Proteomic Analysis Dissects Molecular Mechanisms Underlying Plant Responses to Phosphorus Deficiency

Ming Zhou 1,†, Shengnan Zhu 2,†, Xiaohui Mo 1,†, Qi Guo 1, Yaxue Li 1, Jiang Tian 1,* and Cuiyue Liang 1,*

1 Root Biology Center, State Key Laboratory for Conservation and Utilization of Subtropical Agro-Bioresources, College of Natural Resources and Environment, South China Agricultural University, Guangzhou 510642, China; 14765080801@stu.scau.edu.cn (M.Z.); xhmo@scau.edu.cn (X.M.); guoqi1421@163.com (Q.G.); yaxueli2021@163.com (Y.L.)
2 Life Science and Technology School, Lingnan Normal University, Zhanjiang 524048, China; shnzhu@163.com

* Correspondence: jitian@scau.edu.cn (J.T.); liangcy@scau.edu.cn (C.L.); Tel.: +86-2085283380 (J.T.);
+86-2085280156 (C.L.)
† These authors contributed equally to this work.

Abstract: Phosphorus (P) is an essential nutrient for plant growth. In recent decades, the application of phosphate (Pi) fertilizers has contributed to significant increases in crop yields all over the world. However, low efficiency of P utilization in crops leads to intensive application of Pi fertilizers, which consequently stimulates environmental pollution and exhaustion of P mineral resources. Therefore, in order to strengthen the sustainable development of agriculture, understandings of molecular mechanisms underlying P efficiency in plants are required to develop cultivars with high P utilization efficiency. Recently, a plant Pi-signaling network was established through forward and reverse genetic analysis, with the aid of the application of genomics, transcriptomics, proteomics, metabolomics, and ionomics. Among these, proteomics provides a powerful tool to investigate mechanisms underlying plant responses to Pi availability at the protein level. In this review, we summarize the recent progress of proteomic analysis in the identification of differential proteins that play roles in Pi acquisition, translocation, assimilation, and reutilization in plants. These findings could provide insights into molecular mechanisms underlying Pi acquisition and utilization efficiency, and offer new strategies in genetically engineering cultivars with high P utilization efficiency.

Keywords: phosphorus; proteomics; P use efficiency

1. Introduction

Phosphorus (P) is an essential mineral nutrient for plants, accounting for up to 0.5% of plant dry weight depending on plant species [1]. It is not only an indispensable structural constituent of biomolecules, including deoxyribonucleic acid (DNA), proteins, and phospholipids, but also is a key signal factor that functions in mediating the phosphate (Pi)-signaling network [2–5]. Plants mainly take up P in the form of Pi, including HPO$_4^{2-}$ and H$_2$PO$_4^-$, which is about 0.1–10 µM in soils [6]. Meanwhile, multiple factors exist in soils, limiting Pi availability by directly or indirectly chelating Pi into immobile forms, such as microbial activities and an abundance of cations (e.g., Al$^3+$, Fe$^{2+}$, Ca$^{2+}$). For example, in acid soils, Pi easily reacts with Al$^{3+}$ or Fe$^{2+}$ and becomes sparingly soluble forms (i.e., Al-P and Fe-P) [6]. Low Pi availability and fluctuation severely limits crop yield by adversely affecting root growth, photosynthesis, respiration, and energy transduction [7,8]. To meet the requirement of P in crops, millions of tons of Pi fertilizers are profligately applied to farms worldwide every year. However, due to low P utilization efficiency in crops, applied Pi fertilizers are either fixed by soil particles or leached into the biosphere, leading to environmental pollution and biodiversity loss [4,9,10]. Therefore, a deeper understanding of the molecular mechanisms regarding plant tolerance to low Pi availability is required to...
develop cultivars with high Pi fertilizer utilization efficiency, thus ameliorating the sole reliance on excess Pi fertilizer applications to improve crop yield.

