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ORIGINAL ARTICLE

Polyphosphate-Induced Changes in Transcriptome and Root-Functional Traits Elucidate Enhanced Phosphorus Acquisition Mechanisms and Growth of Durum Wheat

Said Khourchi[1,2,3,4] | Yordan Muhovski[5] | Adrien Blum[2] | Iván Jauregui[6] | Caroline De Clerck[2] | Adnane Bargaz[1] | Pierre Delaplace[2]

[1]Agrobiosciences Program, College of Agriculture and Environmental Sciences, Mohammed VI Polytechnic University (UM6P), Ben Guerir, Morocco | [2]TERRA – Teaching and Research Center, Plant Sciences, Gembloux Agro-Bio Tech, Université de Liège, Gembloux, Belgium | [3]African Sustainable Agriculture Research Institute, Mohammed VI Polytechnic University, Laâyoune, Morocco | [4]African Genome Center, Mohammed VI Polytechnic University, Ben Guerir, Morocco | [5]Department of Life Sciences, Walloon Agricultural Research Centre, Gembloux, Belgium | [6]Department of Sciences, Public University of Navarre, Campus Arrosadía, Pamplona, Spain

Correspondence: Said Khourchi (said.khourchi@um6p.ma) | Pierre Delaplace (pierre.delaplace@uliege.be)

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ABSTRACT

Phosphate (P) fertilization impacts many rhizosphere processes, driving plant P use efficiency. However, less is known about the induced molecular and physiological root-rhizosphere traits in responses to polyphosphates (PolyP), particularly root transcriptome and belowground functional traits responsible for P acquisition. The present study aims to investigate physiological and transcriptomic belowground mechanisms explaining the enhanced durum wheat P acquisition under PolyP (PolyB and PolyC) supply. Root molecular traits were differentially expressed in response to PolyP, where PolyB induced upregulation of OGDH, MDH, and ENO, PAP21 and downregulation of PFK, and LDH genes. The modulation of gene expression can presumably explain the PolyP-induced changes in rhizosphere (root, rhizosphere soil, soil solution) acidification (pH decreased from 8 to 6.3) and acid phosphatase activities, which were concomitant with enhanced rhizosphere soil P availability and shoot Pi content (145% and 36% compared to OrthoP, respectively) along with changes in morphological and transcriptomic root (particularly, the upregulation of AUX1 and ABA transporter genes) traits. These findings provide novel insights that P acquisition from polyphosphates involves the coordinated regulation of genes governing root–rhizosphere processes and root development, ultimately enhancing wheat P acquisition.

1 | Introduction

Polyphosphates (PolyP) are known to be multiple-feature phosphorus (P)-based fertilizers that capable of enhancing soil P-fertility while lowering the soil retention potential of P (Loudari et al. 2022; Khourchi et al. 2022b; Khourchi et al. 2023b). They undergo progressive hydrolysis while being able to chelate micronutrients (Wan et al. 2019; Khourchi et al. 2022b; Khourchi et al. 2023a). In addition to the directly available P-fraction, PolyP fertilizers contain a polymeric P-fraction that needs to be hydrolyzed before its uptake by the roots. Hence, PolyP hydrolysis constitutes a crucial step that determines PolyP-use efficiency in agricultural soils. This hydrolysis can occur depending on the soil physicochemical properties (pH, temperature, moisture, soil texture, etc.) and triggered by the roots and their associated microbes (Chtouki

et al. 2022; Khourchi et al. 2022a, 2023b). Elevated temperature and moisture levels along with low pH have been considered the most important pedoclimatic factors that control PolyP hydrolysis (McBeath et al. 2007; Chtouki et al. 2022; Khourchi et al. 2023a). Previous findings from soil-based experiments often contradict in-vitro observations, highlighting the complexity of key factors driving PolyP behavior in soil environments and limiting our mechanistic understanding of how soil physicochemical properties influence PolyP hydrolysis (Khourchi et al. 2023a). In parallel, maximizing root-rhizosphere processes is an eco-efficient approach that is necessary for mobilization and acquisition of soil nutrients, as well as for enhanced agronomic use efficiency of P fertilizers (Zhang et al. 2010; Shen et al. 2013). This approach may prove to be highly relevant to enhance PolyP use efficiency. Although the increasing evidence suggesting root-rhizosphere activities may play a critical role in mediating P availability from PolyP in synchrony with crop P demand (Khourchi et al. 2023a), assessments of the agronomic efficiency of PolyP have rarely considered the biological potential of belowground traits, limiting our understanding of belowground mechanisms driving PolyP use efficiently in the soil.

Application of PolyP was reported to stimulate specific trade-offs between the morphological and physiological root traits of wheat (Khourchi et al. 2022b), suggesting their potential regulatory role in root growth enabling both PolyP hydrolysis (exudation of P-hydrolyzing enzymes and organic acids) and uptake of released P (induced absorptive root functional traits). While most studies on PolyP (Darch et al. 2016; Gao et al. 2020; Chtouki et al. 2021; Loudari et al. 2022; Khourchi et al. 2022b) are still lack a sound biochemical and functional trait-related mechanistic understanding of how maximizing root-rhizosphere efficiency contributes to the optimization of hydrolysis and use efficiency of PolyP, studies by Khourchi et al. (2022a, 2022b, 2023a, 2023b) have recently provide novel and up-to-date physiological evidence of the active involvement of root-microbe activities in enhancing the agronomic efficiency of PolyP. Therefore, intensifying rhizosphere processes (e.g., acidification, rhizodeposition, root-associated microbiome interactions, etc.) should be investigated as a promising below-ground strategy to enhance PolyP use efficiency.

In this study, we hypothesized that PolyP may trigger transcriptional reprogramming of multiple genes involved in P-acquisition, which subsequently enhanced root–rhizosphere functioning (e.g., phosphatase exudation, rhizosphere acidification, root growth regulation) and consequently improve P uptake and wheat growth performance.

To address this hypothesis, this study aims to (i) assess, for the first time, the induced transcriptomic responses of durum wheat fertilized with two PolyP (potassium PolyP: PolyB and sodium PolyP: PolyC) fertilizers, (ii) assess the role of induced rhizosphere acidification and APase activities (in roots, rhizosphere soil, and soil solution) in P availability from PolyP in the root-rhizosphere microenvironment, (iii) map the potential regulatory role of PolyP in root growth dynamics of wheat and determine its role in enabling better P acquisition from PolyP, and (iv) elucidate the key physiological and transcriptomic mechanisms underlying increased P availability and acquisition from PolyP.

2 | Materials and Methods

2.1 | Experimental Design and Plant Growth Conditions

Rhizobox-based experiments were carried out under controlled conditions to determine the key belowground mechanisms that might be responsible for enhanced P availability and acquisition from PolyP, and consequently, wheat growth performance.

The rhizoboxes (internal dimensions: 58 cm length × 1 cm width × 27 cm depth) were made of polyethylene with a removable Plexiglas transparent panel. The Rhizobox dimensions, the conducted analysis, and other related details are illustrated in Supporting Information S1: Figure S1. The rhizoboxes were filled with 2.3 kg of P-deficient soil (mixed with sand at a ratio of 3(soil):1(sand) v/v) collected from the upper 20 cm of a maize-cultivated field at Estinnes, Hainaut province, Belgium (50°23′27.5″N 4°02′39.4″E). The key physicochemical properties of the substrate characteristics are presented in Supporting Information S1: Table S1. The rhizoboxes were equipped with soil solution samplers (Rhizons, described below) positioned at 10 cm and 40 cm from the top to enable a non-destructive and in-situ method for collecting soil solutions, allowing the analysis of soil solutions at different depths in response to different treatment. Grains of durum wheat (*Triticum turgidum* subsp. *durum* (Desf.), Karim variety) were surface-disinfected by successive immersion in ethanol (70%, 30 s) and sodium hypochlorite (3%, 5 min), followed by several washes using sterile distilled water. One disinfected grain was sown per rhizobox. The transparent sides of rhizoboxes were covered with black sheets to avoid penetration of light into them. Each set of five rhizoboxes was placed adjacently in a plastic box at an angle of 45° to force root growth along the transparent side of rhizobox due to gravitropism.

The rhizoboxes were subsequently placed in a growth chamber under controlled conditions (temperature was maintained at 23°C, relative humidity 60%, a photoperiod of 16/8 h light/dark with a light irradiance of 260 mmol/m^2/s) and their positions were randomized each week. The experiment comprised a randomized complete block design with four treatments and eight replicates per treatment.

The P was applied as PolyC (sodium PolyP), PolyB (potassium PolyP) and OrthoP at a rate of 60 kg P ha^{-1} as described by Khourchi et al. (2022b), along with control plants with no P added (P0). All other nutrients were balanced by applying modified Hoagland nutritive solution as described by Khourchi et al. (2022b). The plants were watered daily to maintain a volumetric water content of 25%–30% using soil moisture probes (METER Group, EC-5).

