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RESEARCH ARTICLE

Acid Phosphatases Activity and Growth of Barley, Oat, Rye and Wheat Plants as Affected by Pi Deficiency

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Abstract:

Objective:

The influence of phosphorus deficit on the growth of plants and acid phosphatases activity in leaves and roots of barley seedlings (*Hordeum vulgare* L.), as well as oat (*Avena sativa* L.), rye (*Secale cereale* L.) and wheat plants (*Triticum vulgare* L.) was studied.

Method:

Plants were cultured three weeks in a nutrient media: complete (control, +P) or without phosphorus (-P). The growth on -P medium significantly affected the inorganic phosphate (Pi) content in plants tissues. Pi deficit decreased shoots growth but ratio of root/shoot was higher for -P plants when compared to control. The root elongation was enhanced under Pi deficiency - in -P oat and barley more intensive elongation was observed than in other plants. On the other hand, inhibition of shoot growth was more pronounced for -P rye and wheat. Pi-deficient plants showed higher activity of acid phosphatases in tissue extracts and in exudates from roots than +P plants.

Result:

Extracellular acid phosphatases activity increased the most for -P rye and wheat plants. Acid phosphatases secretion was intensive in growing parts of Pi-deficient roots. The activity of enzymes secreted by -P roots of all studied plants was higher than intracellular acid phosphatases.

Conclusion:

Our results indicated that wheat is more sensitive to the Pi deficiency at the early stage of growth than other plants, whereas oat is rather resistant to Pi deficit. The results suggested that acid phosphatases played an important role in acclimation of studied crop plants to moderate Pi deficiency.

Keywords: Extracellular phosphatase, Low Pi nutrition, Root, Secretion, Pi mobilization, Phosphate deficiency.

1. INTRODUCTION

Phosphate-limiting condition is common in the soils because phosphorus-containing compounds are mainly insoluble and thus unavailable for plants. Phosphorus is an essential nutrient important in metabolism, plant growth, development and productivity. Plants respond to phosphorus starvation by developing various mechanisms that can increase the Pi availability and uptake from soil as well as Pi mobilization/recycling and transport in plant cells and tissues [1 - 5]. Plants acclimate to Pi deficiency by modifications of growth parameters and metabolism or genes expression and protein production [6 - 11]. One of the common symptoms of phosphate deficiency is the increase of root/shoot ratios which is usually the result of reduction of shoot growth and/or stimulation of root growth [7, 12 - 14].

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Low Pi availability in the soil often affected the root elongation, increase development of lateral roots as well as the number and length of root hairs [15 - 17]. Pi deficiency can regulate many features of anatomy of roots, or root architecture, *e.g.*, aerenchyma formation (*via* ethylene mediation), higher root hair density, different lateral branching or cluster root formation and the total surface area enhance [6, 7, 16, 18]. In response to Pi deficit many plants activate root colonization by mycorrhizal fungi or interaction with rhizosphere bacteria [5, 9, 19]. Probably, all of these changes result in a better opportunity for soil exploration and are under strictly genetic control [20, 21]. Differences in Pi uptake from soil may be due to better growth of roots or high external root efficiency, the simulations indicated that even very small changes in parameters related to the root growth could have significant effects on Pi uptake [21]. In addition, low Pi supply often induces exudation of organic acids and protons from roots to increase Pi availability from insoluble mineral forms of phosphorus in the rhizosphere, or secretion of enzymes hydrolyzing organic esters of phosphorus [4, 5, 22, 23].

Acid phosphatases (EC 3.1.3.2) are important components of the response of plants to Pi limitation [23 - 25]. Acid phosphatases, mainly extracellular isoforms, catalyze the hydrolysis of Pi from phosphate monoesters (present both in soil and plant tissues) and function in the processes of uptake, transport and recycling of Pi. Intracellular acid phosphatases are important for phosphorus scavenging processes and Pi remobilization in plant cells and tissues [4, 14, 23, 25], but their role in plant acclimation to low Pi availability is not always clear [26]. Acid phosphatases are found in intracellular spaces, cell walls and inside cell: in amyloplast, mitochondrium, nucleus, Golgi body and endoplasmic reticulum [23, 25, 27]. Acid phosphatase activity increase under Pi-deficient conditions have been documented for various crop plants, including lupine and clover, barley, oat, rice or wheat [28 - 32]. Some of the genes encoding acid phosphatases, both intra- and extracellular, were found to be upregulated by Pi-deficient condition, and correlated with higher proteins content [5, 14, 29, 33]. Extracellular acid phosphatases, including those secreted by plant roots, can efficiently acquire Pi from organic sources of phosphorus - several studies have demonstrated that Pi deficiency in the soil (or growth medium) increased secretion of acid phosphatases from the roots [29, 30, 33 - 36]. On the other hand, some experiments showed no significant changes in acid phosphatase activities in plants grown under Pi deficiency [26, 28, 30], or indicated that root acid phosphatases are poor indicator of growth of crop plants under low Pi nutrition in different soils [37]. Genotypic variations in activity of acid phosphatases (or secretion) and morphological features under Pi deficiency has been reported for various crop species and cultivars [14, 28, 30, 32, 38, 39].