In recent decades, adaptive mechanisms underlying plant responses to P deprivation have been extensively investigated through forward and reverse genetic analysis, with the aid of the application of proteomics, transcriptomics, metabolomics, and ionomics [11–14]. Among these, proteomic analysis, as a powerful tool, has permitted us to identify numerous differentially accumulated proteins (DAPs) in response to Pi availability, which sheds light on molecular responses of plants to Pi starvation at the protein level, especially at protein modification levels (e.g., phosphorylation, succinylation) [13,15]. For example, a set of DAPs was identified at different P levels in many plant species, such as in Arabidopsis (Arabidopsis thaliana) [16,17], maize (Zea mays) [18,19], tomato (Solanum lycopersicum) [20], rice (Oryza sativa) [21,22], barley (Hordeum vulgare) [23], and soybean (Glycine max) [24,25] (Table 1). In this review, we mainly summarize recent advances in the identification and functional characterization of DAPs in response to Pi starvation in plants through proteomic analysis, and highlight the complex regulatory network underlying Pi acquisition, remobilization, and reutilization at protein levels. Meanwhile, we discuss the advantages and challenges of proteomic analysis to shed light on molecular mechanisms underlying plant adaptations to Pi starvation, and thus contribute to developing crop cultivars with high P efficiency in the future.
Table 1. A list of proteomic analyses of plant responses to phosphorus deficiency.

| Plant Species       | Organ/Tissues | Culture Time before Treatment (d) | Treatment Time (d) | Protein Separation Method                  | Total Protein Number (#) | Number of DAPs (#) | Protein Number Identified by MS Analysis | References |
|---------------------|---------------|-----------------------------------|--------------------|---------------------------------------------|--------------------------|------------------|------------------------------------------|------------|
| Arabidopsis thaliana| Leaves        | 10                                | 7                  | SCX iTRAQ LC-MS/MS                          | 5106                     | 156              | 106                                      | [17]       |
| Zea mays            | Leaves        | 4                                 | 25                 | 2-DE MALDI-TOF/MS/MS/TOF MS                 | 680/592                  | 29/71            | 9/20                                      | [27]       |
| Glycine max         | Leaves        | 5                                 | 14                 | 2D-IEF/SDS-PAGE MALDI-TOF MS               | 55                      | 17               | 7                                        | [28]       |
| Glycine max         | Leaves        | 3                                 | 14                 | SDS-PAGE Gel Digestion LC-MS/MS            | 4219                     | 707              | 267                                      | [29]       |
| Solanum lycopersicum| Leaves        | nd                                | 10                 | 2-DE MALDI-TOF MS/MS/MS/MS                | 600                      | 46               | 31                                        | [20]       |
| Arabidopsis thaliana| Roots         | 10                                | 3                  | 2-DE MALDI-TOF MS                          | 456                      | 30               | nd                                       | [30]       |
| Arabidopsis thaliana| Roots         | 10                                | 3                  | 2-DE iTARQ LC-MS                           | 13,298                   | 356              | 199                                      | [16]       |
| Arabidopsis thaliana| Roots         | nd                                | 14                 | 2-DIGE MALDI-TOF/TOF/TOF                  | 1420                     | 30               | 14                                       | [13]       |
| Zea mays            | Roots         | 24                                | 17                 | 2-DE MALDI-TOF MS                          | 1300                     | 254              | 76                                       | [31]       |
| Zea mays            | Roots         | 24                                | 17                 | 2-DE MALDI-TOF MS                          | 2822                     | 73/95            | 25/24                                    | [19]       |
| Zea mays            | Roots         | nd                                | 10                 | 2-DE MALDI-TOF TOF MS                      | nd                       | 83/325           | 30/246                                    | [33]       |
| Oryza sativa        | Roots         | 3                                 | 80                 | 2-DE MALDI TOF MS                          | 669                      | 34               | nd                                       | [21]       |
| Oryza sativa        | Roots         | 7                                 | 21                 | 2-DE MALDI-TOF MS                          | 140                      | 10               | 2                                        | [34]       |
| Glycine max         | Roots         | 7                                 | 20                 | 2-DIGE                                     | 325                      | 105              | 61                                       | [35]       |
| Glycine max         | Roots         | 3                                 | 9                  | SDS-PAGE TMT                               | 4216                     | 660/133          | 656/127                                   | [36]       |
| Glycine max         | Roots         | nd                                | 10                 | iTRAQ LC-MS/MS                             | nd                       | 71               | 30                                       | [25]       |
| Glycine max         | Roots         | 5                                 | 14                 | iTRAQ                                      | nd                       | 427              | 213                                      | [34]       |
| Triticum aestivum   | Roots         | 14                                | 8                  | iTRAQ                                      | nd                       | 6842             | 323                                      | [37]       |
| Hordeum vulgare     | Roots         | 10                                | 0.25/2             | SDS-PAGE LC-MS/MS/MS/MS                   | nd                       | 697              | nd                                       | [38]       |
| Brassica napus      | Roots         | 20                                | 3                  | 2-phase LC-MS/MS                           | 828                      | 31/40            | 8/28                                     | [39]       |
| Arabidopsis thaliana| Leaves/roots  | 7                                 | 3                  | 2-DIGE MALDI-TOF/TOF MS/MS                 | 88                       | nd               | nd                                       | [40]       |
| Hordeum vulgare     | Leaves/roots  | 17                                | 21                 | 2-DE MALDI-TOF/TOF/TOF/TOF MS             | nd                       | 31               | nd                                       | [23]       |
| Brassica napus      | Leaves/roots  | 20                                | 26                 | 2-DE MALDI-TOF MS                          | 1000                     | 32               | 4/12                                     | [41]       |
| Pinus massoniana    | Seedlings     | 10                                | 58                 | 2-DE MALDI-TOF/TOF/TOF/TOF MS             | nd                       | 98               | 44                                       | [42]       |
| Arabidopsis thaliana| Suspension cells | 7                              | 2                  | 2-DE MALDI-TOF MS                          | 110                      | 46               | 26                                       | [43]       |
| Glycine max         | Nodules       | 5                                 | 25                 | 2-DE MALDI-TOF MS                          | nd                       | 44               | 17                                       | [24]       |