2.2 | Non-Destructive Measurement of Root Growth Dynamics

In this experiment, a "PhotoBox" was constructed as an imaging system, equipped with a rhizobox holder, a Nikon camera, a camera holder, and two LED lights, to non-destructively monitor the wheat root growth over time in response to PolyP

(Alonso-Crespo et al. 2023). The camera was connected to a computer and placed 50 cm away from the transparent lid of the rhizobox. The left and right sides of the lid window were illuminated by LED tubes positioned laterally, allowing uniform lighting of the rhizobox during image acquisition. From 9 days after sowing (DAS), images were taken every 3 days using a digital camera (Nikon D3400, Nikon USA) equipped with a zoom lens (AF-P DX NIKKOR 18–55 mm) set up at 18 mm. The acquired images were first cropped using ImageJ (https://imagej.nih.gov/ij/) to remove the rhizobox borders. Then, convolutional neural network (CNN)-based software, RootPainter, was used to segment the acquired images (Smith et al. 2022). RootPainter was used to create training datasets consisting of 1600 smaller images (704 × 706 pixels) as described by Alonso-Crespo et al. (2023). The training images (> 700 small images) were annotated, and CNN models were trained to enable them to distinguish roots from background (soil) in our images using RootPainter. After annotating more than 700 images and confirming that the trained CNN model (the most accurate one) was able to detect most roots in the images, the training was stopped, and the best model was chosen to segment the cropped images based on the accuracy index (using the "metrics plot" option). Rhizovision Explorer software was used to quantify the morphological root traits such as root length (RL), root surface area (RSA), root volume (RV), and number of branches (RB) from the segmented images (Seethepalli et al. 2021).

2.3 | Collection of Rhizosphere Soil Solutions

The soil solutions were collected using Rhizons samplers (Rhizons MOM, Rhizosphere Research Product, Netherlands) as a non-destructive technique allowing in situ soil solution sampling. Rhizons MOM with a 10 cm porous part (0.12–0.18 μm pore size), 2.5 mm outer diameter, female Luer-lock and 12 cm PVC/PE tubing were used (Seeberg-Elverfeldt et al. 2005). After filling the rhizoboxes with the substrate, two Rhizons were installed per rhizobox at the sides at 10 and 40 cm from the top of rhizobox, allowing sampling of soil solution at different depths. To collect the soil solutions, the 20 mL syringe was connected to the female Luer-lock and a vacuum was created by wedging the syringes with wooden sticks. After 4 h at room temperature (23°C), the collected rhizosphere soil solutions were stored at −20°C for further analysis (Pi content, phosphatase activities, and pH). The soil solutions were collected every 5 days starting from 10 days after sowing.

2.4 | Postharvest Analyses

At harvest (30 DAS), the rhizoboxes were carefully opened by removing the transparent lid. The roots were gently shaken, and soil tightly adhering to the roots was collected as rhizosphere soil and stored at −20°C until further processing. Fresh samples of shoots and roots were stored at −20°C for further analysis including Pi content, APase activities, while fresh roots for transcriptomics were kept at −80°C. The dry weight of shoots (SDW) and roots (RDW) was measured after placing the samples into an oven at 70°C for 72 h.

2.4.1 | Shoot and Root Inorganic P-Content

Fresh shoots and roots (aliquots of 100 mg fresh weight) were ground in liquid nitrogen before extraction with sodium acetate buffer (0.2 M, pH 5.6). The samples were centrifuged at 12 000×*g*, 4°C for 10 min and an aliquot of the supernatant was used to estimate the inorganic P (Pi). Shoot and root Pi content values were determined spectrophotometrically at 880 nm according to Khourchi et al. (2022b).

2.4.2 | Available P in Rhizosphere Soil

An aliquot of rhizosphere soil (0.5 g) was placed into 10 mL of sodium bicarbonate (0.5 M, pH 8.5). The mixture was stirred for 30 min at 150 rpm and filtered using Whatman No.1. An aliquot of 1 mL of rhizosphere soil filtrate was used to determine the available P in rhizosphere soil as described by Khourchi et al. (2022b).

2.4.3 | Acid Phosphatase Activity in Roots and Rhizosphere Soil

The APase activity measurement was performed by quantifying the amount of *p*-nitrophenol (*p*-NP) produced from p-nitrophenyl phosphate (*p*-NPP) according to Bargaz et al. (2017). Fresh rhizosphere soil (125 mg) was placed directly into cold acetate buffer (pH 5.6, 0.2 M), whereas roots (100 mg) were ground in the same buffer. The homogenates were centrifuged at 12 000×*g* for 10 min. An aliquot (125 μL) of the supernatant was used to quantify the APase activity in rhizosphere soil and root of wheat plants in response to PolyP.

2.4.4 | Available P, Acid Phosphatase Activity, and pH Variation in Rhizosphere Soil Solution

The available P content and APase activity in the soil solution were determined using molybdenum blue method and *p*-NPP method, respectively, as described above. The acidification of rhizosphere soil solution in response to PolyP was estimated by measuring the variation of pH across multiple measurement timepoints using a pH meter.

2.4.5 | Extraction of Total RNA From Wheat Root and RNA-Seq Analysis

2.4.5.1 | RNA Extraction and Library Construction. The collected root tissues were ground in liquid nitrogen and the resulting powder was then used for RNA extraction. The extraction and purification of RNA from each sample were performed using NucleoSpin RNA Plant (Macherey-Nagel, Germany), following the manufacturer's instructions. RNA quality (RNA integrity number and 28S/18S) and purity were checked using an Agilent 2100 Bioanalyzer (Agilent RNA 6000 Nano Kit) and NanoDrop, respectively. RNA sequencing and library construction were carried out at Beijing Genomics Institute (BGI), China, at a sequencing depth of 20 million paired end reads. Briefly, oligo-dT beads were used to enrich RNA with

PolyA, followed by fragmentation. The fragmented RNA was used to generate the first strand cDNA using random N6-primed reverse transcription, followed by second strand cDNA synthesis, end repair, end adenylation, and adapter ligation. The resulting product was purified and enriched by polymerase chain reaction (PCR) amplification to yield the final cDNA library. The library was loaded onto a DNBSEQ platform (DNBSEQ Technology) for sequencing.

2.4.5.2 | Bioinformatic Analyses. Low-quality raw RNA-seq reads were filtered using SOAPnuke (version 1.5.6). The filtered reads were stored in FASTQ format. The filtered and high-quality data were used for all downstream analyses. The filtered reads were mapped onto the reference genome of durum wheat (*Triticum turgidum subsp. durum*, Svevo.v1) using HISAT2 (version 2.0.4), followed by novel gene prediction (Martin and Wang 2011; Kim et al. 2015; Maccaferri et al. 2019). Transcript assembly based on the reference genome for each library was performed using StringTie (version 1.0.4), Cuffcompare was used to compare reconstructed transcripts to reference annotation, followed by CPC to predict coding potential of novel transcripts (Martin and Wang 2011).

Bowtie2 (version 2.2.5) and RSEM (version 1.3.3) were used to map clean reads to the genes and transcript and to calculate gene expression, respectively. The differentially expressed genes (DEGs) were identified using DESeq. 2 (version 1.32.0) by comparing PolyB, PolyC, and P0 with the control (OrthoP; representing the totally available source of P that may ensure optimal P uptake). The genes with $|\log2(\text{fold change})| \geq 1$ (with adjusted p-value ≤ 0.05) are considered differentially expressed.

The DEGs were arranged according to official classification, and KEGG (Kyoto Encyclopedia of Genes and Genomes) pathway functional enrichment was performed using phyper, a function of R (version 3.4.2). The terms with FDR ≤ 0.05 (false discovery rate) are considered to be significantly enriched. RNA sequencing data of this study have been deposited at GEO (NCBI) under the accession number GSE277488.

2.5 | Statistical Analysis

Statistical data analyses were performed using Minitab software (version 21.1.0) and R (version 4.4.3). The analysis of variance was used to evaluate the effects of PolyP fertilizers on the wheat above- and below-ground parameters (inorganic P content, root traits, APase activities, dry biomass, and soil-available P), followed by the Tukey post-hoc test to classify the means of the treatments at a 0.05 significance level.

3 | Results

3.1 | Effects of Polyphosphates on Plant Biomass and Pi Content of Wheat Plants Grown in P-Deficient Soil

Application of PolyP significantly increased SDW, RDW, shoot and root Pi contents compared to both controls (OrthoP and P0) under P-deficient soil conditions (Figure 1A–D). For instance, PolyB and PolyC increased SDW by 50% and 110% compared to OrthoP-fertilized and unfertilized plants (Figure 1A). Similarly, PolyB- and PolyC-fertilized plants exhibited shoot Pi content that was 5 and 6 times higher than in unfertilized plants (Figure 1C). Except for SDW, PolyB and PolyC induced similar effects on plant biomass and Pi content. Overall, PolyC presented the best results for improving plant biomass and P uptake.

3.2 | Effects of Polyphosphates on Wheat Root Transcriptome Profile in P-Deficient Soil

To unravel the molecular basis that explains the observed PolyP-induced positive effect on P acquisition and growth performance of durum wheat, a comparative root transcriptome analysis was carried out to determine the DEGs between PolyP (PolyB and PolyC) and the control P treatments (OrthoP and P0) (Figure 2). Out of 5108 genes identified, 445 and 2036 genes were upregulated and downregulated, respectively, in response to PolyB, whereas only 71 upregulated and 113 downregulated genes were obtained in response to PolyC compared to OrthoP. The number of DEGs (both down- and upregulated genes) was significantly higher in the P0_OrthoP comparison (3338 differentially expressed genes).

To further understand the key biological pathways influenced by PolyP, KEGG pathway enrichment was performed in each pairwise group (Figure 3). A total of 33 KEGG pathways were enriched between the three pairwise groups, where metabolic pathways, biosynthesis of secondary metabolites, plant hormonal signal transduction, pyruvate metabolism, MAPK signaling, and ABC transporters are the key PolyP-induced KEGG pathways (Figure 3). According to the KEGG analysis, both PolyP significantly influenced plant metabolism, as the metabolic processes (genes related to plant metabolism) were the most enriched and dominant KEGG pathways in response to PolyP.

