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IC and EŻ wrote the manuscript; EŻ measured intracellular APase isoforms activity and APases in root fragments and cross sections, and provided figures; KZ prepared plant culture and determined the growth parameters, Pi content, enzyme activity, and protein content; AK prepared split-root system culture and measured growth parameters, Pi content, and enzyme activity; IC supervised the work, gave the final corrections and critical revisions

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ORIGINAL RESEARCH PAPER

The effects of diversified phosphorus nutrition on the growth of oat (*Avena sativa* L.) and acid phosphatase activity

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Abstract
We studied the effect of differential phosphorus (P) supply on the development of oat seedlings (*Avena sativa* L. 'Arab') as well as localization and activity of acid phosphatases in tissues and root exudates. Plants were grown for 1–3 weeks on nutrient media with inorganic phosphate (+P, control), reduced Pi (0.1 P), phytic acid (PA) as organic P source, and without P addition (−P), in standard conditions or in a split-root culture system. Phosphate starvation reduced shoot growth but increased root elongation and root/shoot ratio, whereas 0.1 P and PA oat plants had similar growth parameters to +P plants. The growth on −P medium significantly decreased Pi content in all tissues, but only a slight Pi decrease was observed in plants grown on 0.1 P and PA media or various split-root system conditions. Pi starvation led to an increase in acid phosphatase (APase) activity in root exudates when compared to +P, 0.1 P, and PA plant samples. APase activity was especially intensive in root cross sections in rhizodermis and around/in vascular tissues of −P plants. For plants grown on 0.1 P medium and on phytic acid, APase activity did not change when compared to the control. Three major isoforms of APases were detected in plant tissues (similar in all studied conditions, with a higher activity of one isoform under Pi deficit). Generally, lowered Pi content (0.1 P) was not stressful to oat plants for up to 3 weeks of culture. Oat plants grew equally well on nutrient media with Pi and on media with phytate, although phytate was considered not available for other plants. The oat plants activated mainly extracellular APases, but also intracellular enzymes, rather via nonlocal signals, to acquire Pi from external/internal sources under Pi deficiency.

Keywords
APase localization; extracellular acid phosphatase; phytate; Pi deficit; root exudates; split root

Introduction

Phosphorus is a critical macronutrient required for plant development and metabolism, including processes such as organic compound biosynthesis, photosynthesis, respiration, energy transfer, coordination of gene expression and enzymes activity, or signal transduction [1–5]. The P availability is one of the most limiting factors for crop plant growth and yield production, mainly due to its immobilization. Phosphorus in soil exists predominantly as sparingly soluble inorganic phosphates and insoluble organic phosphates, which are not directly available to plants. The available forms of P are $H_2PO_4^-$ and HPO_3^- ions (Pi), usually present in the soil solution in very small concentrations [6]. Plants have evolved various morphological, physiological, and biochemical adaptations to counteract phosphate deficiency. Two main strategies to

enhance P influx and efflux in crop plants are currently being discussed – P acquisition and P utilization. Many processes are involved in both mechanisms, however, the proper root characteristics are most important in P acquisition [7,8]. Changes in root architecture and elongation, increased root hair growth and density, increased root/shoot growth ratio due to the extended root surface area are typical morphological adaptations to Pi starvation [5,9–13]. The reorganization of root system architecture is thought to be a local response of plants to Pi deficiency conditions [12,14]. Recent reports indicated that changes in Pi availability in soil are locally sensed by root tips and root growth is adjusted via modulating division and expansion of cells [14–16]. *LPR1*/*LPR2* (*LOW PHOSPHATE ROOT 1*, *2*) and *PDR2* (*PHOSPHATE DEFICIENCY RESPONSE 2*) gene products are probably key components of root Pi sensing [7,15]. Low Pi supply induces high-affinity phosphate transporters in root cells as well as exudation of various compounds from roots, such as organic acid and protons, which are able to increase Pi availability by mobilization of insoluble Fe, Ca, and Al phosphates, via pH changes in the rhizosphere or utilization of organic esters of P by (non)specific enzyme activities [5,16–18]. Alternatively, plants are developing specialized cluster/proteoid roots or can activate root colonization by mycorrhizal fungi and/or rhizosphere bacteria [5,12,18–21]. Phosphate starvation often affects metabolic processes in shoots, e.g., photosynthesis and respiration or causes alterations in the partitioning of assimilated carbon into sucrose/starch pathways through the regulation of gene expression and/or protein phosphorylation [2,4,22,23]. Most of these reactions of plants are believed to be a systemic response, including metabolic adjustments for more efficient Pi recycling under deficient conditions [14,16,24].

In most cultivated soils, organic P compounds comprise a large fraction of up to 80% of the total P, mainly in the form of phytate and its derivatives, not easily absorbed by crop plants [25,26]. Phytate is a common storage form of P in seeds. A major part of P organic form in the soil is derived from agricultural runoff or fertilizers [25,27]. However, the importance of organic P/phytate for plant nutrition in croplands is still not fully understood. Organic P forms, as an example, are not used as the sole source of P by wheat [28], however, our results showed that barley plants could effectively grow on a medium containing only phytate [10]. The fixation of inositol hexa- and pentaphosphate isomers (phytate) in soil is stronger than orthophosphate anions, thus some plants (or soil microorganisms) developed/improved mobilization processes, such as excretion of organic acid anions or secretion of enzymes like phosphatases/phytases [17,18,21,25].

