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PHOSPHATE SOLUBILIZING MICROORGANISMS AND PHOSPHATASE ACTIVITIES IN THE RHIZOSPHERE SOIL OF ORGANICALLY GROWN WINTER WHEAT CULTIVARS*

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Abstract

In 2014-2016, eleven commercial winter wheat cultivars, released between 2008 and 2011, were organically grown in a field experiment at Osiny Experimental Station (51°28′N, 22°30′E). Each year in the first or second ten days of May, samples of rhizosphere soil were analyzed for numbers of colony forming units (CFU) of phosphate solubilizing microorganisms (PSM) and for acid (AcP) and alkaline phosphatase (AlP) activities. At the same sampling days in the May of each year, winter wheat tillers at shooting (GS 35-40) were collected to determine the total P content in plant tissues, while grain yields were measured at full ripeness of winter wheat. Relationships between the microbial and enzymatic parameters, wheat grain yields and plant P content were also analyzed. Under organic farming the tested winter wheat cultivars differed markedly with respect to their grain yields and P acquisition in particular years, although none of these cultivars showed superior performance across all years. In 2014, significant correlations were found to occur between CFU numbers of PSM and activities of alkaline phosphatase (AlP), between grain yields and AlP activities and between plant P content and PSM numbers. In 2015 and 2016, significant correlations, $r = 0.611$ and $r = 0.630$ respectively, were found only between the CFU numbers of PSM and grain yields of the examined cultivars, indicating that phosphate solubilizing microorganisms and their activity can play an important role in the performance of winter wheat cultivars under organic farming.

Keywords: phosphate solubilizing microorganisms, phosphatases, cultivars, winter wheat, organic farming.

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INTRODUCTION

Phosphorus (P) is the second most essential nutrient, after nitrogen, for plat growth and productivity of arable crops. Although the total content of organic and inorganic pools of this macronutrient in soils may be high, it usually occurs in forms that are relatively unavailable for plant uptake (Sapek 2014, Alori et al. 2017). In conventional or integrated crop management systems, P requirements of agricultural plants are satisfied mainly through the application of mineral phosphate fertilizers, which also build up soil reserves of this nutrient (Shen et al. 2011, Kulczycki 2012). It was estimated 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 (Richardson et al. 2009, Shen et al. 2011, Sapek 2014). P complexes with these metals constitute usually the largest fraction of inorganic phosphate in soils. A considerable portion of soil P is also contained in organic matter (Tarafdar, Claassen 1988, Shen et al. 2011, Sapek 2014). Organic forms of P usually constitute about 50% of the total phosphorus content in most soils, although they may range from as low as 5% to as high as 95%.

Soil microorganisms possessing the ability to liberate phosphates from organic and inorganic pools can also enhance the availability of P to plants (Nautiyal 1999, Zhu et al. 2001, Richardson et al. 2009, Alori et al. 2017). The liberation of organic phosphates by microorganisms is mediated mainly through the production 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 (Tarafdar, Claassen 1988, Böhme, Böhme 2006, Trivedi, Tongmin 2008, Alori et al. 2017). 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 (Hildermann et al. 2010, Martyniuk et al. 2016).

It is well documented that different plant species shape their characteristic rhizosphere and phyllosphere microbiomes (Zhu et al. 2001, Rengel, Marschner 2005, Nelson et al. 2011, Sapkota et al. 2014). Concerning cultivar-specific interactions between plant roots and soil microorganisms facilitating P acquisition by crops, only arbuscular mycorrhizal (AM) fungi have been relatively most widely studied in this respect (Zhu et al. 2001, Richardson et al. 2009, Hildermann et al. 2010). For example, Zhu et al. (2001) reported that although modern winter wheat cultivars had slightly higher percentages of their roots colonized by AM fungi than old cultivars, the opposite was true for mycorrhizal responsiveness (shoot P concentration) of these cultivars.

The aims of this work were: to find out if organically 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, and to analyze relationships between these parameters and P acquisition and grain yields of these cultivars.