The analysis of DEGs revealed that PolyP induced the expression of several genes associated with P acquisition, including phosphatases, phytohormone synthesis, N transporters, organic acid exudation, and numerous genes encoding various transcription factors and signaling molecules (Figure 4). For instance, PolyP significantly upregulated genes related to auxin synthesis (TRITD_2Bv1G246320), auxin transporters (TRITD_4Av1G160490), cytokinin (BGI_novel_G014395), abscisic acid (TRITD_4Bv1G034450), P homeostasis (TRITD_7Av1G277070), malate transporters (TRITD_6Bv1G148310), oxoglutarate dehydrogenase (BGI_novel_G014620), and transcription factor regulating P homeostasis (MYB: TRITD_7Bv1G046440, WRKY: BGI_novel_G006801). Even though PolyC-fertilized roots showed fewer DEGs compared to PolyB, its application significantly upregulated genes related to ethylene, cytokinin, phosphatases, glycolysis enzymes, and MYB transcription factor (Figure 4). As indicated earlier, the metabolic processes pathway was among the most enriched KEGG pathways in response to PolyP. Specifically, PolyP significantly influenced the expression of specific key enzymes involved in glycolysis and tricarboxylic acid cycle (TCA) enzymes. The two PolyP revealed contrasting regulations of glycolysis- and TCA-related genes. Interestingly, PolyB significantly upregulated the enolase (ENO) and 2-oxoglutarate dehydrogenase (OGDH) genes, while glucose-6-phosphate isomerase (GPI), 6-phosphofructokinase

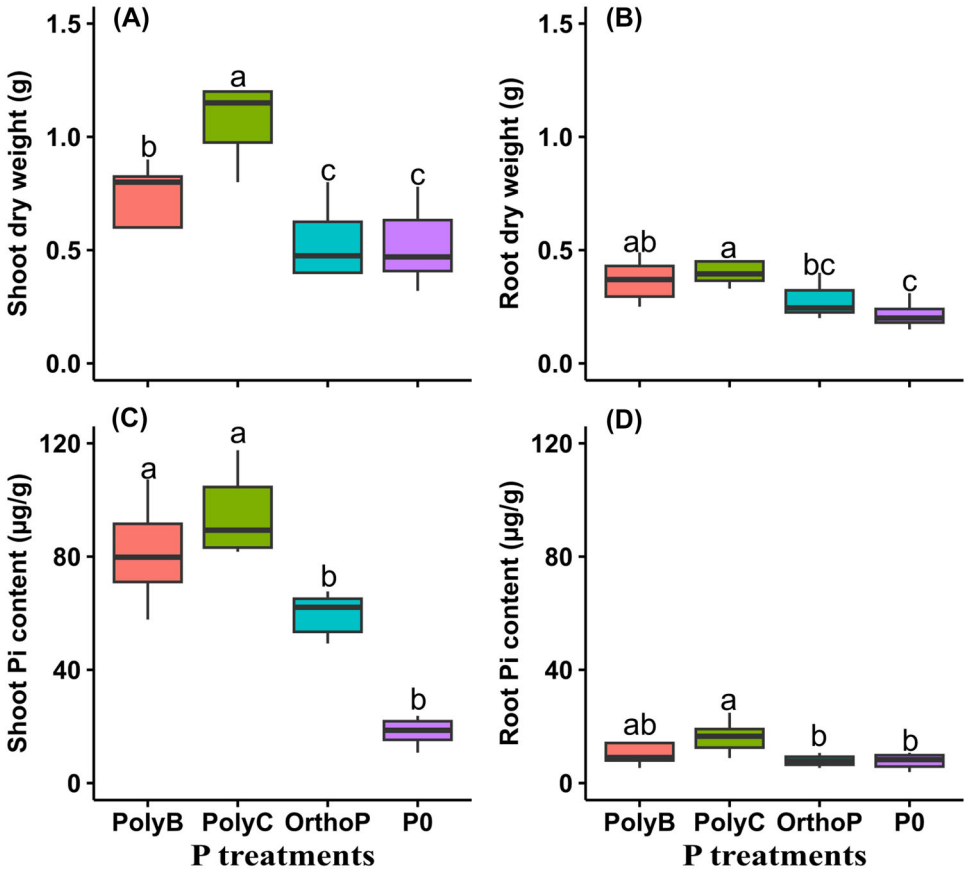


FIGURE 1 | Biomass and phosphorus uptake in wheat plants in response to polyphosphates (PolyB and PolyC) and OrthoP. (A) shoot dry weight (B), root dry weight, (C) shoot inorganic phosphorus content, and (D) root inorganic phosphorus content. Data are mean values ± SD ($n = 8$), different lowercase letters above the bars indicate significant differences ($p < 0.05$) according to Tukey's test. [Color figure can be viewed at wileyonlinelibrary.com]

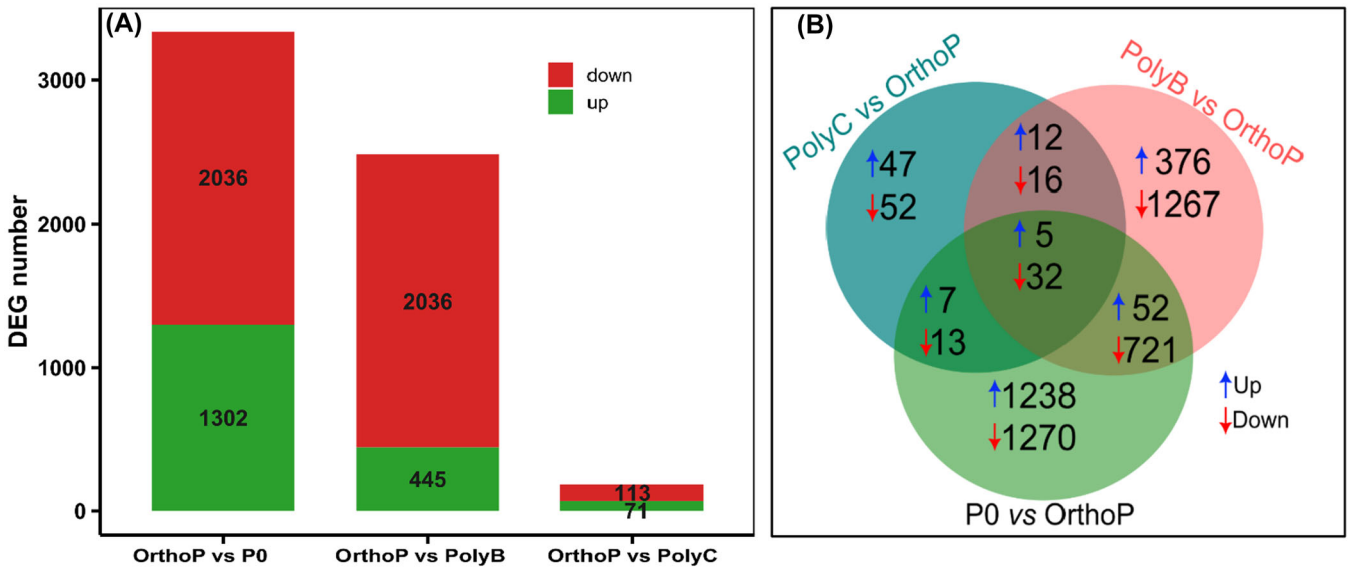


FIGURE 2 | Differential expression of genes (DEGs) in the root of durum wheat plants fertilized with different polyphosphates. (A) The stacked bar plot shows the total number of upregulated (blue arrow) and downregulated (red arrow) DEGs in the roots of PolyP-fertilized and unfertilized durum wheat. (B) The Venn diagrams display the common and unique upregulated and downregulated DEGs in the roots of PolyP-fertilized and unfertilized plants. Differentially expressed genes (DEGs) were identified by comparing the gene expression profiles in PolyP-fertilized and unfertilized plants compared to OrthoP-fertilized plants. [Color figure can be viewed at wileyonlinelibrary.com]