Acid phosphatases (APases; EC 3.1.3.2) are integral components of the plant response to Pi starvation [9,10,29–31]. Extracellular acid phosphatases can be secreted by roots of plants to acquire Pi from organic P sources. Intracellular acid phosphatases are believed to be involved in Pi remobilization from senescing organs, storage tissues, older leaves, or intracellular compartments (mainly vacuoles) to young tissues, especially under Pi deficiency conditions [29,31]. Secreted APases have been purified and well characterized in model plants, such as *Arabidopsis*, lupin, tobacco, or rice [21,32–34] and most of the results indicate an important role of APases during plant growth in response to Pi starvation. However, a negative correlation between the activity of root APase and Pi uptake was also observed under low P stress [28,35]. Thus, a comprehensive understanding of metabolic function of APases is difficult, partly due to their heterogeneity, large number, as well as relative nonspecificity. Acid phosphatases (especially purple acid phosphatases) and phytases have been recently intensively studied due to their usefulness for Pi-efficient crop engineering [24,31,33]. Transgenic plants expressing microbial or plant-derived APase genes (or phytase) often show higher enzyme activity and improved plant growth under low Pi nutrition [26,31,36].

In this study, we investigated the physiological response of oat plants to short-term and medium stress of Pi deficiency during the first weeks of growth, a period critical to tillering and further plant productivity. Oat (*Avena sativa* L.) is an important grain crop in human and animal nutrition or cosmetic industry; it also has a certain therapeutic potential in some diseases (diabetes or cardiovascular disorders) [37]. Oat is usually cultured on poor soils, with a low mineral concentration, but its acclimation mechanisms to such environment are not fully understood. Our previous results indicated that oat cultivars differed in terms of acclimation to Pi starvation [38]. In the present study, the effects of differential phosphorus supply (inorganic and organic P or no P) on oat

growth and acid phosphatase activity and localization were investigated to estimate the role of these enzymes in oat response to Pi deficiency. In addition, by applying split-root system culture we also tend to focus on the induction/secretion of root APases, as a process controlled by the local oat response to external Pi deficiency.

Material and methods

Plant material and culture

Oat seedlings (*Avena sativa* L. 'Arab'), after 7 days of germination (on Petri dishes), were grown hydroponically on nutrient media with contrasting P sources: inorganic phosphate, KH_2PO_4 (+P, control), lowered Pi concentration to 0.1 of the control (0.1 P), phytic acid (PA) as organic source of P, or without phosphate (−P) in conditions similar to that used by Ciereszko et al. [10] and Żebrowska et al. [38]. The control 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), $ZnSO_4$ (0.8 µM), $CuSO_4$ (0.3 µM), H_2MoO_4 (0.1 µM); the 0.1 P medium contained only 0.1 mM KH_2PO_4; to the PA medium instead KH_2PO_4 was added phytic acid (0.1 mM), and to the −P medium – KCl (2 mM) (instead KH_2PO_4). Oat seedlings were cultured in plastic containers (usually 15 seedlings per 5 L of nutrient media, pH 5.2). The culture media were continuously aerated and replaced every 4–5 days. Plants were cultured in a growth chamber with a light period of 16 h (and 8 h of darkness), PAR – 150 µmol m^{-2} s^{-1}, temperature – 23/19°C (day/night), and about 65% relative humidity. Plants were harvested after 1, 2, and 3 weeks of growth on nutrient media. For the measurements, plant tissues/root exudates were collected about 4 h after the commencement of the photoperiod.

Split-root system experiments

Oat seedlings, after germination, were cultured one week on +P nutrient medium (control) and then transferred for 2 weeks to two-compartment containers (2 L) with different nutrient solutions. Two plants were fixed in each double vessel with separated parts of the root system in each compartment. For example, half of the root system was placed on +P medium in one compartment, and the other part of the root system was placed on −P medium in the other compartment, in a similar way to that described by Ciereszko et al. [39]. Separate compartments of the chamber were filled with a complete nutrient medium or with Pi-deficient medium (+P/−P), with +P and phytate solutions (+P/PA), or phytate and −P solutions (PA/−P). As controls, we transferred oat seedlings to similarly divided containers with both parts of roots on +P nutrient medium (or −P medium) (root system was in the same medium). The composition of nutrient solutions and growth conditions were the same as described above, and by Ciereszko et al. [10].

Pi content determinations

Inorganic phosphate (Pi) was determined in plant tissues of all the studied conditions as described before by Ciereszko et al. [9,10]. An aliquot of 0.5 g of roots (half of the system/fragments) or leaves was frozen in liquid N and the tissues were homogenized in 10% (v/v) TCA at 4°C. The homogenate was diluted with 5% TCA, then incubated for 30 min. Pi content was determined in the filtrate using a phosphomolybdate colorimetric assay, as described by Ames [40].