The aim of the study was to compare responses of common cereal plants (barley, oat, rye, wheat) to early phosphate deficiency during growth period important to tiller formation and further productivity. Due to the variability in responses of plant species/cultivars to Pi deficiency, such studies are still necessary. The examination of the intensity of growth and activity of acid phosphatases in different plant tissues could be helpful to estimate the role of these enzymes in acclimation of studied crop plants to Pi deficit.

2. MATERIAL AND METHODS

2.1. Plant Growth Conditions

Seeds of barley (*Hordeum vulgare* L., cv. Rodos) and oat plants (*Avena sativa* L., cv. Bajka), rye (*Secale cereale* L., cv. Dankowskie Zlote) as well as wheat (*Triticum vulgare* L., cv. Henrika) were germination (7 days), after that transferred to separate containers filled with control nutrient medium (+P) or medium without Pi (-P), similar to that described by Ciereszko *et al.* [40]. Pi-sufficient nutrient medium contained: $Ca(NO_3)_2$ (4.4 mM), $MgSO_4$ (2.7 mM), KNO_3 (1.5 mM), KH_2PO_4 (1 mM), Fe-EDTA (76 μM), H_3BO_3 (43 μM), $MnCl_2$ (9 μM), $CuSO_4$ (0.3 μM), $ZnSO_4$ (0.8 μM), H_2MoO_4 (0.1 μM); to the -P medium KCl (2 mM) (instead KH_2PO_4) was added. Plants were cultured in different containers (15 seedlings per about 5 l of nutrient medium). The culture medium was adjusted to pH 5.7 (by adding drops of 1N NaOH), aerated and changed every 4 days. Plants were cultured for one to three weeks in growth chamber under 16 h light period, photon flux density of 130 μmol m^{-2} s^{-1}, temperature of 23/19 °C (day/night), and air humidity about 70%. Cereal plants were cultured 7, 14 and 21 days on various nutrient media (14-, 21- and 28-day old plants, respectively). Samples of tissues (leaves or roots) were collected 4-5 hours after the beginning of photoperiod. Fresh mass and length of shoots and roots were measured after plant harvest, dry mass-after at least 24 h tissue drying at 90C; root diameters were estimated according to [15].

2.2. Phosphate Content Measurements

Inorganic phosphate (Pi) content was determined after homogenization and extraction of tissues (leaves or roots) of

barley, oat, rye and wheat plants (0.5 g samples), cultured 1, 2 and 3 weeks on +P and –P nutrient media, with cold 10% trichloroacetic acid. The phosphomolybdate colorimetric assay, described by Ames [41], was used to Pi determination.

2.3. Extracellular Acid Phosphatases Activity Measurements

Root surface acid phosphatases activity measurements were described before by Ciereszko *et al.* [13, 30]. The whole roots or root "tips" (around 20 mm) were washed in distilled water, blot dried and placed into 10 ml (or 30 ml for larger roots) of substrate (6 mM *p*-nitrophenyl phosphate in 100 mM sodium acetate buffer, pH 5.0) and incubated at 20°C. To ensure linearity, 100 µl aliquots of medium were removed at different intervals for above 2 hours, to each sample 100 µl of 4N NaOH was immediately added (to terminate the reaction) and the absorbancies were read at 410 nm (Cecil CE 2501) and compared to a standard curve with *p*-nitrophenol. Enzymes activity, after 15 min incubations, was presented, as µmol *p*-nitrophenol h^{-1} g^{-1} of fresh weight (FW).

2.4. Intracellular Acid Phosphatases Activity Measurements

For intracellular acid phosphatase activity assay, tissues samples (0.2 g, leaves or roots) were homogenized and extracted in 5 ml of 50 mM Na-acetate buffer, pH 5.0, with 1 mM DTT (dithiothreitol), centrifuged at 12000 g for 10 min at 4°C. Enzyme activities were determined in supernatants (100 µl) after 10 min incubations at 37°C with 6 mM *p*-nitrophenyl phosphate in 100 mM Na-acetate buffer, pH 5.0; reaction was terminated of as described above. The results after 15 min of incubations are presented. The protein content in media for measurements of surface acid phosphatase activity was extremely low, thus both intra- and extracellular enzymes activity was expressed per g FW (µmol *p*-nitrophenol $h^{-1}g^{-1}$FW), similar to Żebrowska *et al.* [14].

2.5. Soluble Proteins Content Determination

The soluble proteins content was determined by the method described by Bradford [42]. The absorbance at 595 nm was measured in enzymatic extract (0.2 ml) after 15 min incubation with the Bradford reagent (Sigma) (2 ml) and compared to a standard curve with BSA.

2.6. Statistical Analysis

All measurements were performed in at least three replicates in four to five series, independent, of experiments and standard deviation (SD) was calculated. The treatments effects were tested by one way analysis of variance. Means were compared between the treatments at the 0.05 probability level (SPSS Statistics).

3. RESULTS

Inorganic phosphate content in leaves and roots of all studied plants decreased significantly already after one week of culture without Pi (Fig. **1**). After 2 weeks culture on nutrient media the Pi content in -P leaves (21-days-old plants) was between 6-13% of that found in control (+P plants). Pi content in root of P-deficient barley and rye was about 7% of control but in -P roots of oat and wheat was about 16% and 14%, respectively, of control. After three weeks of culture in -P conditions, Pi level in leaves of studied plants was about 8-9% of the control. In the -P roots of barley, Pi content decreased to about 4% of control, however in roots of oat and wheat was 7% or in rye - 11% of that found in +P plants (Fig. **1**).