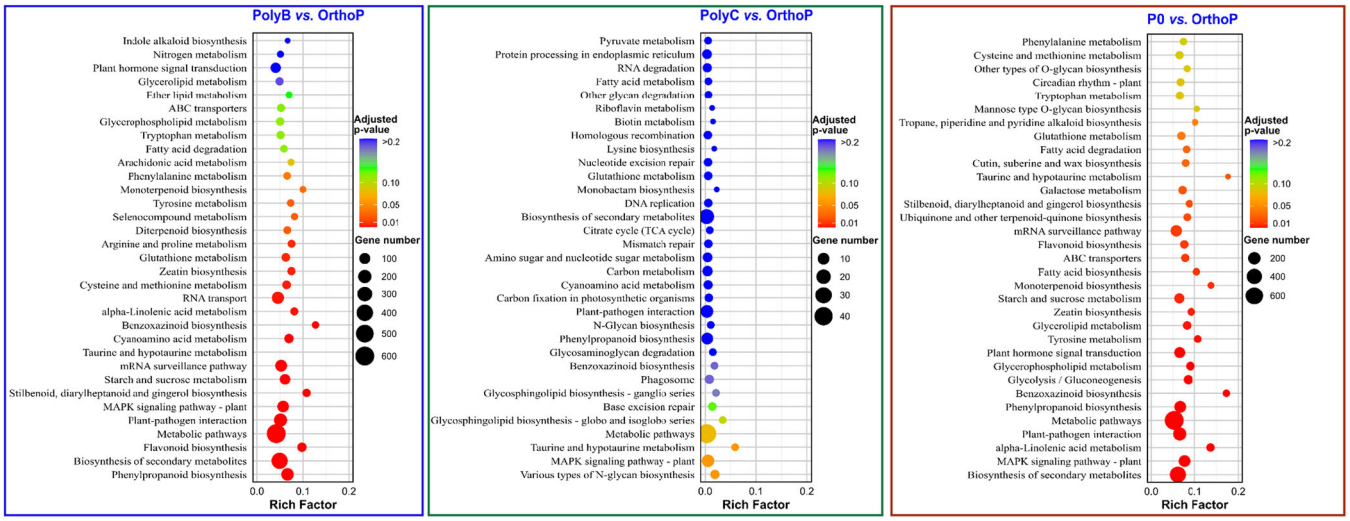
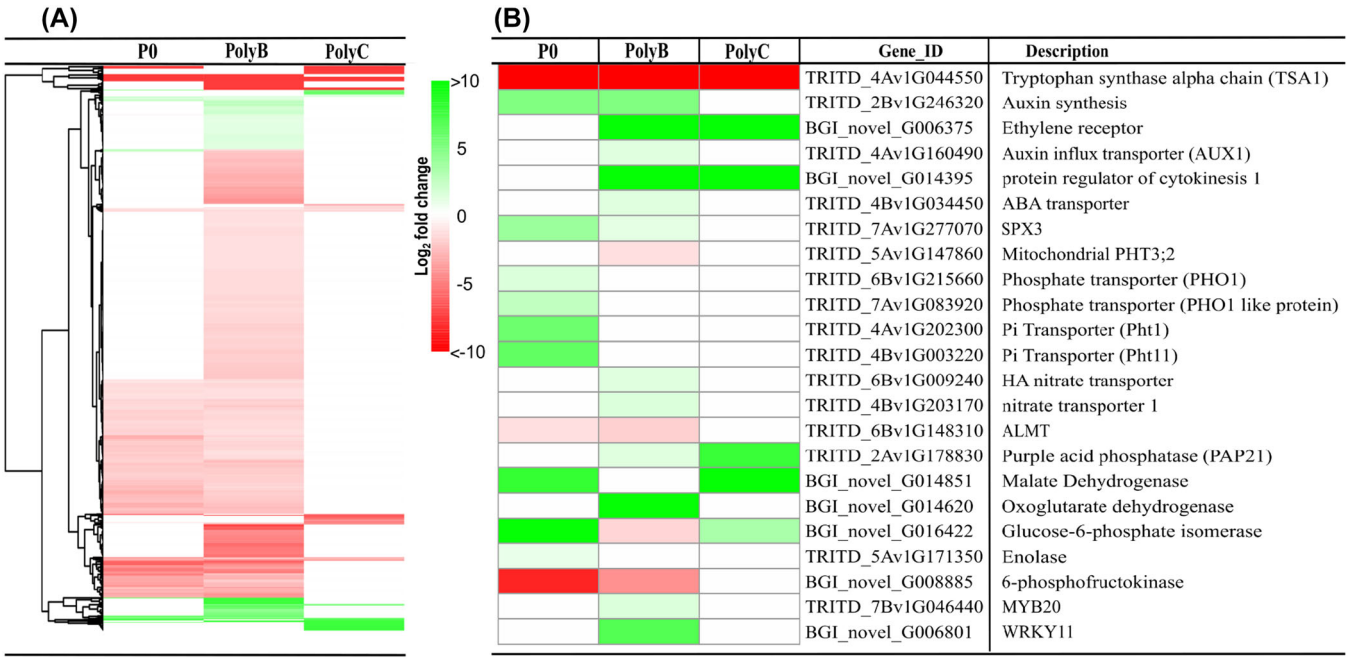


FIGURE 3 | Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways enrichment analysis of differentially expressed genes in roots of wheat plants fertilized with different polyphosphates (PolyB and PolyC) and unfertilized plants. The different colored dots indicate the adjusted *p*-value level, while the size of each dot represents the number of DEGs per KEGG pathway. [Color figure can be viewed at wileyonlinelibrary.com]



P0	PolyB	PolyC	Gene_ID	Description
			TRITD_4Av1G044550	Tryptophan synthase alpha chain (TSA1)
			TRITD_2Bv1G246320	Auxin synthesis
			BGI_novel_G006375	Ethylene receptor
			TRITD_4Av1G160490	Auxin influx transporter (AUX1)
			BGI_novel_G014395	protein regulator of cytokinesis 1
			TRITD_4Bv1G034450	ABA transporter
			TRITD_7Av1G277070	SPX3
			TRITD_5Av1G147860	Mitochondrial PHT3;2
			TRITD_6Bv1G215660	Phosphate transporter (PHO1)
			TRITD_7Av1G083920	Phosphate transporter (PHO1 like protein)
			TRITD_4Av1G202300	Pi Transporter (Pht1)
			TRITD_4Bv1G003220	Pi Transporter (Pht11)
			TRITD_6Bv1G009240	HA nitrate transporter
			TRITD_4Bv1G203170	nitrate transporter 1
			TRITD_6Bv1G148310	ALMT
			TRITD_2Av1G178830	Purple acid phosphatase (PAP21)
			BGI_novel_G014851	Malate Dehydrogenase
			BGI_novel_G014620	Oxoglutarate dehydrogenase
			BGI_novel_G016422	Glucose-6-phosphate isomerase
			TRITD_5Av1G171350	Enolase
			BGI_novel_G008885	6-phosphofructokinase
			TRITD_7Bv1G046440	MYB20
			BGI_novel_G006801	WRKY11

FIGURE 4 | Heat map illustrating the DEGs analysis in the roots of PolyP-fertilized and unfertilized durum wheat compared to OrthoP. (A) Heatmap showing the clustering of DEGs in roots of PolyP-fertilized and unfertilized wheat plants. (B) Heatmap of selected differentially expressed genes involved to phytohormone production (related to root growth modulation), organic acid exudation (key enzymes in citric acid cycle), P-hydrolyzing enzymes (phosphatases), nutrient transporters (phosphate and nitrogen), and transcription factors related to P homeostasis and P acquisition. The red and green colors indicate the downregulated and upregulated genes, respectively, in response to PolyP and P0 compared to OrthoP. Different color intensity indicates different levels of expression (log2 Fold Change), while white represents no significant difference. [Color figure can be viewed at wileyonlinelibrary.com]

(PFK), triosephosphate isomerase (TPI), phosphoglycerate mutase (PGAM), pyruvate decarboxylase (PDC), alcohol dehydrogenase (ADH), lactate dehydrogenase (LDH), isocitrate dehydrogenase (IDH), aconitate hydratase (ACON), and citrate synthase (CS) were significantly downregulated in response to PolyB (Figure 5). However, the total number of PolyC-induced genes was significantly lower than that for PolyB, with PolyC only down- and upregulating TPI and OGDH genes, respectively (Figure 5).

3.3 | Effects of Polyphosphates on Root Growth Dynamics of Wheat Plants Grown in P-Deficient Soil

Application of PolyP induced significant modifications of root growth dynamics, as indicated by changes in morphological root traits across multiple measurement timepoints, which varied depending on the source of P. Enhanced root