Secreted and extracellular acid phosphatase activity measurements

For the root surface and secreted acid phosphatase assay, whole roots (or half of the root system or fragments of about 1.5-cm-long root tips and mature part of roots) were

washed in distilled water, blot dried and placed into 50 mL (or 30 mL) of incubation media with a substrate mixture (6 mM *p*-nitrophenyl phosphate – pNPP – and 1 mM DTT in 50 mM Na acetate buffer, pH 5.0) and incubated at 25°C. 200-µL aliquots of the reaction medium were collected at different intervals (for 2 h), then 200 µL of 4 M NaOH was added (to stop reaction) and the absorbance was read at 410 nm (Cecil CE 2501). The results are presented after 15 min of incubation; enzyme activity was expressed as µmol pNP (*p*-nitrophenyl) min^{-1} g^{-1} FW [9,41].

In vivo staining for APase activity

Acid phosphatase activity was also determined by in vivo activity stain as described by Żebrowska et al. [38]. Therefore, intact roots (after 1 week of growth on nutrient media) were rinsed in 0.1 mM Na acetate buffer (pH 5.0) and embedded in 2% agarose with a substrate mixture (0.2% 1-naphthyl phosphate, 0.2% Fast Blue B in 100 mM Na acetate buffer, pH 5.0). Dark purple color (after 24 h incubation) indicated APase activity in the roots or root exudates, as compared to the control (heat-killed root tissue).

Localization of APase activity in root cross sections

Free-hand oat root cross sections were incubated in a mixture with substrate (0.2% 1-naphthyl phosphate, 0.2% Fast Blue B in 100 mM Na acetate buffer, pH 5.0) and subsequently (20 min) washed in distilled water. Sections were imaged with a light microscope (Olympus BX41) as described by Żebrowska et al. [38]. Dark brown or purple color of the product indicated enzyme activity in the tissue.

Intracellular APase activity measurements

Intracellular acid phosphatase activities were determined in extracts from shoots and roots (also half of the root-split system or fragments) similar to the method described by Ciereszko et al. [9,10,41]. Samples (0.5 g) were ground in liquid N, then the extraction buffer (50 mM Na acetate buffer, 1 mM DTT, pH 5.0) was added and subsequently the extracts were centrifuged at 12,000 rpm for 10 min at 4°C. Enzyme activity was determined after 5–60 min of incubation with 6 mM *p*-nitrophenyl phosphate (in 100 mM Na acetate buffer, pH 5.0) at 37°C, after that the reaction was terminated (with NaOH). The results are presented after 15 min incubation. Soluble protein contents in the extracts were measured according to Bradford [42].

Analysis of intracellular APase isoforms

Root and shoot tissues were ground in a liquid nitrogen-chilled mortar, then 4 mL extraction buffer per 1 g tissue was added (100 mM; 2 mM EDTA, 5 mM DTT, 20 mM $CaCl_2$), and 60 mg polyvinylpolypyrrolidone (PVPP) per 1 g tissue, as described by Żebrowska et al. [38]. The tissue/buffer/PVPP solution was gently mixed at 4°C for 60 minutes and then centrifuged at 10,000 rpm and the pellet was discarded. Equal amounts of protein (10 µg for various shoots per lane and 6 µg for roots) were loaded on to a discontinuous native PAGE [5% (w/v) stacking gel, 10% (w/v) resolving gel]; proteins were quantified according to the method described by Bradford [42]. Electrophoresis was run on a Hoefer SE260 mini gel system (Amersham) at 4°C. Approximate masses of APase isoforms were estimated using Full Range Rainbow Molecular Weight Markers (Amersham). Native gels were washed (0.1 mM Na acetate buffer) and poured with 4-methylumbelliferyl phosphate in 100 mM Na acetate and 1% agarose (w/v). The fluorescence of methylumbelliferone liberated by APase activity was visualized and documented under UV light (Gel Doc 2000, Quantity One version 4.1; Bio Rad).

Statistical analysis

All assays were performed in at least three replicates in three to five independent series of experiments, and standard deviation (*SD*) was calculated. Treatment effects were tested using one-way analysis of variance (ANOVA), and post hoc Tukey's test was conducted. Means were compared between treatments at the 0.05 probability level ($p < 0.05$) (SPSS Statistics).

Results

Plant growth and Pi content in tissues

Oat plants showed Pi-deficiency symptoms after 2–3 weeks of culture on –P medium (Fig. 1). Shoot height of –P plants was lower than that of other plants (74–79% of the control after 2–3 weeks). However, root elongation was generally enhanced by Pi deficiency (133–142% of the control after 2–3 weeks) (Fig. 1A). Shoot fresh weight in –P plants was significantly lower (55% and 42% of the control after 2 and 3 weeks of growth, respectively), root fresh weight was similar or higher (e.g., 139% of the control for plants grown 3 weeks on –P medium) (Fig. 1B). A similar pattern was observed for dry weight of shoots and roots (data not shown). No significant differences were observed in growth parameters of 0.1 P and PA when compared to the control plants (Fig. 1A–C). The ratio of root to shoot fresh and dry mass was always higher in –P plants (especially in the 3rd week of culture) than in +P plants, whereas for 0.1 P and PA plants, the ratio was similar to the control (Fig. 1C). Exposure of half of the root system to phosphate-deficient nutrient medium or PA medium (split-root system) also did not significantly affect shoot and root growth parameters (Tab. 1). Water content in tissues was similar in all studied plants, under all P treatments (data not shown).