* Two genotypes were used in the studies. nd, not described.
2. Morphological, Physiological, and Biochemical Responses of Plants to Pi Starvation

Phosphorus deficiency has many deleterious effects on plant growth, as reflected by a significant decrease in plant biomass and yield [4,45,46]. It is well known that plants have evolved a set of morphological, physiological, and molecular strategies to adapt to P deficiency conditions. These adaptive strategies could enhance plant capability to acquire Pi from soils and restrict Pi consumption in plant organisms or increase plant internal Pi reutilization [9,47–49]. Among plant morphological responses, modification of root morphology and architecture is generally considered to play a key role in controlling plant Pi acquisition efficiency, such as the formation of shallower root architecture and increases in both lateral root length and root hair density [50,51]. Interestingly, in white lupin and several species of the Proteaceae family, the formation of cluster roots termed “proteoid roots” was observed, which could enhance root–soil contact and thus facilitate plants to acquire more Pi from soils [52–54]. Accompanied by changes in root morphology and architecture, physiological and biochemical responses in plant roots could strengthen the capability of roots to mobilize sparingly soluble P in soils, including increased organic acid synthesis and exudation to mobilize inorganic-P forms (e.g., Al-P, Fe-P, Ca-P), and enhance root-associated purple acid phosphatase activity to hydrolyze organic-P forms (e.g., phytate-P, ATP) [9,55,56]. Meanwhile, plants could form beneficial symbiosis with soil microorganisms, such as arbuscular mycorrhizal (AM), to improve Pi acquisition [57–61].

In addition to changes in root traits, Pi availability also significantly triggers leaf/shoot morphology remodeling. For example, leaf angles (i.e., erectness, inclination) and tiller numbers are regulated by P deficiency in rice [62–64]. Moreover, accompanied by changes in shoot morphology, a group of physiological and biochemical processes in response to P deficiency was also influenced. For example, leaf color turned from green to purple or dark-green in most plants under low-P conditions, which was mainly caused by anthocyanin accumulations, along with the reduced chlorophyll contents [28,29,65]. Meanwhile, increased starch content and decreased sucrose content in leaves were widely observed in most plants [66,67]. Therefore, identification and functional characterization of the DAPs related to morphological, physiological, and biochemical responses in plants could help us to further improve Pi fertilizer utilization efficiency in crops [68,69].