The growth of studied plants was significantly affected by Pi deficiency, especially after three weeks of growth (Fig. **2**). The fresh mass of shoots after one week growth on -P nutrient medium was between 73%-82% of control plants; the fresh mass of +P and -P roots after one week culture was similar, with exception of barley (64% of control) (Table **1**). Shoot mass of Pi-deficient cereals decreased significantly after two weeks of culture and was 70%, 58%, 35% and 51% of control for -P barley, oat, rye and wheat, respectively; however root fresh mass of -P and +P plants was similar (Tables **1**-**4**). The differences in growth parameters were higher after three weeks of culture when the shoot fresh weight was about 54%, 26%, 13% and 23% of control, respectively, for -P barley, oat, rye and wheat. Additionally, the reduction of -P root mass was observed and was 67%, 69% and 49% of control, respectively, for -P oat, rye and wheat (Tables **2**-**4**). However, the ratio of root/shoot for fresh mass was always significantly higher in -P plants than in +P plants, *e.g.* up to 3-5-fold for rye (Table **3**). The shoot dry masses of Pi-deficient cereal plants were lower when compared to phosphate-sufficient plant, in a similar way like fresh weight of shoots (Tables **1**-**4**). The dry masses of roots were similar in younger +P and -P plants, however after 3 weeks of culture under Pi-deficient condition the root dry weight of rye and wheat was about 65% of the control (Tables **3**, **4**). The decrease in shoot mass of -P crop plants

was accompanied by a decrease in shoot height; on the other hand, root length of Pi-deficient plants increased by about 20% (for 21 days old barley and 28 days old wheat), by 28 and 38% (for 14- and 21 days old rye) or even by 39% (for 28 days old oat) when compared to control, even despite mass drop (Tables **1**-**4**). The increase of root length of studied -P plants was probably at the cost of decrease of root diameters. The ratio of root/shoot length was always higher in -P plants than in +P plants, especially after 2-3 weeks of culture (Tables **1**-**4**).

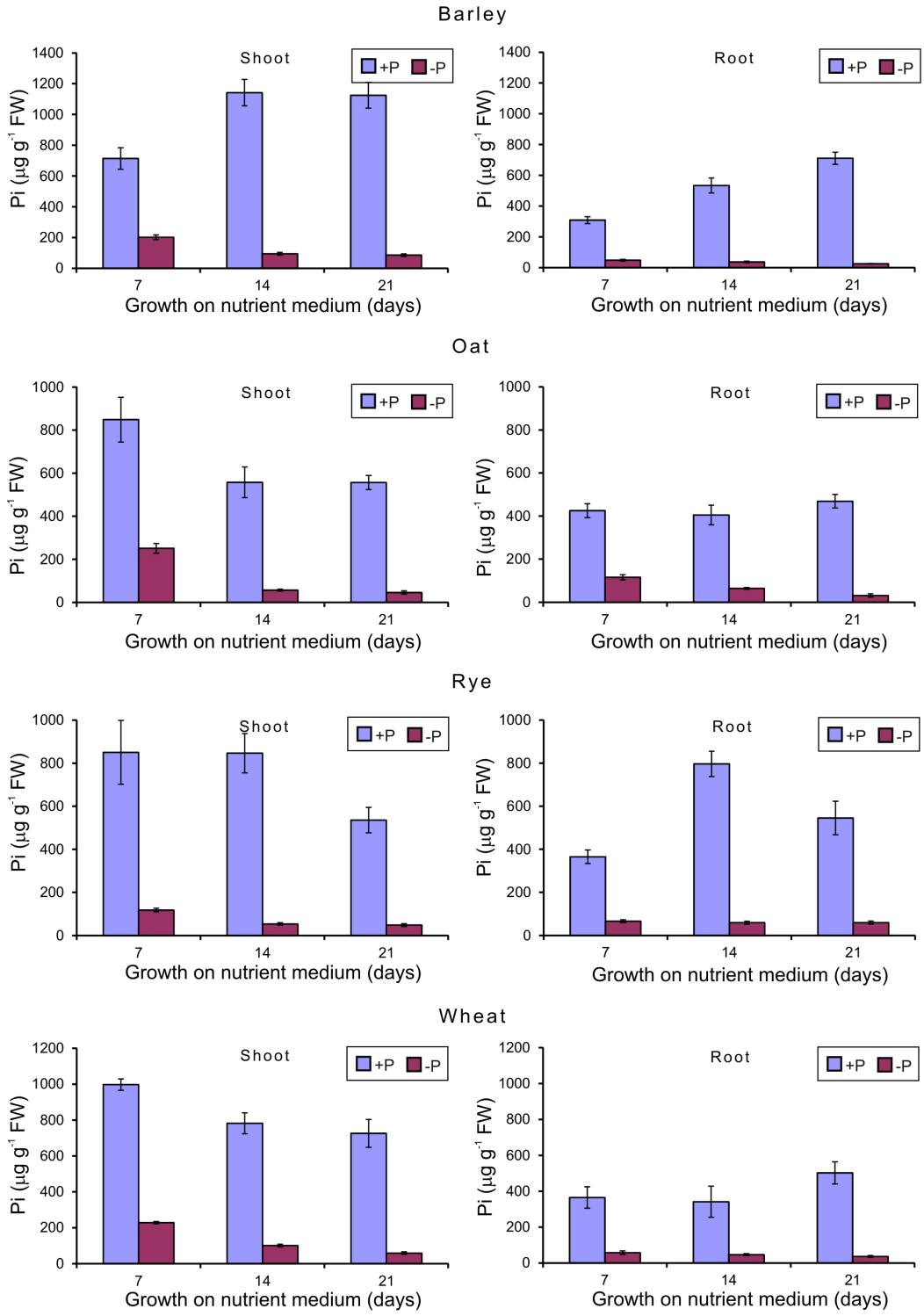

Fig. (1). Pi content in shoots and roots of barley, oat, rye and wheat plants grown for 1, 2 and 3 weeks in phosphate-sufficient (+P) or phosphate-deficient (-P) nutrient medium (means ± SD); all differences between treatments are statistically important at $p<0.05$.