Inorganic phosphate content, both in shoots and roots, was significantly lower already after 1 week of culture on –P medium (up to 11% of the control in shoots, and 15% in roots) (Fig. 1D). Pi level in shoots and roots during next 2 weeks of culture was about 7% of the control. Pi content in shoots of 0.1 P plants decreased already after 1 week of culture (86% of the control) and during next 2 weeks was 51–34% of the control, whereas root Pi content decreased after 2–3 weeks (57–32% of the control). Pi level in PA plants decreased to 71% of the control in shoots and 60% in roots, after 2–3 weeks of culture (Fig. 1D). The exposure of half

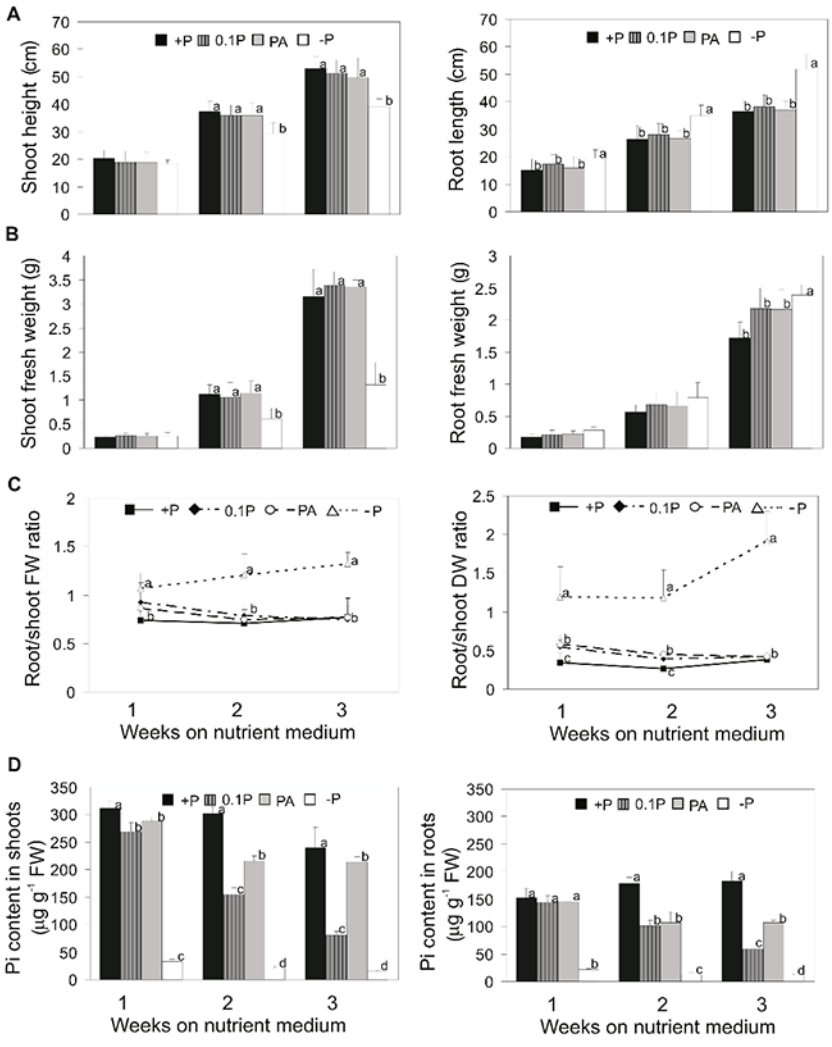

Fig. 1 Growth parameters: shoot height, root length (**A**), fresh mass of shoots and roots (**B**), root to shoot ratio of fresh mass (**C**), and inorganic phosphate content (**D**) of oat cultured on nutrient media: phosphate-sufficient (+P), with reduced Pi content (0.1 P), with phytate (PA), or without P (–P). Means ±*SD* values are indicated. Different letters indicate significant differences ($p < 0.05$) among the treatments (post hoc Tukey's test).

Tab. 1 Growth parameters, Pi content, and acid phosphatase activities in root exudates and tissues of oat (*Avena sativa* L. 'Arab') plants cultured for 2 weeks in split-root system conditions on phosphate-sufficient media (+P/+P, control) or with half of the root system in a complete nutrient medium and half in a medium without Pi (+P/−P), with half of the root system in +P and half in a medium with phytate (+P/PA), or with halves of the roots in phytate and −P solutions (PA/−P).