3. DAPs Reveal Complex Responses of Plants to P Deficiency

3.1. Identification of DAPs in Plant Leaves/Shoots

3.1.1. Differential Proteins Related to Photosynthesis and Carbon Metabolism

Photosynthesis is a light-harvesting process whose intensity is mainly dependent on the generation rate of NADPH and ATP, as well as ribulose-1,5-diphosphate (RuBP) carboxylation [70–72]. Reduced accumulations of proteins associated with the photosynthesis process have been observed in plants under P deficiency conditions through differential proteomic analysis, such as proteins related to light energy absorption, electron transfer, and transformation to generate ATP and NADPH. For example, the differential accumulations of a series of proteins were identified, including chlorophyll a/b binding protein in ramie (Boehmeria nivea), ferredoxin (Fdx) in soybean, as well as ferredoxin-nitrite reductase (FNR) and alpha/beta subunit of the ATP synthetase in maize (Figure 1) [18,26,28,29]. Meanwhile, a set of DAPs exhibiting decreased accumulations were found to be involved in rubisco carboxylation reaction (RuBisco), the conversion of ATP and NADPH process, including NADP-malate dehydrogenase (NADP-MDH), pyruvate orthophosphate dikinase (PPDK), phosphoenolpyruvate carboxylase (PEPC), transketolase isoform, Rubisco activase A (RCA), and the large and small subunit of Rubisco [18,26,27,40,41]. Therefore, decreased accumulations of the proteins strongly suggest that Pi starvation could reduce photosynthesis in plants.
A set of proteins closely related to carbon and energy metabolism were found to be up- or down-regulated by Pi starvation in plant leaves (Figure 1). For example, some proteins associated with the glycolysis process were up-regulated, such as hexokinase (HXK) in ramie leaves, fructose-1,6-bisphosphate aldolase (FBP aldoase), glyceraldehyde-3-phosphate dehydrogenase (GAPDH), phosphoglycerate kinase (PGK), and enolase1 in leaves of both maize and Arabidopsis [18,26,43]. Moreover, the increased accumulations of proteins involved the tricarboxylic acid cycle (TCA) were also found to be affected by Pi deprivation in maize leaves, such as succinyl-CoA synthetase (SCS) and succinate dehydrogenase (SDH) [18]. Additionally, the abundance of MDH and isocitrate dehydrogenase (IDH) in maize leaves was also increased, along with the up-regulation of several enzymes involved in the pentose phosphate pathway (PPP), such as 6-phosphogluconolactonase (6-PGLS) and 6-phosphogluconate dehydrogenase (6-PGDG), which are involved in the conversion of glucose-6-phosphate to NADPH and reducing equivalents.

