	50	33	27
II	1,9	0,9	3,7	-0,5	23	8	65	27
III	6,3	5,2	4,3	3,0	41	49	53	33
IV	10,2	8,2	9,6	8,8	68	29	38	40
V	13,4	12,6	15,5	14,1	171	109	72	59
VI	15,7	16,8	19,8	17,2	99	29	28	62
VII	20,6	19,8	20,0	19,4	56	52	87	83
VIII	18,4	22,4	18,7	18,6	106	4	42	68

Source: own elaboration / Źródło: opracowanie własne

Table 2. Numbers (CFU × 10^7)of phosphate solubilizing microorganisms (PSM) and activities of acid (AcP) and alkaline (AlP) phosphatase in the rhizosphere soil of winter wheat depending on the farming system (organic and conventional) and cultivar in the years 2014-2016

Tab. 2. Liczebność (CFU × 10^7) mikroorganizmów rozpuszczających fosforany (PSM) i aktywność fosfatazy kwaśnej (AcP) i zasadowej (AlP) w glebie ryzosferowej pszenicy ozimej w zależności of systemu uprawy (ekologiczny i konwencjonalny) i odmiany w latach 2014-2016

Cultivar (B)	PSM		AcP		AlP	
	Farming system (A)		Farming system (A)		Farming system (A)	
	Organic	Conventional	Organic	Conventional	Organic	Conventional
	2014					
Arkadia	10.33	12.33	93.9	54.8	41.4	18.0
Bamberka	14.0	7.67	92.2	75.4	40.5	25.7
Jantarka	7.67	9.00	91.5	81.0	41.1	13.2
Sailor	11.33	10.0	89.6	47.0	40.4	8.0
Mean	10.83	9.75	91.8	64.6	40.9	16.2
	2015					
Arkadia	5.33	6.67	53.4	70.4	48.6	20.6
Bamberka	1.33	5.00	55.7	66.5	52.1	20.4
Jantarka	9.67	9.00	63.7	58.3	45.9	24.5
Sailor	1.33	5.00	59.1	65.1	54.7	23.0
Mean	4.42	6.42	58.0	65.1	50.3	22.1
	2016					
Arkadia	5.33	3.00	77.1	70.4	55.6	14.2
Bamberka	9.67	4.00	77.1	66.5	52.9	13.0
Jantarka	6.33	3.67	80.7	58.3	46.2	25.9
Sailor	5.33	3.0	78.7	65.1	44.7	30.7
Mean	6.70	3.42	78.4	59.3	49.9	21.0
Overall mean	7.31	6.53	76.1	64.1	47.0	19.8
LSD	A = n.s.; B = n.s.; B/A/B = n.s. B/A/years = n.s.		A = n.s.; B = n.s.; B/A/B = n.s. B/A/years = signif. (p = 0.05)		A = 2.63; B = n.s.; B/A/B = n.s. B/A/years = n.s.	

Source: own elaboration / Źródło: opracowanie własne

Table 3. Water content (%) and pH (in H_2O) of rhizosphere soil of organically and conventionally grown winter wheat cultivars in May 2014-2016

Tab. 3. Wilgotność (%) o pH (w H_2O) gleby ryzosferowej odmian pszenicy ozimej uprawianej w systemie ekologicznym i konwencjonalnym w maju 2014-2016

Cultivar	Water content		pH	
	Organic	Conventional	Organic	Conventional
	2014			
Arkadia	10.1	10.3	6.6	6.3
Bamberka	11.4	10.4	6.1	6.0
Jantarka	9.5	9.4	6.4	6.3
Sailor	9.8	9.9	6.4	6.2
Mean	10.2	10	6.4	6.1
	2015			
Arkadia	9.3	9.5	6.2	5.6
Bamberka	10.0	9.0	6.0	5.8
Jantarka	9.2	9.0	6.1	5.8
Sailor	9.1	9.1	6.2	6.0
Mean	9.4	9.2	6.1	5.8
	2016			
Arkadia	8.8	5.6	5.9	5.6
Bamberka	8.6	6.0	5.8	5.8
Jantarka	8.5	6.0	6.1	6.0
Sailor	8.8	5.4	6.3	6.1
Mean	8.7	6.0	6.0	5.9

Source: own elaboration / Źródło: opracowanie własne

4. Conclusions

1. Numbers of phosphate solubilizing microorganisms (PSM) and activity of acid phosphatase in the rhizosphere soil of the tested cultivars were not significantly influenced by the farming systems used nor by the cultivars.
2. On the other hand, in all years included in this study the activity of alkaline phosphatase (AlP) was significantly higher in the rhizosphere soil of all winter wheat cultivars grown according to the organic farming system than in soil under these cultivars grown in the conventional system.
3. It is suggested that the activity of AlP could be used as a reliable biological indicator of beneficial effect of organic farming on soil quality.

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