Parameter	Shoot	Roots		Shoot	Roots		Shoot	Roots		Shoot	Roots	
	+P/+P	+P	+P	+P/−P	+P	−P	+P/PA	+P	PA	PA/−P	PA	−P
Fresh mass (g)	2.95 ±0.68	0.58 ±0.14	0.59 ±0.2	3.73 ±1.2	0.86 ±0.2	0.93 ±0.3	3.61 ±0.8	0.71 ±0.2	0.92 ±0.4	3.56 ±0.8	0.93 ±0.5	0.93 ±0.3
Pi content (µg g^{-1} FW)	738 [a] ±261	658 [a] ±142	726 [a] ±59	717 [a] ±143	865 [a] ±126	371 [b] ±45	771 [a] ±59	1,029 [a] ±118	708 [b] ±103	581 [b] ±44	245 [a] ±33	213 [a] ±43
Extracellular APase (µmol pNP g^{-1} FW min^{-1})	-	1.05 [a] ±0.45	0.77 [a] ±0.28	-	0.94 [a] ±0.30	1.15 [a] ±0.31	-	1.31 [a] ±0.75	1.128 [a] ±0.6	-	1.48 [b] ±0.6	2.03 [a] ±0.9
Intracellular APase (µmol pNP g^{-1} FW min^{-1})	9.67 [a] ±1.76	0.55 [a] ±0.23	0.43 [a] ±0.1	9.55 [a] ±1.25	0.48 [a] ±0.15	0.44 [a] ±0.14	8.78 [a] ±2.47	0.65 [a] ±0.14	0.84 [a] ±0.21	7.87 [a] ±2.88	0.50 [b] ±0.08	1.03 [a] ±0.47

Means ±*SD*. Different letters indicate significant differences ($p < 0.05$) among the treatments (post hoc Tukey's test).

of the root system to −P nutrient medium (split-root system conditions) decreased Pi content mainly in −P parts of roots, shoot Pi level was not significantly affected, except PA/−P oat tissues (Tab. 1).

Acid phosphatase secretion, localization, and activity

Staining in vivo for the activity of root surface acid phosphatase (after 1 week of oat culture on +P, 0.1 P, PA, and −P nutrient media) showed higher enzyme secretion by intact −P roots, in comparison to +P plants, whereas the secretion of APases in 0.1 P and PA plants was similar to the control (Fig. 2A). Cellular localization/activity of acid phosphatases was estimated in cross sections of oat roots in all studied conditions. The activity of APases was especially intensive in rhizodermis and vascular tissues of young roots of −P plants (Fig. 2B,C).

Extracellular acid phosphatase activity, root-bound and secreted from the roots to the medium was also the highest for −P plants, whereas 0.1 P and PA plants showed similar activities as the control (Fig. 3). APase activity was twofold higher than in the control after 1 week of growth on −P nutrient medium, and after next 2–3 weeks of culture – about 2.4-fold higher than on +P (Fig. 3). APase activity associated with young, growing zones of roots of −P oat plants (after 2 weeks of culture) was much higher than in +P, 0.1 P, and PA plants (2.8, 3.3, and 3.8 times), whereas enzyme activity in mature parts of roots was lower than in root tips in all studied conditions (Tab. 2). In contrast, the exposure of half

Fig. 2 In vivo staining for acid phosphatase activity in roots (**A**) and root cross sections (**B**) of oat cultured on nutrient media: phosphate-sufficient (+P), with reduced Pi content (0.1 P), with phytate (PA), or without P (−P). Images of rhizodermis and vascular tissues of −P plant roots (**C**). Dark color indicates acid phosphatase activity in the roots and root exudates (as compared to heat-killed tissue – the control).

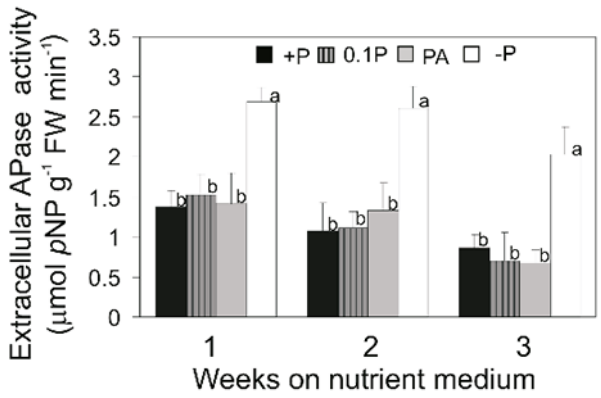

Fig. 3 Extracellular acid phosphatase activity (µmol pNP g^{-1} FW min^{-1}) secreted by intact roots of oat cultured on nutrient media: phosphate-sufficient (+P), with reduced Pi content (0.1 P), with phytate (PA), or without P (−P). Means ±*SD* values are indicated. Different letters indicate significant differences ($p < 0.05$) among the treatments (post hoc Tukey's test).

Tab. 2 Acid phosphatase (APase) activities associated with root tips and mature parts of oat roots (*Avena sativa* L. 'Arab') grown for 2 weeks on nutrient media: phosphate-sufficient (+P), with reduced Pi content (0.1 P), with phytate (PA), or without P (−P).

	APase activity (µmol pNP g^{-1} FW min^{-1})			
	+P	**0.1 P**	**PA**	**−P**
Root tips	1.46 ^{b} ±0.24	1.23 ^{b} ±0.23	1.07 ^{b} ±0.14	4.05 ^{a} ±0.44
Mature parts of root	1.19 ^{b} ±0.11	1.03 ^{b} ±0.03	0.80 ^{b} ±0.07	2.73 ^{a} ±0.34
Whole root system	1.44 ^{b} ±0.05	1.31 ^{b} ±0.14	1.25 ^{b} ±0.23	3.14 ^{a} ±0.20

Means ±*SD*. Different letters indicate significant differences ($p < 0.05$) among the treatments (post hoc Tukey's test).

of the root system to −P nutrient medium (split-root system conditions), when the other part of the root system was in +P solution, did not significantly affect extracellular APase activity, except PA/−P plants (Tab. 1).