MATERIAL AND METHODS

The study was based on a long-term field experiment established in 1994 on a Haplic Luvisol (loamy sand) in Osiny Experimental Station (51°28′N, 22°30′E), which belongs to the Institute of Soil Science and Plant Cultivation, Pulawy, Poland. Some basic properties of this soil are as follows: C org. – 8.2 g kg^{-1}; N tot. – 1.0 g kg^{-1}; pH(KCl) – 5.9; P, K and Mg (mg kg^{-1}) – 37.5, 84.7 and 54.5, 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 system were used. This 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. In 2014, 2015 and 2016 eleven commercial winter wheat cultivars (Table 2), released during 2008-2011, were grown on winter wheat fields. In the absence of winter wheat cultivars bred for the purpose of organic agriculture, conventionally bred cultivars were chosen for

Table 1

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

Month	Temperature (°C)				Precipitation (mm)			
	2013/2014	2014/2015	2015/2016	average 1988-2017	2013/2014	2014/2015	2015/2016	average 1988-2017
Sept	11.8	14.7	15.3	13.5	58	16	126	58
Oct	10.2	10.0	7.0	8.3	8	19	30	41
Nov	5.6	4.7	5.2	3.5	55	30	47	39
Dec	2.0	0.6	4.0	-0.4	14	55	25	31
Jan	-2.2	0.6	-3.4	-1.7	46	50	33	27
Feb	1.9	0.9	3.7	-0.5	23	8	65	27
March	6.3	5.2	4.3	3.0	41	49	53	33
April	10.2	8.2	9.6	8.8	68	29	38	40
May	13.4	12.6	15.5	14.1	171	109	72	59
June	15.7	16.8	19.8	17.2	99	29	28	62
July	20.6	19.8	20.0	19.4	56	52	87	83
Aug	18.4	22.4	18.7	18.6	106	4	42	68

Table 2

Grain yields (t ha^{-1}), number of ears m^{-2} and plant phosphorus (P) content (g kg^{-1}) of organically grown winter wheat cultivars in 2014-2016

Cultivar	2014			2015			2016		
	grain yield	number of ears	P content	grain yield	number of ears	P content	grain yield	number of ears	P content
Arkadia	3.92 ± 0.56*ab**	293 ± 50*abc*	2.34 ± 0.16*bc*	6.11 ± 0.59*a*	482 ± 46*ab*	2.32 ± 0.16*c*	6.70 ± 0.47*c*	391 ± 38*c*	2.51 ± 0.16*b*
Bamberka	3.69 ±0.74*ab*	282 ± 65*abc*	2.54 ± 0.18*e*	7.99 ± 0.21*cde*	474 ± 27*ab*	2.14 ± 0.15*ab*	6.05 ± 0.45*ab*	333 ± 37*a*	2.82 ± 0.18*c*
Banderola	4.31 ± 0.43*abc*	270 ± 44*abc*	2.24 ± 0.16*b*	8.09 ± 0.23*de*	515 ± 35*b*	2.23 ± 0.14*b*	5.85 ± 0.32*a*	339 ± 36*ab*	2.82 ± 0.17*c*
Jantarka	4.54 ± 0.73*abc*	285 ± 48*abc*	2.23 ± 0.16*b*	7.86 ± 0.36*cde*	462 ± 49*ab*	2.21 ± 0.13*b*	7.34 ± 0.37*d*	426 ± 39*c*	2.43 ± 0.15*b*
Julius	4.64 ± 0.63*bc*	282 ± 29*abc*	2.43 ± 0.17*cd*	7.83 ± 0.14*cde*	528 ± 22*b*	2.43 ± 0.16*d*	6.60 ± 0.38*bc*	417 ± 22*c*	2.72 ± 0.16*c*
Ozon	3.27 ± 0.46*a*	236 ± 64*a*	2.44 ± 0.17*cde*	8.18 ± 0.29*e*	440 ± 33*a*	2.43 ± 0.17*d*	6.38 ± 0.38*abc*	381 ± 41*bc*	2.81 ± 0.18*c*
Muszelka	4.43 ± 0.35*abc*	314 ± 32*abc*	2.32 ± 0.16*c*	8.70 ± 0.35*f*	510 ± 62*b*	2.52 ± 0.18*d*	5.88 ± 0.34*a*	303 ± 43*a*	3.00 ± 0.19*d*
Ostroga	4.62 ± 0.25*bc*	351 ± 30*c*	2.64 ± 0.18*e*	7.22 ± 0.33*b*	483 ± 63*ab*	2.21 ± 0.15*b*	6.81 ± 0.24*cd*	390 ± 49*c*	2.80 ± 0.18*c*
Sailor	4.25 ± 0.65*abc*	264 ± 76*ab*	2.22 ± 0.15*b*	7.91 ± 0.39*cde*	489 ± 26*ab*	2.10 ± 0.13*a*	6.85 ± 0.31*cd*	420 ± 26*c*	2.42 ± 0.18*b*
Skagen	4.71 ± 0.37*bc*	320 ± 55*abc*	2.10 ± 0.14*a*	7.52 ± 0.19*bc*	474 ± 36*ab*	2.34 ± 0.16*c*	6.82 ± 0.33*cd*	412 ± 25*c*	2.51 ± 0.16*b*
Smuga	5.27± 0.32*c*	326 ± 69*bc*	2.81 ± 0.21*f*	7.62 ± 0.37*bcd*	460 ± 35*ab*	2.42 ± 0.17*cd*	6.74 ± 0.37*c*	397 ± 22*c*	2.10 ± 0.14*a*
Mean	4.32 ± 0.50	293 ± 51	2.39 ± 0.17	7.73 ± 0.31	483 ± 39	2.30 ± 0.15	6.55 ± 0.32	382 ± 34	2.63 ± 0.17