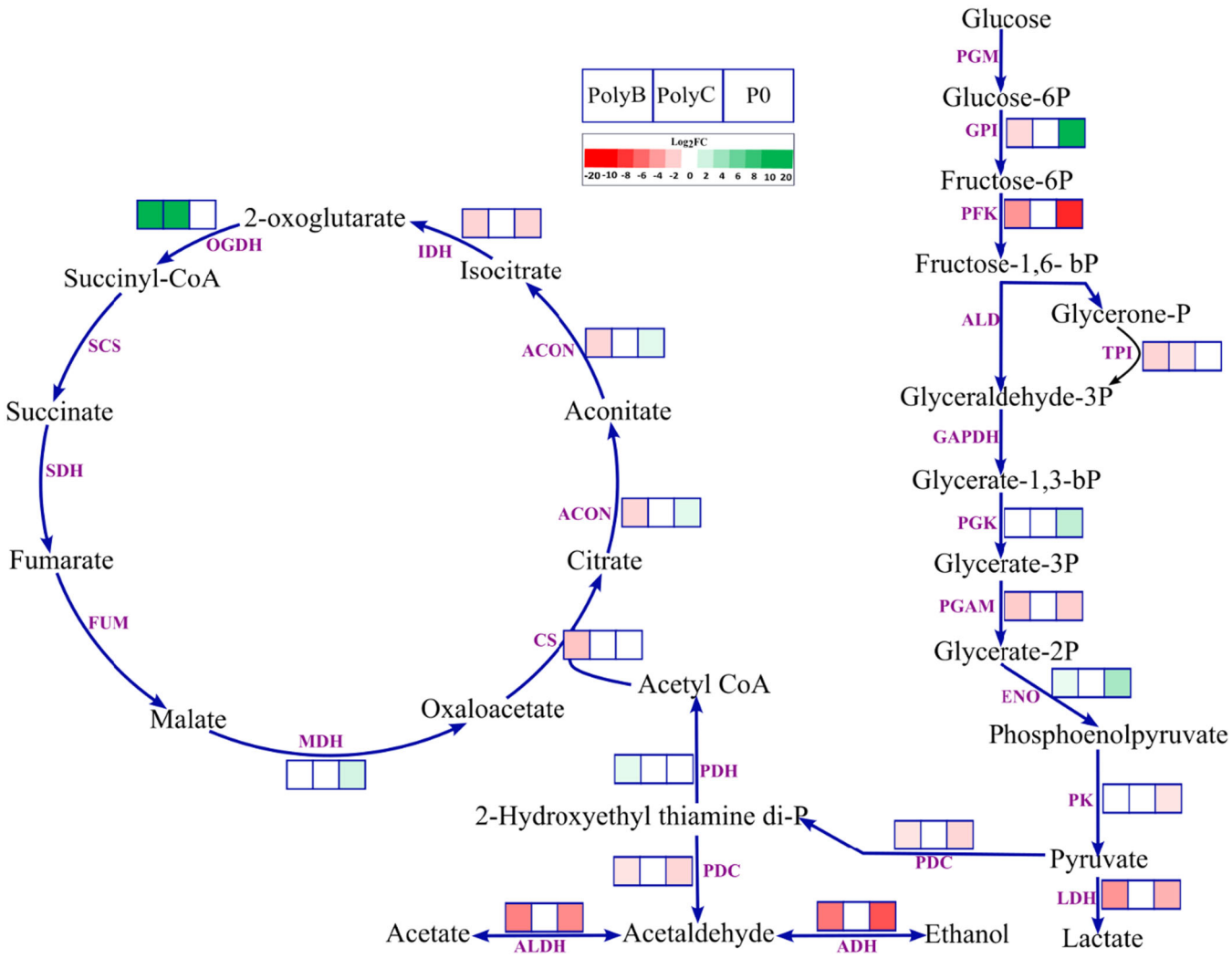


FIGURE 5 | Schematic representation of differentially expressed genes (DEGs) involved primary metabolic pathways (glycolysis and citrate cycle) occurring in wheat roots in response to polyphosphate (PolyB and PolyC). Red and blue colors indicate downregulated and upregulated genes, respectively, in response to PolyB, PolyC, P0 compared to OrthoP. The intensity of color (red/blue) indicates the expression level of the genes in response to different treatments compared to control (OrthoP). [Color figure can be viewed at wileyonlinelibrary.com]

morphological traits were observed under PolyC compared to other treatments (Figure 6). At harvest (30 DAS), PolyC significantly increased RL (75%), RSA (73%), RV (71%), and RB (85%) compared to unfertilized plants (Figure 6A). In addition, both PolyB and OrthoP induced similar root growth dynamic patterns, while the unfertilized plants showed the lowest values for the measured morphological root traits. The root images confirmed that PolyP (especially PolyC) significantly enhanced root morphological traits across multiple measurement timepoints, compared to other treatments (Figure 6B).

3.4 | Effects of Polyphosphates on Soil P Availability and APase Activities in Roots and Rhizosphere Soil of Wheat Plants Grown in P-Deficient Soil

Application of PolyP significantly enhanced soil P availability and APase activities in root and rhizosphere soil compared to OrthoP and P0 treatments, with the highest values being obtained under PolyB (Figure 7A–C). PolyB and OrthoP significantly enhanced the rhizosphere soil available P compared to P0. Though the difference was not significant, the rhizosphere soil available P was 1.5 times higher under PolyB than under OrthoP, a readily available source of P (Figure 7A).

In parallel, the PolyP significantly influence APase activity in both roots and rhizosphere soil (Figure 7BC). A clear difference was noted in the rhizosphere soil, in which the PolyB induced the highest rhizosphere soil APase activity. For instance, the rhizosphere soil APase activity increased by 63% under PolyB compared to unfertilized plants. Moreover, both PolyP, along with P0 treatments, significantly increased root APase activity compared to OrthoP. PolyB and PolyC induced significant increase in root APase activity by 25% and 35%, respectively, compared to OrthoP treatment. However, the unfertilized plants exhibited the highest root APase activity, as a typical P deficiency response.

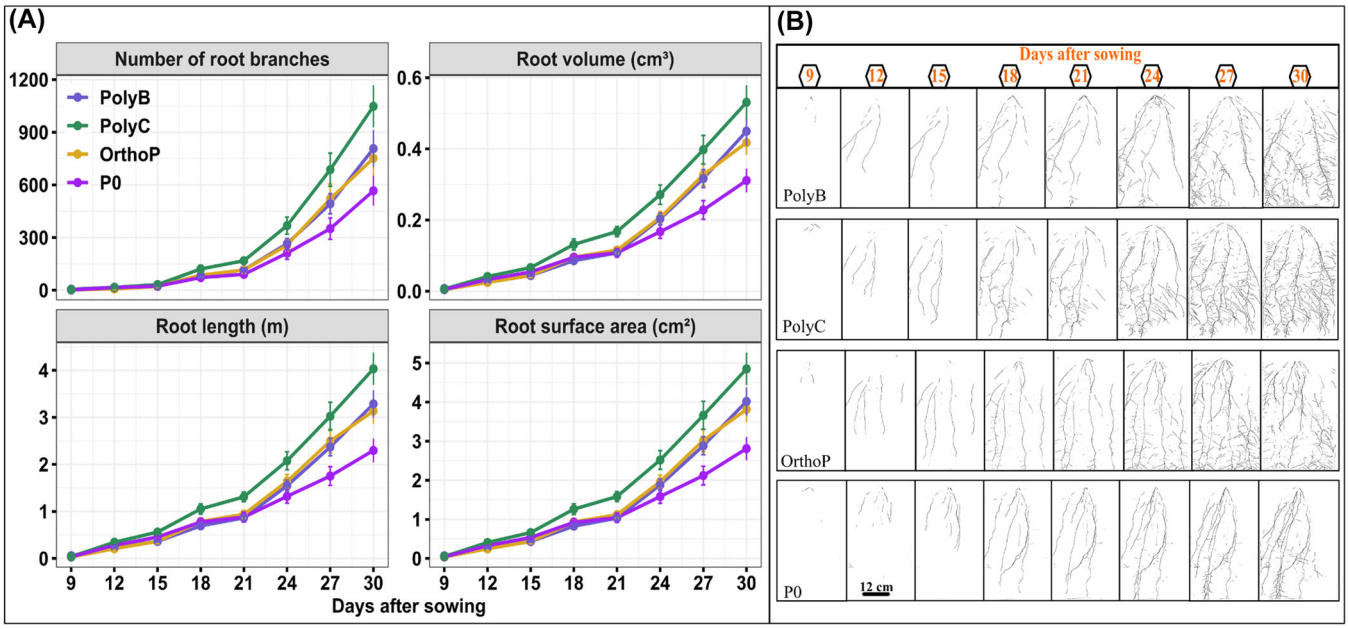


FIGURE 6 | Dynamic measurement of morphological traits of wheat roots in response to polyphosphates (PolyB and PolyC) and OrthoP. (A) Temporal changes in root morphological traits—including total root length (RL), root surface area (RSA), root volume (RV), and number of branches (RB)—measured every 3 days up to 30 days after sowing (DAS) in response to polyphosphates (PolyB and PolyC), Orthophosphate (OrthoP), and unfertilized control (P0). (B) Representative segmented root images (generated using RootPainter software) illustrating the dynamic root growth patterns of PolyP-fertilized, OrthoP-fertilized and unfertilized wheat plants over the 30-day period. Data are mean values ± SD ($n = 8$), different lowercase letters above the bars indicate significant differences ($p < 0.05$) according to Tukey's test. [Color figure can be viewed at wileyonlinelibrary.com]

3.5 | Effects of Polyphosphates on P Availability, APase Activity, and Acidification of Soil Solutions of Wheat Plants Grown Under P-Deficient Soil

In addition to rhizosphere soil, the soil solution analysis revealed that PolyP induced a significant increase in available P, variation of pH, and APase activity in the soil solution compared to the OrthoP and P0 treatments (Figure 8A–F). Overall, this increase (especially for P availability and APase activity, as well as acidification) was significantly higher for soil solutions collected from the upper 10 cm of the rhizobox than from the deeper part (40 cm depth). For instance, at 15 DAS, PolyB resulted in 34 mg/L of available P in the soil solution, which is more than 2 times higher than for OrthoP-fertilized plants. In the upper 10 cm of the rhizobox, the P availability in the soil solution under PolyP (PolyB and PolyC) was significantly higher than for other treatments during the experiment, except at 10 DAS where OrthoP induced the highest available P content in the soil solution.

The same trend of variation was observed for APase in the soil solution under PolyB and PolyC (Figure 8CD), especially for the upper 10 cm of the rhizobox. For example, at 25 DAS timepoint, PolyB and PolyC induced a significant increase in rhizosphere soil solution APase activity (98% and 172%, respectively) compared to OrthoP-fertilized and unfertilized plants (Figure 8CD).

Regarding rhizosphere acidification, both PolyP induced a noticeable—but transient—acidification of rhizosphere soil solution compared to OrthoP and P0 treatments (Figure 8EF). As with the changes in APase and available P content above, the rhizosphere soil solution acidification was significantly induced by both PolyB and PolyC, especially in the upper 10 cm of the rhizobox. Specifically, the lowest pH values of rhizosphere soil solution (6.3–6.8) were noted between 15 and 25 DAS under both PolyB and PolyC, while rhizosphere soil solution pH ranged from 7.6 to 7.8 under OrthoP and P0 treatments.