Table 1. Growth parameters of barley plants (*Hordeum vulgare* L.) cultured 3 weeks on phosphate-sufficient (+P) or phosphate-deficient (-P) nutrient medium. Means ± SD.

Parameters	7 days		14 days		21 days	
	+P	-P	+P	-P	+P	-P
Shoot Fresh Weight (g)	0.33 ± 0.05	0.24 ± 0.04*	0.71 ± 0.2	0.50 ± 0.09*	1.36 ± 0.16	0.74 ± 0.14*
Roots Fresh Weigth (g)	0.28 ± 0.06	0.18 ± 0.04*	0.44 ± 0.14	0.54 ± 0.08	0.87 ± 0.17	0.77 ± 0.15
Root/Shoot	0.85	0.75	0.62	1.08	0.64	1.04
Shoot Dry Weight (mg)	39 ± 6	31 ± 5*	87 ± 23	68 ± 12*	180 ± 21	103 ± 20*
Roots Dry Weigth (mg)	41 ± 13	21 ± 4*	31 ± 10	47 ± 7*	67 ± 12	69 ± 10
Shoot Height (cm)	20.8 ± 2.4	18.0 ± 1.8*	29.4 ± 3.9	27.2 ± 1.7	40.3 ± 3.8	34.5 ± 3.6*
Root Length (cm)	12.2 ± 1.5	15.2 ± 2.3*	28.8 ± 4.2	33.5 ± 3.4*	48 ± 5.4	52.1 ± 4.3
Root/Shoot	0.59	0.84	0.99	1.23	1.19	1.51
Mean Root Diameter (mm)	0.85	0.61	0.70	0.72	0.76	0.69

* Significantly different at 0.05

Table 2. Growth parameters of oat (*Avena sativa* L.) plants cultured 3 weeks on phosphate-sufficient (+P) or phosphate-deficient (-P) nutrient medium. Means ± SD.

Parameters	7 days		14 days		21 days	
	+P	-P	+P	-P	+P	-P
Shoot Fresh Weight (g)	0.28 ± 0.03	0.23 ± 0.07	1.02 ± 0.15	0.59 ± 0.08*	2.43 ± 0.52	0.62 ± 0.14*
Roots Fresh Weigth (g)	0.16 ± 0.03	0.16 ± 0.02	0.56 ± 0.14	0.64 ± 0.1	0.97 ± 0.18	0.65 ± 0.14*
Root/Shoot	0.57	0.69	0.55	1.08	0.4	1.05
Shoot Dry Weight (mg)	27 ± 3	24 ± 5	93 ± 13	70 ± 11*	220 ± 49	92 ± 22*
Roots Dry Weigth (mg)	13 ± 3	14 ± 3	34 ± 7	50 ± 8*	60 ± 11	57 ± 8
Shoot Height (cm)	21.5 ± 3.1	18.9 ± 2.5 *	35.9 ± 3.2	30.2 ± 3.3*	51.6 ± 1.9	32.1 ± 5.0*
Root Length (cm)	13.2 ± 1.3	15.2 ± 2.6*	31.1 ± 4.4	32.1 ± 3.8	35.8 ± 7.9	49.7 ± 9.2*
Root/Shoot	0.61	0.8	0.87	1.06	0.69	1.55
Mean Root Diameter (mm)	0.62	0.59	0.79	0.76	0.93	0.64

* Significantly different at 0.05

Table 3. Growth parameters of rye (*Secale cereale* L.) plants cultured 3 weeks on phosphate-sufficient (+P) or phosphate-deficient (-P) nutrient medium. Means ± SD.

Parameters	7 days		14 days		21 days	
	+P	-P	+P	-P	+P	-P
Shoot Fresh Weight (g)	0.16 ± 0.02	0.12 ± 0.02*	0.52 ± 0.1	0.18 ± 0.03*	1.56 ± 0.11	0.2 ± 0.05*
Roots Fresh Weigth (g)	0.1 ± 0.02	0.09 ± 0.02	0.22 ± 0.06	0.22 ± 0.06	0.49 ± 0.1	0.34 ± 0.07*
Root/Shoot	0.62	0.75	0.42	1.22	0.31	1.7
Shoot Dry Weight (mg)	19 ± 3	15 ± 3*	57± 10	25 ± 6*	164 ± 17	31 ± 7*
Roots Dry Weigth (mg)	9 ± 2	9 ± 2	16 ± 4	19 ± 5	42 ± 9	28 ± 5*
Shoot Height (cm)	13.9 ± 1.4	14.3 ± 1.7	23.3 ± 3.7	17.8 ± 2.1*	32.9± 2.9	20.8 ± 4.2*
Root Length (cm)	15.2 ± 1.7	19.5 ± 2.9*	23.9 ± 5.5	33.1 ± 7.1*	39.8 ± 2.9	44.3± 2.9*
Root/Shoot	1.09	1.36	1	1.86	1.21	2.13
Mean Root Diameter (mm)	0.47	0.45	0.54	0.46	0.63	0.50

* Significantly different at 0.05

Table 4. Growth parameters of wheat (*Triticum vulgare* L.) plants cultured 3 weeks on phosphate-sufficient (+P) or phosphate-deficient (-P) nutrient medium. Means ± SD.