Intracellular APase activity, estimated in extracts from both shoots and roots, was higher than extracellular APase activity in all P treatments (Fig. 4). Increased APase activity in shoots of −P plants was observed only after 1 week of culture (20% higher in comparison to +P plants). Plants cultured on 0.1 P medium for 1–2 weeks showed lower APase activities (74% and 60% that of the control). A decrease in enzyme activity was also observed for PA plants grown for 1 and 3 weeks (86% and 80% that of the control) (Fig. 4A). Pi deficiency enhanced root APase activity, especially after 2–3 weeks in −P culture (40% and 30% higher compared to the control). A significant increase of APase activity (40% higher) was also observed in 0.1 P plants after 3 weeks of culture (Fig. 4B). The growth of plants for 2 weeks in split-root system conditions generally did not significantly affect APase activity, however, the exposure of half of the root system to −P nutrient medium (while other part of root was in PA solution) increased twice the activity of intracellular APases (Tab. 1). Our preliminary experiments with short-term split-root system conditions (2- and 5-day culture) also did not show any modification in APases activity (data not shown).

Soluble protein contents in enzymatic extracts from roots of all the studied plants were affected by different P sources (Fig. 5). After 2 weeks of plant growth on −P nutrient medium, the protein content in roots was 50% higher when compared to the control. However, plants grown on 0.1 P, PA, and without phosphate showed lowered protein content in comparison to +P plants (except −P plants grown for 2 weeks, in which the protein content was 30% higher than in the control) (Fig. 5). Generally, soluble protein content in shoots was about twofold higher than in roots (Fig. 5).

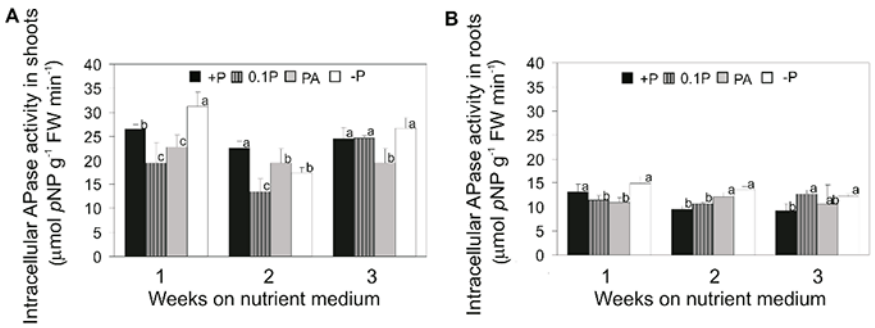

Fig. 4 Intracellular acid phosphatase activity (µmol pNP g^{-1} FW min^{-1}) in shoots (**A**) and roots (**B**) of oat cultured on nutrient media: phosphate-sufficient (+P), with reduced Pi content (0.1 P), with phytate (PA), or without P (−P). Means ±*SD* values are indicated. Different letters indicate significant differences ($p < 0.05$) among the treatments (post hoc Tukey's test).

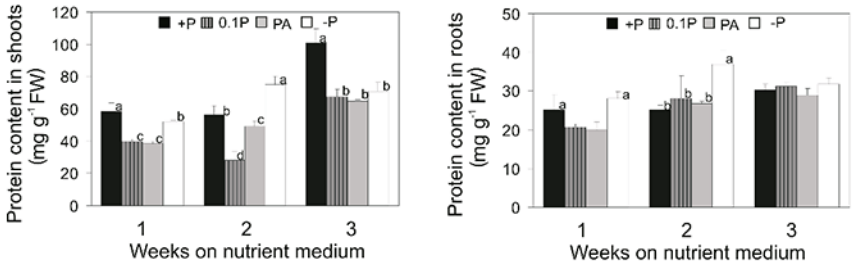

Fig. 5 Soluble protein contents in the extracts from shoots and roots of oat cultured on nutrient media: phosphate-sufficient (+P), with reduced Pi content (0.1 P), with phytate (PA), or without P (−P). Means ±*SD* values are indicated. Different letters indicate significant differences ($p < 0.05$) among the treatments (post hoc Tukey's test).