4 | Discussion

To the best of our knowledge, this study represents the first of its kind, providing a comprehensive analysis of plant responses to PolyP with a focus on physiological and molecular functional traits likely to be responsible for enhanced P uptake by durum wheat. The key findings highlighted, for the first time, that PolyP induced up- and downregulation (compared to OrthoP) of genes encoding key enzymes in the TCA cycle and glycolysis, which presumably explains the induced rhizosphere acidification and elevated APase activity in both soil solutions and rhizosphere soil of PolyP-fertilized plants. This molecular regulation led to consistent physiological changes as increased APase activity was noted in roots, rhizosphere soil, and rhizosphere soil solution (Figure 8), which might consequently enhance P availability from PolyP to a similar level to OrthoP. Accordingly, PolyP-fertilized plants exhibited strongly stimulated root-rhizosphere (acidification and P-hydrolyzing enzyme activities) as the main mechanism driving PolyP hydrolysis and P availability at the root-rhizosphere interface (Khourchi et al. 2023b, 2023a). In line with this, the overexpression of some genes encoding phytohormones (known for their regulatory roles in root growth) coupled with the upregulation of

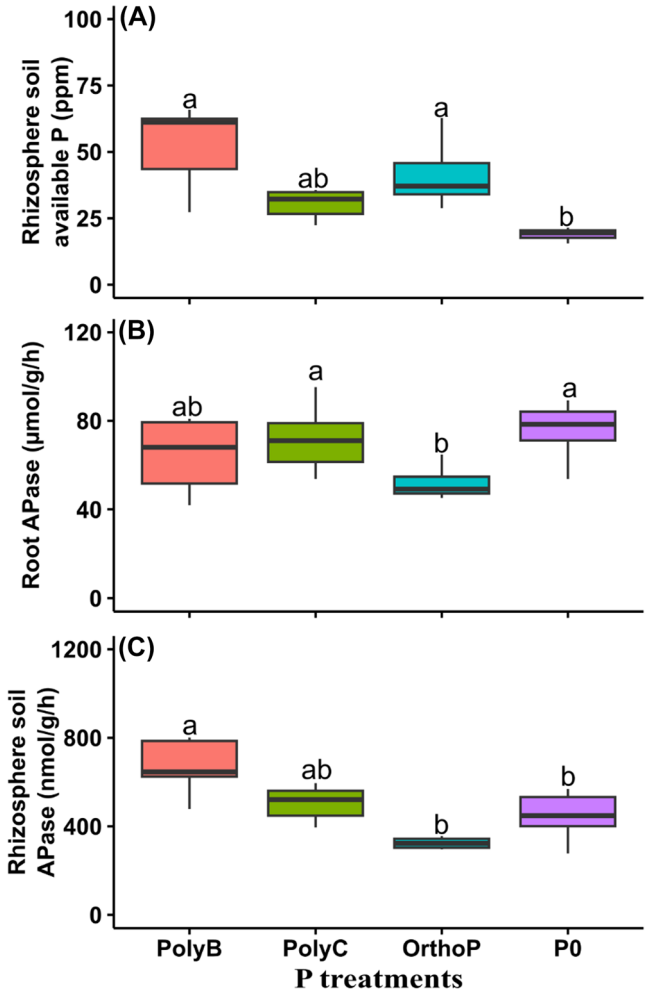


FIGURE 7 | Phosphorus availability and phosphatases activities in the root-rhizosphere interface of wheat plants in response to polyphosphates (PolyB and PolyC) and OrthoP. (A) Available phosphorus content in the rhizosphere soil of wheat plants in responses polyphosphates and OrthoP. Acid phosphatase activity in the roots (B) and rhizosphere soil (C) of wheat plants in response (PolyB and PolyC) and OrthoP. Data are mean values ± SD ($n = 8$), different lowercase letters above the bars indicate significant differences ($p < 0.05$) according to Tukey's test. [Color figure can be viewed at wileyonlinelibrary.com]

SPX3 gene (known as regulator of P homeostasis) might explain the enhanced P uptake and shoot biomass in response to PolyP. Moreover, the contrasting responses between the two PolyP could be attributed to different mechanisms driving the hydrolysis of each PolyP, where PolyC remains less responsive to hydrolysis (long-chain PolyP) compared to PolyB (short-chain PolyP) (Khourchi et al. 2022a, 2023a).

Altogether, by assessing both transcriptomic and physiological root traits, new evidence unraveling the stimulatory effects of PolyP from root-rhizosphere processes to plant P acquisition can be exploited to deepen research on crop responses to PolyP. Our findings suggest that PolyP induced up- and down-regulation (compared to OrthoP) of genes encoding TCA-enzymes (an important source of organic acids that are exuded into the rhizosphere) and phosphatases (known for their involvement in PolyP hydrolysis) as key mechanisms explaining the enhanced P availability from PolyP (Figure 9).

4.1 | Polyphosphate-Induced Regulation of Genes Related to Root-Rhizosphere Processes Explains the Enhanced P Availability, P Acquisition, and Growth of Polyp-Fertilized Wheat

In this study, our findings highlight that the two PolyP induce significant changes in root architecture, which potentially explains the increased P allocation to wheat shoots. The transcriptomic analysis demonstrated that, compared to OrthoP, PolyP significantly upregulated the expression of phytohormone-related genes (e.g., AUX1, auxin synthesis, cytokinin, ABA transporter, etc.), which suggests that PolyP can influence the root architecture through inducing significant changes in the synthesis of phytohormones and their balance at root level. Despite the scarcity of studies investigating molecular root responses to PolyP, our findings were consistent with previous studies highlighting that AUX1 ensured shoot-to-root auxin transport and modulated root elongation and lateral root initiation (Gruber et al. 2013; Jensen et al. 2024). Similarly, aux1 loss-of-function mutations lead to a significant reduction in the number of lateral roots (Laskowski et al. 2008). In addition, P sensing cross-talks with several phytohormones (auxin, cytokinin, gibberellins, etc.), thereby conferring developmental plasticity of RSA traits that allow better P use efficiency (López-Bucio et al. 2003; Ding et al. 2008; Balliu and Sallaku 2017). The role of the AUX1-mediated influx transporter in controlling the seminal root angle in rice was reported by Giri et al. (2018), a pioneering study that highlights the need for deeper investigation into phytohormone balance and its influence on root architectural traits. Taken together, our findings suggest that wheat roots sense PolyP, modulating the genes encoding phytohormones, such as AUX1, to improve root architecture, allowing enhanced P uptake. However, it is still unclear as to how plant roots sense changes in P availability (especially under PolyP) and subsequently trigger specific phytohormone-related transcriptional changes responsible for efficient P-absorptive root systems.

Beyond PolyP regulating role in root architecture, our findings along with the literature suggest that PolyP may induce a significant increase in P-hydrolyzing enzymes that are actively involved in PolyP hydrolysis (Tiessen 2008; Darch et al. 2016; Huang et al. 2018; Khourchi et al. 2022b, 2023a). In line with this, our RNA-seq analyses indicated that PolyP, especially PolyB, induced upregulation of the PAP21 gene encoding APase, which confirms the increased APase activity in root-rhizosphere interface of PolyB-fertilized plants (Figures 7–8). These findings highlighted for the first time that the induced increase of APase activity in the root and rhizosphere soil could be presumably explained by the ability of PolyP to modulate the expression of PAP genes, given the key role of these genes in enhancing P utilization and homeostasis in roots, nodules, and rhizosphere soil (Roussis et al. 2003; Severin et al. 2010; Dionisio et al. 2011; Li et al. 2012). For instance, a recent study on wheat found that 57 genes of the PAP family were highly expressed in response to P starvation, where TaPAP31-4A transgene in *A. thaliana* promoted plant growth under P deficiency by enhancing exudation of APase (Hou et al. 2025). Additionally, the overexpression of PAP enzymes gene in soybean (Gm*PAP21)* and rice (*OsPAP10c*) plants significantly enhanced exudation of APase (up to 10 times in the growth media), APase activity in the plant tissues (up to 5 times) under P sufficient and P deficient conditions, which consequently