Parameters	7 days		14 days		21 days	
	+P	-P	+P	-P	+P	-P
Shoot Fresh Weight (g)	0.22 ± 0.03	0.18 ± 0.03*	0.69 ± 0.11	0.35 ± 0.04*	1.77 ± 0.2	0.41 ± 0.06*
Roots Fresh Weigth (g)	0.13 ± 0.02	0.16 ± 0.04	0.48 ± 0.08	0.4 ± 0.05*	0.95 ± 0.2	0.47 ± 0.07*
Root/Shoot	0.59	0.89	0.69	1.14	0.54	1.15

(Table 4) contd.....

Parameters	7 days		14 days		21 days	
	+P	-P	+P	-P	+P	-P
Shoot Dry Weight (mg)	29 ± 3	23 ± 3*	85± 13	52 ± 4*	208 ± 36	69 ± 7*
Roots Dry Weigth (mg)	15 ± 1	19 ± 4*	40 ± 6	41 ± 5	83 ± 20	54 ± 6*
Shoot Height (cm)	17.9 ± 1.3	18.3 ± 1.1	33.4 ± 2.2	30.1± 2.4*	42.8 ± 1.4	30.8 ± 3.6*
Root Length (cm)	13 ± 3.8	15.3 ± 3.4	21.8 ± 4	25.9 ± 2.1*	39.7 ± 5.6	33.1 ± 4*
Root/Shoot	0.73	0.84	0.65	0.86	0.93	1.07
Mean Root Diameter (mm)	0.57	0.58	0.84	0.70	0.87	0.68

* Significantly different at 0.05

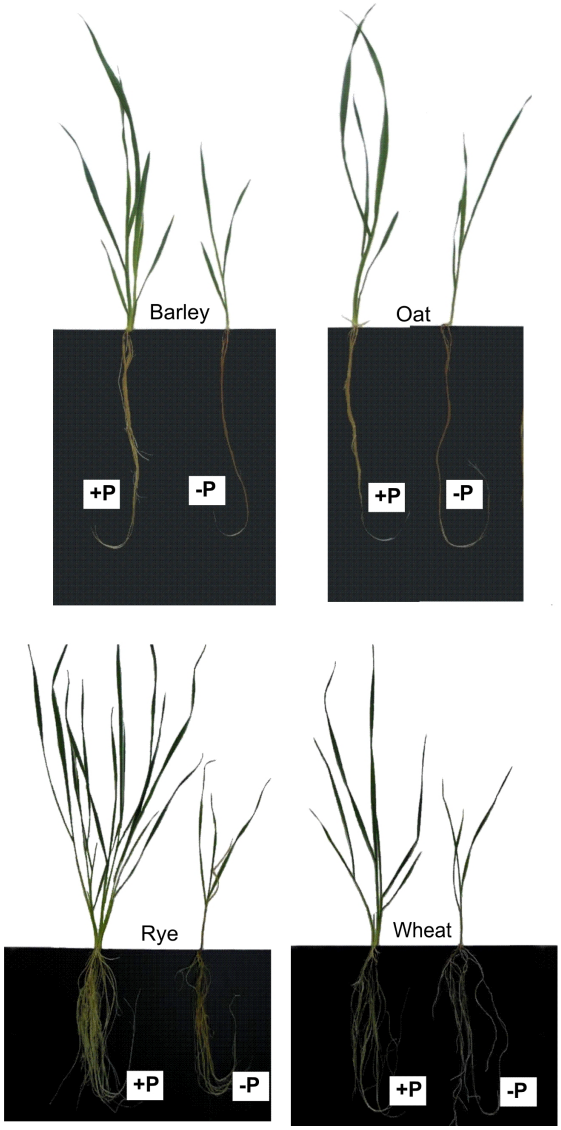

Fig. (2). Barley, oat, rye and wheat plants after 3 weeks of culture on complete phosphate-sufficient nutrient medium (+P), and phosphate-deficient (-P) nutrient medium.

The activity of intracellular acid phosphatases in shoots and roots generally increased in all studied plants under Pi deficiency, especially after 2-3 weeks of culture on nutrient medium; however, this occurred to a lesser extent than increase of extracellular phosphatases activity (Tables **5**-**8**). After three weeks of culture the activity of acid phosphatase in extracts from leaves of -P barley and oat plants was higher by about 20% and 50%, respectively, as compared with +P plants (Tables **5**, **6**). However, the activity of internal acid phosphatases in -P shoots of rye and wheat increased significantly already after 2 weeks culture by about 1.9 and 1.3-fold, whereas after 3 weeks - by 2.5-fold and 2.3-fold,

respectively, when compared to control plants (Tables **7**, **8**). After 14 days of plant growth on nutrient media, the activity of internal acid phosphatases in -P root of rye increased by about 85%, but in wheat and barley roots - by 35% and 27%, respectively, as compared to +P plants (Tables **6**-**8**). After 21 days of culture, intracellular acid phosphatases activity was enhanced by about 1.4-fold for -P roots of barley and oat (Tables **5**-**6**), and even 2.7-fold for -P rye (Table **7**), but was similar in -P and +P roots of wheat (Table **8**). Soluble proteins content in enzymatic extracts from leaves and roots was generally not affected by Pi deficit, except that found in shoots of barley and wheat plants, grown 1-2 weeks on –P medium and shoots of oat and rye cultured 3 weeks (Table **9**); in all experimental conditions soluble proteins content in roots was much lower than in leaves.