* Values (±SD) in columns followed be the same letter are not significantly different.

their high yielding potential and good tolerance to foliar fungal diseases. Each cultivar was sown (4.5 million grains ha^{-1}) in 4 replicated and randomized micro-plots (35 m^2). No N mineral fertilizers are used in the organic system, but regarding phosphorus 150 kg ha^{-1} of powdered phosphate rock is applied once per rotation. With respect to K fertilization, the winter wheat field was treated with 150 kg ha^{-1} 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.

Samples of the rhizosphere soil were collected in the first or second ten days of May 2014, 2015 and 2016 by digging out about 15 plants with soil adhering to their roots. Only loose soil clods were removed in the field and the remaining soil with roots was placed in plastic bags. In the laboratory, plants were separated and gently shaken to remove soil not adhering to the roots, a step followed by vigorous shaking to collect only soil closely attached to the roots. Moist, sieved (2 mm) soil samples were refrigerated (4°C). Within one week, soil samples were analyzed for: acid (AcP) [EC 3.1.3.1.] and alkaline phosphatase (AlP) [EC 3.1.3.2.], using *p*-nitrophenyl phosphate (PNP) as the substrate according to TABATABAI, BREMNER method (1969), and for numbers of colony forming units (CFUs) of phosphate solubilizing microorganisms (PSM) using the soil dilution-plate method. Rhizosphere soil samples weighing 10 g were suspended in 90 cm^3 of sterile water and following vigorous shaking (15 min, 120 rpm) the suspension was further diluted up to 10^{-7}. From the last two dilutions (10^{-6} - 10^{-7}), 0.1 cm^3 was pipetted and spread onto the Pikowskya agar medium in Petri plates, which was replicated four times. The medium had the following composition (dm^3): glucose, 10 g; $Ca_3(PO_4)_2$, 2,5 g; $(NH_4)_2SO_4$, 0.5 g; NaCl, 0.2 g; $MgSO_4 \cdot 7H_2O$, 0.1 g; KCl, 0.2 g; yeast extract, 0.5 g; $MnSO_4 \cdot H_2O$, 0.002 g; and $FeSO_4 \cdot 7H_2O$, 0.002 g; bacto-agar, 15 g. After 7 days of incubation at 28°C, microbial CFU with clear zones around colonies were enumerated.