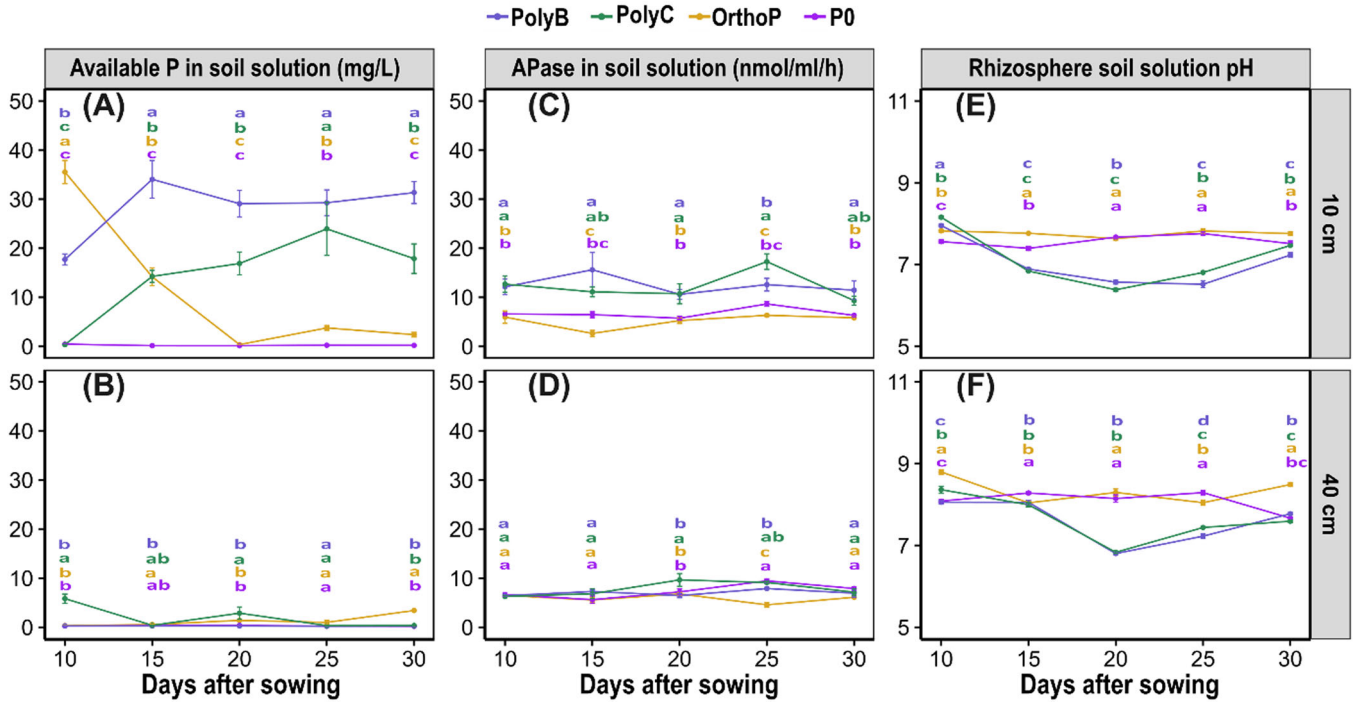


FIGURE 8 | Temporal and spatial variations in phosphorus availability, rhizosphere acidification, and acid phosphatase activity in rhizosphere soil solutions of wheat plants grown in rhizoboxes under different phosphorus treatments. Soil solutions were sampled using Rhizons samplers from the upper layer (10 cm from the top) and deeper layer (40 cm from the top) of the rhizobox. (A,B) Phosphorus availability in the rhizosphere soil solution at the upper and deeper layers of the root system profile in response to different P treatments. (B,C) Acid phosphatase activity in the rhizosphere soil solution at the upper and deeper layers of the root system profile in response to different P treatments. (E-F) pH of the rhizosphere soil solution (rhizosphere acidification) at the upper and deeper layers of the root system profile in response to different P treatments. Data are mean values ± SD ($n = 8$), different lowercase letters above the bars indicate significant differences ($p < 0.05$) according to Tukey's test. [Color figure can be viewed at wileyonlinelibrary.com]

resulted in enhanced P content, biomass, lateral root and root hairs growth (Lu et al. 2016; Li et al. 2017; Mehra et al. 2017). Similarly, a recent study by Guo et al. (2025) found that GmPAP23 from soybean was able to hydrolyze several P-containing compounds (which could be the case with PolyP) and significantly enhanced internal P use efficiency under P starvation. Although none of these studies focus on polyphosphates, several previous findings combining omics approaches and physiological assessments highlighted the importance of the PAP genes in regulating P availability and acquisition under both deficient and sufficient P conditions (Kaida et al. 2009; Liang et al. 2010; Tran et al. 2010; Lu et al. 2016; Bhadouria et al. 2017; Deng et al. 2020).

Concomitantly, acidification is a well-recognized mechanism governing PolyP hydrolysis in vitro, yet no studies have investigated PolyP-mediated rhizosphere acidification and its role in facilitating phosphorus uptake. In that regard, our findings provide strong evidence that PolyP are likely to induce significant metabolic adaptations that promote rhizosphere acidification, including the modulation of key genes involved in the citrate cycle, a known source of organic acid exudation. Specifically, upregulated the expression of OGDHC and PDH genes and downregulated IDH, ACON, and CS genes in response to PolyB, support the observed significant acidification in the rhizosphere soil solution (Figure 8EF) and the enhanced P availability (Figures 7A, 8AB) and uptake (Figure 1CD) under PolyP. PolyP-induced rhizosphere acidification could be attributed to the exudation of organic acids and protons that are known for their involvement in PolyP hydrolysis (Khourchi et al. 2022a, 2023a). Despite the lack of studies investigating the molecular responses of crops to PolyP, previous studies confirmed that plants have evolved several alternative glycolytic pathways and modified the expression of citrate cycle enzymes, enabling efficient use of P under contrasting P supply (Wu et al. 2003; Plaxton and Tran 2011; Chen et al. 2013). For instance, findings integrating metabolomics and transcriptomics revealed that phosphorus deficiency led to the upregulation of several genes (*GmPS3*, *GmPHT*, *GmPLDP*, *GmSPX*, and *GmSQD*) involved in metabolism and P uptake, which was accompanied by a significant accumulation of various metabolites, particularly organic acids such as malonic acid, glycolic acid, and gluconic acid (Li et al. 2023). A study by Condori-Apfata et al. (2021) found that 2-ogdh2 mutant lines have a significant decrease in the nitrate level and heterogeneous changes in individual amino acids, suggesting the importance of 2-OGDH activity for nitrogen metabolism in plants. In line with this, PolyP upregulated the expression of 2-OGDH, suggesting that the positive effect of PolyP can be extended to improved N uptake (evidenced by upregulation the expression of nitrate transporters, Figure 4).

The upregulation of genes encoding high-affinity transporters was one of the most important adaptive plant responses to ensure better exploration of the soil solution under limited P availability. This was in line with our findings showing that P0

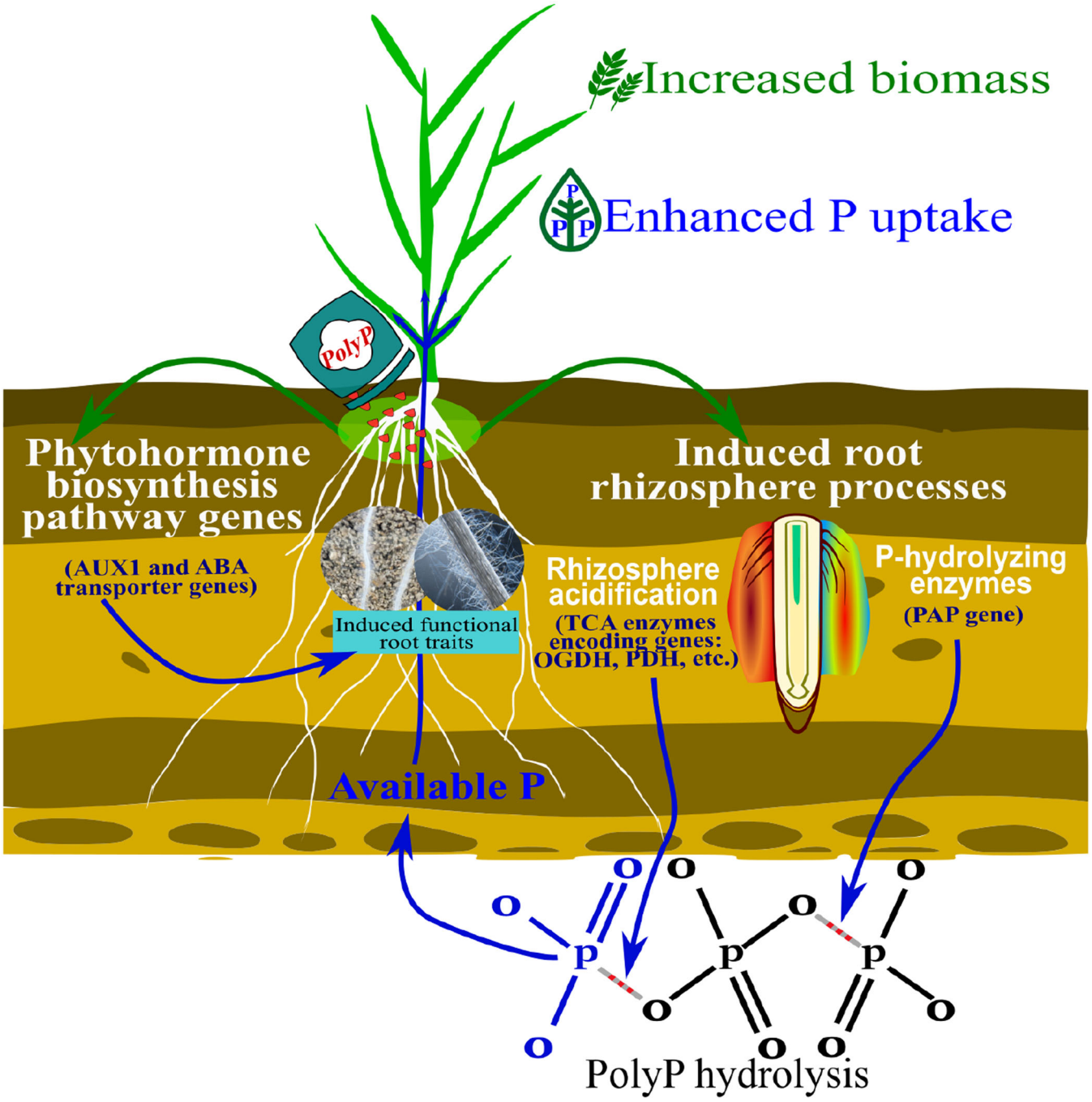


FIGURE 9 | Conceptual diagram illustrating how polyphosphates induce rhizosphere acidification, phosphatase activity, and root growth modulation as key mechanisms explaining the enhanced phosphorus availability and acquisition by durum wheat. [Color figure can be viewed at wileyonlinelibrary.com]

treatment (P-deficiency condition) expressed significant upregulation of PHO1, Pht1 and Pht11 genes encoding high-affinity Pi transporters, while the SPX3 gene (known for its regulatory role in P homeostasis) was upregulated in response to PolyB. Even though there are certain parallels, the overexpression of P transporter genes (PHO1, Pht1, and Pht11) under P0 treatment as opposed to PolyP (no differential expression) could support the hypothesis that plants' adaptive responses to PolyP differ from those of P-deficiency conditions. Furthermore, upregulation of MYB and WRKY transcription factors in response to PolyP indicate their key regulatory roles in maintaining P homeostasis, which confirms the important role of MYB and WRKY transcription factors in the expression of genes encoding for P transporters, P starvation, and root architecture (Hernández et al. 2007; Miao et al. 2009; Nilsson et al. 2010; Sun et al. 2018).