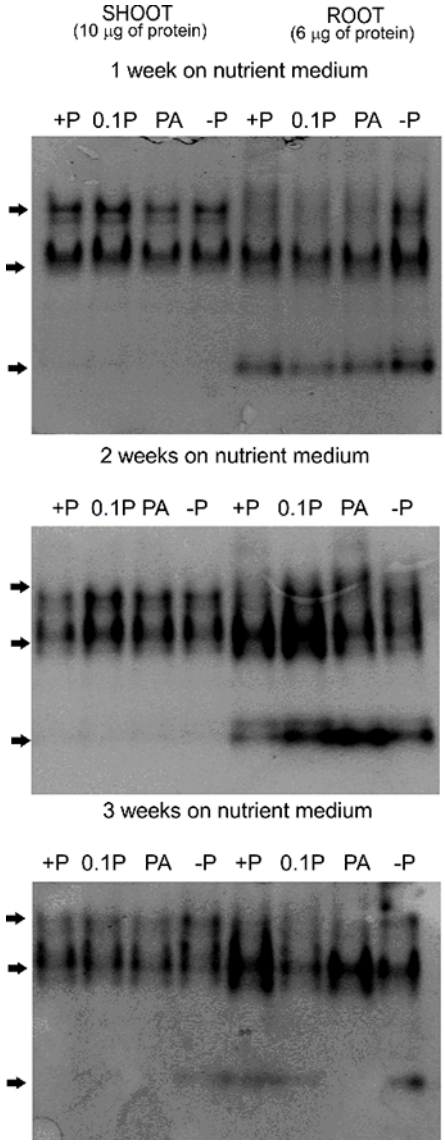

Fig. 6 Detection of APase isoforms in shoot extracts (10 µg protein per lane) and root extracts (6 µg protein per lane) from oat plants cultured for 1–3 weeks in complete nutrient media: phosphate-sufficient (+P), with reduced Pi content (0.1 P), with phytate (PA), or without P (−P). Protein extracts from both shoots and roots were run on native discontinuous PAGE and stained for APase activity using 4-methylumbelliferyl phosphate and visualized under UV light.

To examine APase isoforms, proteins isolated from oat shoots and roots, grown on +P, 0.1 P, PA, and −P nutrient media, were separated on native PAGE and stained for APase activity (as described in "Material and methods" section). At least three major APase isoforms with different mobility on the gel were detected in tissue extracts (approximately: 95 kDa, 70 kDa, and 27 kDa) in all studied growth conditions (Fig. 6), the obtained results are in agreement with our previous studies [38]. One of these isoforms (about 27 kDa) was induced by Pi deficiency in −P root extracts, particularly after 1 or 3 weeks of culture (higher activity than in the control was also observed for 0.1 P and PA plants after a 2-week growth). The isoformic pattern of APases remained essentially unchanged during 1–3 weeks of plant growth (Fig. 6).

Discussion

Effects of Pi deficiency and organic P on oat growth

The lack of Pi in the culture media significantly affected the growth of oat plants (*Avena sativa* L.) and acid phosphatase activity in the tissues and root exudates. Shoot mass was reduced, but root elongation was not changed and, in some conditions, even stimulated; in addition, high ratios of root/shoot mass (or length) were observed for −P plants (Fig. 1, Tab. 1). Modifications of oat growth parameters were observed already at the early and intermediate stages of Pi deficiency (2–3 weeks of culture); the effects of longer Pi starvation have not been investigated in this study. Our previous study indicated that the increase of root elongation and root/shoot ratio was an important adaptive response of oat plants to Pi deficiency and that different oat cultivars demonstrated various growth modifications consistent with a low Pi content in tissues [38]. The growth of oat 'Arab' proved to be less sensitive to low-Pi stress than other studied cultivars, but this cultivar was characterized by the highest extracellular APase activity under Pi-starvation conditions [38]. In the latter article, we described only oat responses to the growth on nutrient media that did not contain Pi, thus the current experiments were also performed for oat plants cultured on media containing low amounts of inorganic phosphate (0.1 P) or organic source of P (PA). However, such modifications of nutrient media did

not significantly influence growth parameters of oat plants during 3 weeks of culture (Fig. 1). Probably, the observed decrease of Pi level in tissues was not sufficiently drastic to cause growth retardation. Additionally, we have obtained similar results for three barley cultivars grown in almost identical conditions [10]. In our studies, we did not compare the details of root characteristics under different growth conditions, perhaps it should be investigated in future experiments. The increase of root to shoot ratio, branched root systems or longer root hairs and their higher density are common plant responses to a low external level of Pi. Even small changes in root elongation, root diameter, and density of root hairs are thought to be significant for better exploration of soil and might enhance Pi uptake [11,12] (see also [26] and studies cited therein). Several studies have addressed the function played by phytohormones in root architecture modification and the role of transcription factors, such as MYB62, WRKY75, PHR1, ZAT6, or miRNAs in growth responses to Pi deficiency [3,7,8,12].

Split-root experiments, in which the plants were cultured with halves of divided root system in high phosphate (+P) and the other without Pi or in PA medium, have shown that the growth of oat plants (as well as Pi content in tissues) was similar to the control (Tab. 1). Split-root system techniques were used in several studies to investigate the impact of an uneven distribution of nutrients, e.g., nitrogen or P on ions uptake by roots and other root features, as dependent on local signals [11,15,16,24,39] (see also [43] and studies cited therein). Our results suggested that local signal of relatively short-term Pi deficiency in about half of the divided root system was not sufficient to affect significantly oat growth or Pi content in plant tissues in our experimental conditions (Tab. 1). This indicates that oat plants could be better adapted to low-Pi stress than other crop plants and develop other efficient acclimation mechanisms to respond rapidly to the lack of mineral nutrient.