At the same sampling days in May of each year, winter wheat tillers at shooting (GS 35-40) were collected to determine total P content in plant tissues using continuous flow analysis (CFA) spectrometry following mineralization of plant material in concentrated H_2SO_4 and H_2O_2 (in accordance with PN-EN ISP 15681-2:2006P). At full ripeness, winter wheat cultivars were harvested to measure grain yields and some yield components.

The data were subjected to the analysis of variance (ANOVA) using the FR-ANALWAR software based on Microsoft Excel, with significance of differences assessed by the Tukey's test at $p < 0.05$. Pearson's correlation coefficients were calculated to evaluate interactions between the studied parameters.

RESULTS AND DISCUSSION

Variable weather conditions, both with respect to temperature and precipitation (Table 1), during growing seasons in the period of 2013-2016 were mainly responsible for relatively large variability in grain yields of winter wheat cultivars grown in this period (Table 2). For example, the lowest grain yields (3.27-5.27 t ha^{-1}) were obtained in the 2013/2014 growing season with low autumn rainfall, particularly in October of 2013, which resulted in low plant ear densities (Table 2) and, as a consequence, in severe weed infestation of all winter wheat cultivars. Problems with weed pressure in organically managed crops, particularly at low plant densities, are well documented (Kolb, Gallandt 2012, Feledyn-Szewczyk et al. 2013, Jończyk, Stalenga 2016). On the other hand, the maximum grain yields (6.11-8.70 t ha^{-1}) were obtained in 2015, mainly owing to the dense winter wheat canopies formed after mild winter and minimal frost killing of plants in the 2014/2015 growing season (Table 2).

In 2014, cv. Smuga was the best yielding variety while significantly lower grain yield were produced by the cultivars Ozon, Bamberka and Arkadia, although differences between the cultivars were most often insignificant due to the marked variability of the yields in that year (Table 2). In 2015, the maximum grain yields exceeding 8 t ha^{-1} were obtained from three cultivars: Muszelka, Ozon and Banderola, but only Muszelka yielded significantly higher than all the other cultivars sown in 2015 and this cultivar also had the highest content of plant P (Table 2). In 2015, cv. Arkadia was the worst yielding winter wheat and its leaves showed the strongest infestation with *Puccinia strifirmis* among the tested cultivars (Jończyk, Stalenga 2016). In 2016, it was only the cultivar Jantarka that yielded over 7.3 t ha^{-1} and this cultivar had the highest number of ears m^{-2} in the same year. Slightly lower grain yields were produced by the cultivars Sailor, Sklagen and Ostroga, and the lowest yields in this year were obtained from the following cultivars: Banderola, Muszelka and Bamberka (Table 2). The worst yielding cultivars in 2016 had the lowest ear densities and this possibly explains why these cultivars contained the highest amounts of P in their shoots (Table 2), which also resulted in a negative correlation between grain yields and plant P content (Table 3). The above results indicate that under organic farming the tested winter wheat cultivars differed markedly with respect to their grain yields and P acquisition in particular years, although none of these cultivars showed superior performance across all years (Table 2).

Winter wheat cultivars grown in our experiment also differed significantly with respect to PSM numbers and phosphatases activities in their rhizosphere soil (Table 4). Generally, the maximum numbers of PSM occurred in 2014 and the minimum PSM numbers were counted in 2016, and these differences were most likely connected with weather conditions, particularly with rainfalls, which were most abundant in May of 2014 (Table 1).

Table 3

Correlation coefficients between numbers of phosphate solubilizing microorganisms (PSM), activities of acid (AcP) and alkaline (AlP) phosphatases, plant P content and grain yields of organically grown winter wheat cultivars in 2014-2016

Cultivar	2014			Plant P	2015			Plant P	2016			Plant P
	PSM number	AcP	AlP		PSM number	AcP	AlP		PSM number	AcP	AlP	
PSM number	X				X				X			
Acid phosphatases	0.534	X			0.413	X			-0.168	X		
Alkaline phosphatases	0.745*	-0.383	X		-0.235	-0.299	X		-0.207	-0.314	X	
Plant P	0.626*	-0.494	0.565	X	0.161	-0.154	-0.494	X	-0.308	-0.002	0.002	X
Grain yield	0.458	-0.317	0.611*	0.188	0.619*	0.143	-0.341	0.176	0.630*	0.047	-0.188	-0.699**