Table 5. Intracellular and extracellular acid phosphatase activities in leaves and roots of barley (*Hordeum vulgare* L.) plants cultured 3 weeks on phosphate-sufficient (+P) or phosphate-deficient (-P) nutrient medium. Means ± SD.

Parameters	7 days		14 days		21 days	
	+P	-P	+P	-P	+P	-P
	(µmol g^{-1} FW h^{-1})					
Leaf Acid Phosphatases	323 ± 44	360 ± 41.7	396 ± 15.7	411 ± 56.6	444 ± 49.4	533 ± 48*
Root Intracellular Acid phosphatases	113 ± 11	122 ± 15	90.4 ± 4	115 ± 7.8*	136 ± 15.5	190 ± 17.2*
Extracellular Acid Phosphatase: Intact Root	67.5 ± 5	100 ± 6.7*	55 ± 4.6	80.4 ± 3.1*	31.8 ± 1.2	75 ± 3*
Extracellular Acid Phosphatase: Root Tips	119 ± 5	201 ± 8*	85.5 ± 3.5	187 ± 4.4*	110 ± 8.2	193 ± 15.6*

* Significantly different at 0.05

Table 6. Intracellular and extracellular acid phosphatase activities in leaves and roots of oat (*Avena sativa* L.) plants cultured 3 weeks on phosphate-sufficient (+P) or phosphate-deficient (-P) nutrient medium. Means ± SD.

Parameters	7 days		14 days		21 days	
	+P	-P	+P	-P	+P	-P
	(µmol g^{-1} FW h^{-1})					
Leaf Acid Phosphatases	124 ± 8.4	125 ± 13.5	166 ± 12.8	157 ± 18	154 ± 21.2	234 ± 15.7*
Root Intracellular Acid Phosphatases	40.1 ± 8	47.3 ± 4.2*	81.4 ± 14.3	91.9 ± 16	87 ± 15.3	124 ± 5.4*
Extracellular Acid Phosphatase: Intact Root	19.4 ± 5.2	22.3 ± 3.7	16.5 ± 1.5	22.7 ± 1.1*	21.1 ± 5.1	48.7 ± 2.1*
Extracellular Acid Phosphatase: Root Tips	31.8 ± 4.3	31.8 ± 3.1	29.4 ± 7.4	79.8 ± 7.8*	38.4 ± 1.7	108 ± 2.3*

* Significantly different at 0.05

Table 7. Intracellular and extracellular acid phosphatase activities in leaves and roots of rye (*Secale cereale* L.) plants cultured 3 weeks on phosphate-sufficient (+P) or phosphate-deficient (-P) nutrient medium. Means ± SD.

Parameters	7 days		14 days		21 days	
	+P	-P	+P	-P	+P	-P
	(µmol g^{-1} FW h^{-1})					
Leaf Acid Phosphatases	115.3 ± 7.6	141 ± 17.9*	126 ± 18.8	237 ± 10.6*	197 ± 18.6	497 ± 61.2*
Root Intracellular Acid Phosphatases	61.1 ± 3.3	63 ± 3.1	85.7 ± 6.1	158 ± 13.6*	91.9 ± 10.5	248 ± 25.4*
Extracellular Acid Phosphatase: Intact Root	48.5 ± 6.4	75.8 ± 3.9*	49.9 ± 3.5	119 ± 6.9*	30.5 ± 5.9	145 ± 11*
Extracellular Acid Phosphatase: Root Tips	140.6 ± 7.2	141.7 ± 7.3	111 ± 9.5	261 ± 14.5*	76 ± 4.1	277 ± 30*

* Significantly different at 0.05

Table 8. Intracellular and extracellular acid phosphatase activities in leaves and roots of wheat (*Triticum vulgare* L.) plants cultured 1-3 weeks on phosphate-sufficient (+P) or phosphate-deficient (-P) nutrient medium. Means ± SD.