Modification of acid phosphatase activity and localization as an important oat plant response to Pi depletion

Pi content in the roots of oat plants grown for 3 weeks in a phosphate-deficient medium (−P, whole root exposed) dropped to 7% of the control value and a significant increase of APase secretion from the roots was observed in these conditions (Fig. 1, Fig. 3, Tab. 2). A decrease of Pi level in oat tissues observed in 0.1 P and PA plants probably was not sufficient to cause modifications in APase activity (Fig. 1–Fig. 3). The extracellular APase activity was higher in young root tips than mature parts of the roots in all tested growth conditions, but was particularly high in −P oat plants (Tab. 2). The enhanced activity of extracellular APase was observed already after 1 week of culture on −P media (Fig. 2, Fig. 3), which could indicate that enzyme secretion is an early response of oat plants to Pi deficiency. Secretion of APases from roots or the induction of root-surface-bound enzymes can be a major oat plant mechanism facilitating Pi availability by breakdown of different organic P sources in soil [31]. Histochemical visualization of APases in oat root transverse sections showed the highest enzyme activity in the rhizodermis and vascular tissue of −P plants, which was consistent with our previous results [38]. High activity of APases in the root epidermis suggested that some of these enzymes are secreted from oat roots to the ground. Indeed, in vivo APase activity staining of oat roots showed intensive enzyme secretion from intact −P roots (Fig. 2). The results of APase activity estimations, root-associated and secreted, are usually in agreement with the visualization of enzyme activity on transverse root sections. However, oat culture in split-root system conditions, when half of the root system was exposed to −P nutrient medium (or PA), while other part of the root system was in +P solution, did not significantly affect the activity of extracellular APases, which can suggest that the induction and secretion of APases from oat roots is not a local response to Pi depletion in the ground. As indicated by other studies, APase determination in situ on cluster roots of white lupine showed that the activity of extracellular APases steadily increased with root development from meristematic, juvenile, mature to senescent stages under Pi deficiency, but the activity of intracellular APases remained relatively constant [44]. LASAP2 was considered to be a major form of exudated APases, easily released from the roots of white lupine into the rhizosphere [45]. AtPAP25 secreted by the roots of *Arabidopsis* seedlings, the cell wall-targeted purple APase, was shown to be critical for

plant acclimation to P deprivation [46]. Overexpression of OsPAP10a, root-associated purple acid phosphatase, was reported to increase extracellular organic P utilization by rice roots [36]. Activity of AtPAP10, a major Pi starvation-induced APase associated with the root surface in *Arabidopsis* is probably regulated both through local and systemic signaling under Pi starvation [24]. In a recent study, Lu et al. [33] identified a novel secreted purple APase in rice (OsPAP10c) and investigated its role in organic P utilization – *OsPAP10c* overexpression significantly increased APase activity both in leaves and roots, but especially on the root surface and in culture media. Studies by Wang et al. [47] indicated that the quantity of rhizosphere APase had positive correlations with P fractions available to plants and Pi uptake by the plants in low-Pi soils; these authors also stated that both *Brassica napus* and *Avena sativa* were good candidates to study P utilization.

Some reports indicated that also other root exudates, such as organic acids, are important for crop plants under Pi deficiency [16–18,26,48]. However, the decrease of pH in the nutrient media (and rhizosphere) was not observed in our experimental conditions (data not shown) [49], which suggested that oat plants did not respond to Pi starvation via increased exudation of protons or organic acids from the roots. Wang et al. [47] suggested that the effects of organic anions in the rhizosphere varied among the plant species and these exudates appeared to play minor roles in improving Pi uptake and the availability in soil.

Intracellular APase activity in shoots increased mainly at the beginning of Pi deficit in oat plants grown on –P medium, whereas enzymatic activity in Pi-deficient roots was also high during longer culture periods (Fig. 4). Three major APase isoforms were detected in extracts from oat shoot and root tissues under all growth conditions; one isoform (about 27 kDa) was induced by Pi deficiency, particularly in root extracts (Fig. 6), similar to results by [38]; no significant differences in isoform patterns between the control and 0.1 P and PA oat plants were observed. Three APase isoforms were also detected in barley shoot, however, four APase isoforms were detected in the roots and two isoforms (about 80 kDa and 27 kDa) were induced under Pi deficiency [10]. The response of 29 purple APases was studied at the transcriptional level and 11 genes were upregulated by Pi starvation in *Arabidopsis* [3] (also [50] and studies cited therein). One of Pi starvation-inducible APase isoforms was characterized as AtACP5/AtPAP17 around 34 kDa monomeric protein, in addition the strong expression of *BnPAP17-2* and *BnPAP17-3* was detected in vascular tissues suggesting their roles in Pi absorption and transport [50]. In rice, several APase isoforms corresponding to OsPAP10c, rice novel secreted purple APase, were identified using in-gel activity assays [33]. *OsPAP10c* overexpression increased the accumulation of four APase isoforms in transgenic rice plants; in addition, the results suggested that OsPAP10c could form different homo- or heteromeric protein complexes [33].

Conclusions

Our study indicates that phytate can provide a good source of P for sustained growth of oat (*Avena sativa* L.) in phytate-rich environments. Oat plants grow equally well on a medium with Pi and on a medium where Pi is replaced with phytate. The results indicate that Pi deficiency mainly induces the activity of extracellular phosphatases, also those secreted by oat roots to the ground. Moreover, Pi deprivation increases intracellular APase activity in oat roots, but to a lesser extent. Not all isoforms of APases detected in oat tissues are induced under Pi deficiency. Growth changes and APase activity modifications in response to low-Pi stress occur probably via nonlocal signals, as suggested by the results obtained from the split-root system culture, when half of the root system was exposed to –P nutrient medium.

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