** significant at $p \leq 0.01$, * significant at $p \leq 0.05$

Table 4

Numbers (CFUs · 10^7 g^{-1} soil DW) of phosphate solubilizing microorganisms (PSM) and activities of acid (AcP) and alkaline (AlP) phosphatases (μg *p*NP g^{-1} DW h^{-1}) in the rhizosphere soil of organically grown winter wheat cultivars in 2014-2016

Cultivar	2014			2015			2016		
	PSM	AcP	AlP	PSM	AcP	AlP	PSM	AcP	AlP
Arkadia	8.3±4.5*ab**	57.6±1.5*ab*	41.5±1.1*a*	1.7±0.5*a*	53.4±3.1*a*	48.6±2.1*c*	0.7±0.6*a*	74.0±2.4*a*	55.6±5.7*b*
Bamberka	14.0±1.0*b*	61.0±2.1*ab*	40.5±2.7*a*	5.7±2.9*b*	55.7±2.0*a*	53.1±0.5*d*	2.3±1.5*ab*	77.1±3.2*ab*	52.9±3.8*ab*
Banderola	14.0±5.3*b*	59.3±2.9*ab*	57.7±2.7*b*	2.6±1.1*ab*	63.9±2.7*b*	36.8±0.5*ab*	1.3±0.6*ab*	79.6±3.0*ab*	48.0±2.1*ab*
Jantarka	7.3±3.5*ab*	63.5±3.1*b*	41.1±0.5*a*	5.7±4.0*b*	63.7±3.0*b*	45.9±2.5*c*	7.3±3.1*d*	80.7±3.3*ab*	46.2±1.7*a*
Julius	11.0±2.6*ab*	54.5±1.2*ab*	46.0±4.6*a*	4.7±1.2*ab*	59.9±4.3*ab*	33.2±1.3*a*	1.7±1.1*ab*	75.0±2.2*a*	49.5±0.5*ab*
Ozon	9.0±1.0*ab*	60.0±2.3*ab*	40.9±3.7*a*	5.6±3.2*b*	64.3±3.0*b*	32.8±1.5*a*	2.3±1.1*ab*	85.1±2.9*bc*	52.4±3.7*ab*
Muszelka	5.3±3.7*a*	59.5±0. *ab*	40.7±1.8*a*	6.3±1.5*b*	59.6±1.7*ab*	39.8±2.5*b*	0.3±0.2*a*	79.1±1.2*ab*	45.5±1.2*a*
Ostroga	11.3±3.0*ab*	62.8±2.9*ab*	58.1±1.2*b*	6.0±2.6*b*	75.2±2.1*c*	36.6±1.3*ab*	6.3±3.2*cd*	74.5±2.7*a*	47.1±2.9*ab*
Sailor	10.3±5.1*ab*	61.8±2.6*ab*	40.4±2.2*a*	4.0±2.0*ab*	59.1±0.9*ab*	54.7±2.3*d*	2.0±0.7*ab*	78.5±1.1*ab*	44.7±1.3*a*
Skagen	11.0±5.0*ab*	61.8±3.1*ab*	43.7±4.7*a*	3.7±1.5*ab*	59.7±0.9*ab*	33.5±0.7*a*	1.0±0.0*a*	90.1±3.3*c*	45.9±1.5*a*
Smuga	23.3±4.9*c*	53.4±1.1*a*	63.0±1.5*b*	4.6±2.7*ab*	55.0±2.4*a*	33.6±1.9*a*	4.0±1.0*bc*	76.7±2.3*ab*	50.2±4.4*ab*
Mean	11.3±3.6	59.6±2.1	46.7±2.4	4.6±2.1	60.9±2.4	40.8±1.6	2.7±1.2	79.1±2.5	48.9±2.6

* Values (±SD) in columns followed be the same letter are not significantly different.