Parameters	7 days		14 days		21 days	
	+P	-P	+P	-P	+P	-P
	(μmol g^{-1} FW h^{-1})					
Leaf Acid Phosphatases	147 ± 20	156 ± 12.8	205 ± 18.8	260 ± 28.8*	216 ± 11.5	506 ± 54.6*
Root Intracellular Acid Phosphatases	49.5 ± 8.7	61.3 ± 12.9*	71.6 ± 11.4	96.9 ± 7.1*	123 ± 7.2	122 ± 13.6
Extracellular Acid Phosphatase: Intact Root	30.2 ± 3.5	54.3 ± 3.4*	18 ± 1.9	50.8 ± 8.5*	21.8 ± 1.6	77.2 ± 6.4*
Extracellular Acid Phosphatase: Root Tips	91.9 ± 6.5	165 ± 6.7*	61.5 ± 1	126 ± 5.9*	30.6 ± 3.6	155.3 ± 4.2*

* Significantly different at 0.05

Extracellular acid phosphatase activity increased already after one week of culture on -P nutrient medium in intact roots of barley, rye and wheat, by about 50% or 80% when compared to +P roots (Tables **5**, **7**, **8**). The increase of enzyme activity, secreted by intact -P roots after 2 week-culture, was about 1.5- and 1.4-fold for barley and oat (Tables **5**, **6**), 2.4-fold for rye (Table **7**), 2.8-fold for wheat (Table **8**), as compared to control. After 3 weeks of culture the increase of extracellular phosphatase activity in -P roots was: 2.4-fold for barley, 2.3-fold for oat, 4.8-fold for rye and 3.5-fold for wheat as compared to the control (Tables **5-8**). The acid phosphatases activity (and secretion) was intensive in young, growing parts of -P roots of all crop plants (*e.g.*, even up to 4-5 times more, for rye and wheat) whereas activity of extracellular phosphatases in the mature parts of roots was lower (Tables **5-8** and data not shown). When compared the studied plants, the highest activity of root surface enzymes was observed for wheat and rye, after 21 days of culture on -P medium (especially in root tips of plants).

Table 9. Soluble protein content in extracts from leaves and roots of barley, oat, rye and wheat plants grown for 3 weeks on phosphate-sufficient (+P) or phosphate-deficient (-P) nutrient medium. Means ± SD values are indicated. *Differences statistically important at 0.05.

	Proteins Content In Shoots						Proteins Content In Roots					
	(mg g^{-1}FW)											
	Growth On Nutrient Medium (days)											
Plant	7		14		21		7		14		21	
	+ P	- P	+ P	- P	+ P	- P	+ P	- P	+ P	- P	+ P	- P
Barley	5.97 ±0.6	7.92* ±0.9	5.9 ±0.5	8.1* ±0.6	11.2 ±0.7	10.4 ±0.7	1.23 ±0.15	1.74 ±0.2	0.67 ±0.2	0.65 ±0.2	1.4 ±0.1	1.3 ±0.2
Oat	6.4 ±0.4	6.9 ±0.6	6.2 ±0.5	7.8 ±0.6	4.3 ±0.3	9.1* ±0.7	1.36 ±0.2	1.26 ±0.2	0.8 ±0.1	0.7 ±0.2	1.08 ±0.2	0.96 ±0.2
Rye	6.3 ±0.7	8.8 ±1.2	4.9 ±0.6	5.8 ±1.1	3.6 ±0.4	7.1* ±0.9	1.28 ±0.1	0.95 ±0.2	1.2 ±0.1	0.75 ±0.2	0.97 ±0.2	0.66 ±0.15
Wheat	6.1 ±0.8	8.3* ±0.7	7.5 ±1.1	6.8 ±0.5	9.6 ±0.9	9.1 ±0.8	1.33 ±0.2	1.37 ±0.2	0.99 ±0.1	0.68 ±0.1	1.5 ±0.3	0.53* ±0.1

4. DISCUSSION

The growth of barley, oat, rye and wheat plants for three weeks on Pi-deficient nutrient media resulted in lower Pi content in tissues, changed characteristics of growth (mainly root to shoot ratios increase) and had significant effect on acid phosphatases activity increase in tissues and root exudates.

Pi deficiency significantly reduced shoots growth of all studied plants, especially after 3-weeks culture, the –P plants were also characterized by lower formation of tillers. When compared the growth parameters of studied cereal plants, some conclusion could be made, *e.g.* the inhibition of shoot growth was more pronounced for rye and wheat, cultured on Pi-deficient medium than other crop plants (Tables **1-4**). Our other experiments indicated that Pi deficiency, after 2-3 weeks culture, affected also leaves area and intensity of photosynthesis and assimilate production in crop plants [13, 40 and data not published]. Generally, the early Pi-deficiency had no significant effect on fresh and dry mass of roots but a tendency to enhanced elongation of roots was observed for all studied plants. More intensive elongation of the roots was observed for -P oat (and barley) than other plants, especially after 3 weeks growth on nutrient medium.

The length of roots of –P plants increased, compared with control, already after one week of culture on nutrient media without Pi and differences were similar (or higher - for rye) until about 14-days-culture; however after that the root growth was relatively slower and after 21 days of culture the root length was similar (for barley, rye) or lower (for wheat) (Tables **1**-**4**).

Our previous results indicated that transfer of -P cucumber plants to full nutrient media did not change the slope of curve of root growth, the elongation of roots after such transfer was more similar to Pi-deficient plants than to +P plants, which might indicate that a signal coming from Pi starvation caused nonreversible reaction of plant, in this case - the initial increase of root length [13]. The stimulation of root elongation is one of plant responses to low Pi level, important for exploring and searching of available Pi, mainly at the beginning of stress condition. However, as an effect of prolonged Pi starvation the significant reduction of root growth was also observed [6, 7, 12, 13, 43]. It was indicated that growth of maize root was also enhanced shortly after beginning of Pi deficit, although it was reduced when low-Pi conditions were prolonged [12, 15]. The elongation rate of axial roots was maintained but density of some laterals was not affected, however the emergence of new axial roots was drastically reduced, probably due to lower availability of carbohydrates [7, 17]. It was suggested that, under Pi starvation, a decrease of ATP content might be a limiting factor for plant biomass production and that the increase of root mass or length of -P plants was the result of better relative growth rate but only at the beginning of culture [43]. In addition, Pi deficiency could regulate other features of root architecture/ anatomy of crop plants, similar to those reported by [6, 7, 16, 18, 21], often resulted in a greater exploration of the soil and better root efficiency and higher Pi uptake, however we did not observed such features in our studies.