Importantly, the two PolyP exhibited contrasting transcriptomic and morphophysiological responses, supporting the importance of considering the intrinsic properties of each PolyP—where long-chain PolyP (PolyC) was reported to induce rhizosphere acidification and APase activity in the rhizosphere soil to a lesser extent. Additionally, a previous study showed that short-chain PolyP (such as PolyB) are more sensitive to enzymatic

(phosphatases and polyphosphatases) and acidic (organic acids and protons) hydrolysis by both roots and associated microbes, compared to long-chain PolyP (such as PolyC) (Khourchi et al. 2022b, 2023a).

4.2 | Polyphosphates Induced Significant Trade-Offs in Morphophysiological Root Traits to Enhance Biomass and P Acquisition in Durum Wheat

The application of PolyP significantly enhanced plant biomass compared to OrthoP and P0 treatments (Figure 1). Both PolyP, particularly PolyC, significantly enhanced shoot biomass and shoot Pi content. This is in keeping with several previous studies showing that PolyP resulted in enhanced P uptake and plant biomass in wheat and chickpea (Loudari et al. 2022; El-Mejjaouy et al. 2022; Chtouki et al. 2022; Khourchi et al. 2022b). The beneficial effects of PolyP on plant biomass and P acquisition could be attributed to the potential progressive hydrolysis of PolyP (Khourchi et al. 2022a, 2023a). Using alkaline P-deficient soil, these studies suggested that roots and associated microbes can enhance PolyP use efficiency by acidifying the rhizosphere (protons and organic acid production) and producing P-hydrolyzing enzymes (e.g., APase and pyrophosphatases) as well as via inducing functional root traits responsible for enhanced P uptake (Aallam et al. 2021; Janati et al. 2021; Khourchi et al. 2022a; Benmrid et al. 2023; Benmrid et al. 2024).

PolyP significantly influence root growth dynamics by modulating morphological and physiological root traits that are likely to be involved in P acquisition. PolyP displayed contrasting effects on morphophysiological traits depending on the growth conditions (Figure 6). Overall, the three P fertilizers significantly improved morphological root traits compared to unfertilized plants. Specifically, PolyC significantly increased all morphological root traits (RL, RSA, RV, RSA, and RB) and root APase activity compared to other treatments (Figures 6 and 7). The root growth dynamics analysis indicates that PolyC resulted in a well-developed and branched root system starting from 21 DAS compared to other P sources and unfertilized plants. However, PolyB promoted a significant increase in APase activity, especially in rhizosphere soil, rather than enhanced morphological root traits. Additionally, PolyB and OrthoP treatments had similar root patterns in terms of morphological and physiological root traits (Figure 6). In line with our findings, Khourchi et al. (2022b) reported that PolyP enhanced P acquisition through influencing the trade-offs between the morphological and physiological traits. The root stimulation effects of PolyP can explain the enhanced plant growth and P uptake.

The increased root and rhizosphere APase activity under both PolyP contributed to enhanced rhizosphere P availability. For instance, PolyB significantly enhanced available P by 196% compared to unfertilized plants, indicating the considerable contribution of roots to PolyP hydrolysis through the activity of exuded APase (Khourchi et al. 2023a). In that regard, previous studies revealed that APase activity was significantly increased in response to PolyP, highlighting the pivotal role of P-hydrolyzing enzymes in PolyP hydrolysis (Dick 1985; Dick and Tabatabai 1986; Wang et al. 2019). Our findings are consistent with previous studies supporting the root-stimulating effects of PolyP (Loudari et al. 2022; Khourchi et al. 2022b, 2023a; Bourak et al. 2023).

4.3 | Enhanced Rhizosphere Acidification and Phosphatase Activity in Rhizosphere Soil Solution as Potential Mechanisms Driving P Availability From Polyphosphates

The P availability in soil solution and PolyP hydrolysis processes in soil are highly determinant for efficient P acquisition from PolyP. In this context, our findings indicate that PolyP induced a significant increase in APase activity and acidification of the rhizosphere soil solution, which consequently leads to enhanced P availability in the rhizosphere soil solution (Figure 8), especially in the upper parts of the rhizobox. In the rhizosphere soil solution, increased rhizosphere acidification and APase activity in the upper part of the rhizobox explained the augmented P availability here compared to the deeper part, where lower P availability, acidification, and APase activities were noted. These differences could be explained by the spatial distribution of root biomass, alongside the PolyP' mobility and distribution throughout the rhizobox.

From 15 to 25 DAS, the available P in the rhizosphere soil solution was more than 100 times higher under both PolyP than in unfertilized plants. The P availability in the rhizosphere soil solution under both PolyP increased to 5 times higher than under OrthoP between 20 and 30 DAS. Likewise, between 15 and 25 DAS, PolyB and PolyC significantly increased APase activity in the rhizosphere soil solution (more than 1.5 times higher than OrthoP and unfertilized plants), suggesting that root-exuded P-hydrolyzing enzymes are one of the key drivers of PolyP hydrolysis leading to continuous enrichment of soil solutions with available P required for plant growth (Darch et al. 2016; Wang et al. 2019; Khourchi et al. 2023a).

Application of PolyP induced significant acidification of the rhizosphere soil solution, with soil solution pH dropping from 7.7 to 7.8 under OrthoP and P0 treatments to 6.3–6.5 under PolyB and PolyC. This acidification could be a result of organic acids and proton exudation by PolyP-fertilized roots, which are presumably involved in PolyP hydrolysis. Our findings were consistent with previous studies reporting that medium acidification strongly facilitates PolyP hydrolysis (McBeath et al. 2007; Khourchi et al. 2022a, 2023a). Thus, our findings confirmed that plants may facilitate PolyP hydrolysis in the vicinity of their roots by exuding large amounts of organic acids and protons as one of the key physiological mechanisms responsible for enhanced P availability and acquisition from PolyP. All these findings are consistent with previous studies mentioning that acidification is involved in PolyP hydrolysis and facilitation of P acquisition by plants (Darch et al. 2016; Wang et al. 2019; Gao et al. 2020; Khourchi et al. 2022b, 2022a, 2023a). However, investigating the key genes responsible for rhizosphere acidification, phosphatase exudation, and other molecular mechanisms involved in PolyP hydrolysis has, to date, been absent from the literature.

5 | Conclusions

The present study provides novel insights into the main belowground physiological and molecular mechanisms driving PolyP use efficiency at the root-rhizosphere interface and confirms the significant biological potential of root activities in enabling progressive PolyP hydrolysis and acquisition and synchronization with plant P requirements (Figure 9). Specifically, the current findings provide strong evidence that PolyP-fertilized plants enhance their root-rhizosphere processes that are directly involved in PolyP hydrolysis, and this was confirmed at the physiological level (enhanced rhizosphere acidification and exudation of P-hydrolyzing enzymes) and molecular level (modulating the expression of key genes encoding organic acids and phosphatase exudation). In parallel, our findings present compelling indications that PolyP can regulate root growth to acquire more P from PolyP, which is clearly explained by the regulation of the expression of genes involved in phytohormone biosynthesis pathways (known for their regulatory roles in root growth) as well as other transcription factors (e.g., MYB20 and WRKY11) regulating the expression of key genes involved in P uptake. Interestingly, these responses were PolyP-type dependent, with most responses noted in response to PolyB compared to PolyC. This difference can be explained by the intrinsic properties of each PolyP, essentially linked to chain length, where short-chain PolyP (PolyB) are easily susceptible to acidic and enzymatic hydrolysis compared to long-chain PolyP (PolyC).

Altogether, the present study is the first of its kind to provide novel physiological and molecular evidence that root-induced rhizosphere processes (e.g., rhizosphere acidification, P-hydrolyzing enzymes, exudation of carboxylates and protons) along with PolyP-induced specific root growth are presumably PolyP-specific adaptive responses in durum wheat, facilitating P availability and acquisition from PolyP. Guided by the findings of this pioneering study, more studies combining metabolomics, transcriptomics, and proteomics, coupled with crop genotypes exhibiting contrasting P-use efficiencies, are highly needed to investigate the potential role of PolyP in optimizing root-rhizosphere functioning, which will support the advantageous effects of PolyP and encourage their use as an efficient source of P for both soil fertility and crop nutrition. Specifically, metabolomics analyses will help to decipher the biochemical pathways influenced by PolyP, while reverse genetics approaches, such as gene overexpression and gene silencing, will be essential to validate key regulatory genes responsible for the observed physiological and transcriptomic changes. These approaches will provide deeper mechanistic insights into PolyP use efficiency and contribute to their use in large-scale agriculture.

Investigating the temporal dynamics of gene expression at different growth stages will further elucidate the key mechanisms driving the advantageous effects of PolyP through different growth stages. Additionally, given the role of rhizosphere microbes in PolyP hydrolysis and P availability, as well as their involvement in promoting growth, similar studies combining PolyP and beneficial rhizosphere microbes are needed to better explore root-microbe interactions for enhanced PolyP use efficiency.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

All data generated and/or analyzed during this study are included in this paper and its Supporting Information files. The raw data of transcriptome sequencing used in this paper has been deposited to the Gene Expression Omnibus (GEO) database under accession GSE277488.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.