The mean moisture content of soil samples collected from the root zone of the studied cultivars amounted to 10.6%, 9.8% and 8.7% in May of 2014, 2015 and 2016, respectively (data not shown).

The cultivar Smuga, the best yielding one in 2014, also contained the highest amounts of phosphorus (Table 2) and the rhizosphere soil of this cultivar was colonized by the largest population of phosphate solubilizing microorganisms (PSM) and showed the highest activity of alkaline phosphatase (Table 4). In the same year, a highly significant correlation ($p \leq 0.01$) occurred between PSM numbers and activities of alkaline phosphatase (AlP) in the rhizosphere soil of the studied cultivars (Table 3). This high correlation between the number PSM and the AlP activity would confirm results of previous studies showing that this enzyme in the soil environment is produced by soil microorganisms, not by plant roots (Tarafdar, Claassen 1988). Moreover, Böhme, Böhme (2006) reported significant correlations between microbial biomass C and Alp activities in the rhizosphere soil of spring barley and sugar beet grown in pots. It is worth noticing that of the studied parameters calculated across the years 2014-2016 only the mean values of PSM and AlP gave positive and relatively high (r = 0.429), although not significant, correlation coefficient (Table 5). However in our study, which was based on a field experiment, no significant relationships between AlP and PSM were found in 2015 and 2016, indicating that under field conditions factors such as weather conditions and weeds infestations might disturb this relationship, as well as the relationships between the mean values of the tested parameters (Table 5). In 2014 significant at $p \leq 0.05$ correlations were also found between grain yields and AlP activities and between plant P contents and PSM numbers (Table 3). These correlations would indicate that both microbial factors play an important role in P acquisition by winter wheat grown under organic plant management system, although this relationship is diminished by the fact that in the following two years no such relationships occurred. In 2015 Muszelka gave the maximum grain yield and Arkadia the lowest one, and similarly, the highest and lower number of PSM were found in the rhizosphere soil of these cultivars respectively (Table 4). In 2016 Jantarka was the best yielding cultivar while significantly lower

Table 5

Correlation coefficients between mean (for 2014-2016) numbers of phosphate solubilizing microorganisms (PSM), activities of acid (AcP) and alkaline (AlP) phosphatases, plant P content and grain yields of organically grown winter wheat cultivars

Item	PSM number	AcP	AlP	Plant P
PSM number	X			
AcP	-0.091	X		
AlP	0.429	-0.448	X	
Plant P	-0.018	-0.105	0.020	X
Grain yield	0.272	0.203	-0.272	0.189

grain yields were obtained in the case of Banderola and Muszelka. In this year the highest and the lowest numbers of PSM were found in the rhizosphere of Jantarka and Muszelka (Table 4). KUNDU et al. (2009) reported that populations of PSM in the rhizosphere soil of three plant species (chickpea, mustard and wheat) differed significantly. Results of our study indicate that also at the cultivar level plants can shape numbers and activities of PSM in their rhizosphere. In 2014 a relatively high correlation (r = 0.458) occurred, although not statistically significant, between the numbers of PSM and grain yields of the examined winter wheat cultivars, however in 2015 and 2016 significant correlations, r = 0.611 and r = 0.630 respectively, were found between these parameters (Table 3), indicating that phosphate solubilizing microorganisms can play an important role in the growth and performance of winter wheat cultivars cultivated according to the organic farming system.

CONCLUSIONS

The results indicate that under organic farming the tested winter wheat cultivars differed markedly with respect to their grain yields and P acquisition in particular years, although none of these cultivars showed superior performance across all years. In 2014, significant correlations were found to occur between CFUs of PSM and activities of alkaline phosphatase (AlP), between grain yields and AlP activities and between plant P content and PSM numbers. In 2015 and 2016, significant correlations, r = 0.611 and r = 0.630 respectively, were found only between the CFUs numbers of PSM and grain yields of the examined cultivars, indicating that phosphate solubilizing microorganisms and their activity can play an important role in the performance of winter wheat cultivars under organic farming.

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