Change in acid phosphatases activity is rather a common reaction of plant on phosphorus starvation, facilitated Pi availability by hydrolyses of organic sources of phosphorus in soil or inside plant cell [5, 23]. Intracellular acid phosphatases are probably involved in greater recycling of organic P, mainly in the vacuole; whereas acid phosphatases secreted from roots have a role in breakdown of organic forms of phosphorus in the rhizosphere [23 - 25]. The increase of root surface phosphatases activity was often correlated with the decrease of phosphorus level in leaves, as observed for white clover genotypes [28]. However, other experiments showed negative relationship between acid phosphatase activity and efficiency of Pi uptake under phosphate deficit [26, 32]. Enzyme activity is dependent on the plant species, duration of Pi deficiency and may differ, even between crop cultivars, *e.g.* barley, rice, maize or oat genotypes [14, 15, 30, 31, 39]. Significant differences were found, *e.g.*, in activity of soil acid phosphatases under low-Pi availability in the rhizosphere of roots of five barley cultivars [39], however study with other cultivars have shown more similar responses to Pi depletion [30]. As indicated by our previous results, oat varieties may use different forms of acid phosphatases to acquire Pi from the soil or internal sources under Pi starvation [14]. When compared the studied cereal plants, the increase of extracellular acid phosphatases activity was the highest for Pi-deficient rye and wheat, after 2-3 weeks of culture and enzymes secretion was the most intensive in young, growing zones of -P roots (Tables **5**-**8**). In addition, the increase of activity of extracellular acid phosphatases was higher than intracellular enzymes (Tables **5**-**8**). Histochemical visualization of acid phosphatases in oat and barley roots demonstrated the highest enzymes activity in the rhizodermis and vascular tissue of -P plants [14, 30]. In the present study we used in experiments the older cereal varieties, plants which are currently not in use in intensive agriculture. However, the important traits like the ability to increase acid phosphatase production and activity might be useful in breeding and selection of the future-plants. Especially the ability of rye (wheat and perhaps oat) to increase acid phosphatase activity/secretion and better growth under low-Pi conditions are interesting and should be investigated more in details. The knowledge of acclimation mechanism to Pi deficit may be useful to culture the chosen varieties of crop plants, especially when lack of inexpensive phosphorus will cause a potential crisis in agriculture [44, 45].

The induction of acid phosphatase production in roots and secretion could be huge, *e.g.*, under Pi-deficient conditions enzyme secretion from roots of lupine increased up to 20 times, when compared to Pi-sufficient conditions [46]. The increase of acid phosphatases activity in root extracts and exudates of Pi-deficient white lupine was most pronounced in the proteoid region and proteoid-root-specific phosphatases secretion often coincided with organic acids exudation as well as root development [47]. However, in our experimental conditions not observed changes of pH in the -P nutrient media indicated, that studied cereal plants not respond to Pi starvation *via* increased exudation of protons or organic acids from roots (data not shown). Recent studies by [48] indicated that the effects of organic anions in the rhizosphere could be varied among plant species and they play minor roles in improving phosphorus availability and Pi uptake.

The root-associated acid phosphatases pool increased when Pi was limiting and several enzyme isoforms were

secreted from roots of *Arabidopsis*; however, as an activity, only one of them increased specifically as response to low external phosphorus level [33]. Three to four acid phosphatase isoforms were detected in oat and barley tissues but only one unique isoform was strongly induced by moderate Pi deficiency [14, 30]. In rice, several acid phosphatase isoforms were identified corresponding to novel secreted purple phosphatase, *OsPAP10c* overexpression increased the accumulation of four isoforms of acid phosphatases in transgenic plants [49]. Recently, several studies demonstrated that transgenic plants with higher expression of acid phosphatase genes, including a purple phosphatase genes, had improved Pi acquisition and better biomass production [45, 49 - 51], thus contribute to a better understanding of acid phosphatases function in plants.

CONCLUSION

The responses of rye, wheat, oat and barley to phosphorus starvation were similar to those observed for other crop plants. Pi deficiency, at a moderate level, significantly affected the growth of shoots of the studied crop plants, this was followed by enhanced activity of acid phosphatases both in -P root extracts and those secreted by roots. More prolonged low Pi-stress strongly reduced shoot growth of all studied plants, however root elongation growth was not affected or even enhanced. The crop plant acclimation to Pi deficit is dependent both on duration of stress condition, and plant species/cultivar ability, *e.g.*, wheat is more sensitive to lack of Pi than oat or barley. The efficient acclimation of growth and metabolic processes of cereal plants to moderate Pi deficiency conditions are necessary to appropriately respond to changes of environment and survive on low-Pi soil.

LIST OF ABBREVIATIONS

Pi	=	Inorganic phosphate
+P	=	Plants phosphate-sufficient plants (control)
-P	=	Plants - phosphate-deficient plants

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

No Animals/Humans were used for studies that are base of this research.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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