Figure 1. A model of integration of different adaptive strategies to P deficiency regulated by DAPs in shoots or leaves. Red color indicates proteins with increased accumulations in plants under low-P conditions; blue color indicates proteins with decreased accumulations in plants under low-P conditions; brown color suggests proteins exhibiting either up-regulated or down-regulated accumulations under low-P conditions; dashed lines indicate multiple steps; AA5GT, anthocyanidin 5-O-glucosyltransferase; ACC, 1-aminoacyclopropane-1-carboxylate; ADP, adenosine diphosphate; ANS, anthocyanidin synthase; APX, ascorbate peroxidase; Arg, arginine; ADC, arginine decarboxylase; ASA, ascorbic acid; ATP, adenosine triphosphate; CoA, coenzyme A; DFR, dihydroflavonol-4-reductase; DHAR, dehydroascorbate reductase; E4P, erythritol-4-phosphate; F6P, fructose-6-phosphate; FBP aldoase, fructose-1,6-bisphosphate aldolase; FBP, fructose-1,6-bisphosphate; Fdx, ferredoxin; G1P, glucose-1-phosphate; G3P, glyceraldehyde-3-phosphate; G6P, glucose-6-phosphate; G6PDH, glyceraldehyde-3-phosphate dehydrogenase; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GR, glutathione reductase; GSH, reduced glutathione; GSSG, oxidized glutathione; GST, glutathione-S-transferase; GAPDH1, 6-phosphogluconate dehydrogenase; GAPDH2, 6-phosphogluconate dehydrogenase; hexokinase; HXK, hexokinase; LDOX, leucoanthocyanidin dioxygenase; MDA, malondialdehyde; MDH, monodehydroascorbate reductase; MDHA, monodehydroascorbate reductase; Met, methionine; NADP, nicotinamide adenine dinucleotide phosphate; NADPH, nicotinamide adenine dinucleotide phosphate; OAA, oxaloacetate; PEP, phosphoenolpyruvate; PEPC, phosphoenolpyruvate carboxylase; PGA, 3-phosphoglycerate; PG, phosphoglycerate kinase; Pi, inorganic phosphate; PP, pyruvate orthophosphate dikinase; RCA, ribulose bisphosphate carboxylase/oxygenase activase; Ru5P, ribulose-5-phosphate; RuBP, ribulose-1,5-bisphosphate; SAM, S-adenosyl methionine; SCS, succinyl-CoA synthetase; SDH, succinate dehydrogenase; SOX, succinyl-CoA synthetase; SQDH, sulfuroxidases; SQDH2, sulfuroxidases; UDP, uridine diphosphate; UDPG, uridine diphosphoglucose; UDP-SQ, UDP-sulfoquinovosyl.
hydrogenase (SDH) [18]. Additionally, the abundance of MDH and isocitrate dehydrogenase (IDH) was found to be down-regulated in leaves of maize and ramie, respectively [26,27]. Meanwhile, other carbon metabolic processes were also influenced by Pi starvation in plants, as reflected by the identification of related DAPs (Figure 1). For example, the 6-phosphogluconolactonase (6-PGLS) and 6-phosphogluconate dehydrogenase (6-PGDG), associated with the pentose phosphate pathway (PPP), were up-regulated under P deficiency conditions in maize and barley [18,23]. In contrast, triose-phosphate isomerase (TPI) and 6-PGDG were down-regulated in leaves of rape and maize, respectively [18,27,41]. These results indicate that carbon and energy metabolic processes, like glycolysis, TCA cycle, and gluconeogenesis, are significantly influenced in plants under low-P conditions.

3.1.2. Pi Starvation Responsive Proteins Related to Remodeling Lipid Membranes

One of the vital adaptive strategies to low Pi availability in plants is to improve internal P utilization efficiency by remodeling lipid membranes [73]. Under low-Pi stress, phospholipids, accounting for 30% of total organophosphate in plants, were replaced by specific non-phosphorous lipids, such as sulfoquinovosyl diacylglycerol (SQDG) [74]. Consistently, a set of DAPs was identified to function in remodeling lipid membranes. For example, in maize and Arabidopsis leaves, increased accumulations of UDP-sulfoquinovose synthase (SQD1) were observed, which were suggested to function in releasing sulfoquinovose for SQDG production [18,27,75]. Meanwhile, increased accumulations of SQDG synthase (SQD2) were also observed under low-P conditions, and SQDs were suggested to accelerate the replacement of phospholipid glycerate (PG) by SQDG in plant cell plasma membranes [17,75]. Additionally, some proteins, including lipid-transfer protein, lipoxygenase, and 3-phosphoglycerate dehydrogenase, were also observed to be up-regulated in Arabidopsis leaves [17]. These results support the hypothesis that changes in the phospholipid metabolism could further accelerate the conversion of intracellular organic P forms, and thus improve P utilization efficiency under low-P conditions.

3.1.3. Proteins Involved Anthocyanin, Polyamine, and Reactive Oxygen Species (ROS) Metabolisms

It is generally believed that increases in anthocyanin synthesis in shoots or leaves could protect plants from ROS damage under low-P conditions [76–79]. Anthocyanins are products generated from the phenylpropanoid and flavonoid metabolic pathways, regulated by multiple enzymes, such as chalcone synthase (CHS) and dihydroflavonol-4-reductase (DFR) [80]. DAPs functioning in anthocyanin synthesis have been found in Arabidopsis leaves through proteomic analysis, including 4-coumarate:CoA ligase 1 (4-CL1), DFR, leucoanthocyanidin dioxygenase (LDOX), anthocyanidin synthase (ANS), anthocyanidin 5-O-glucosyltransferase (AA5GT), phenylalanine ammonia-lyases (PALs), and glutathione-S-transferase F12 (GSTF12) [17,75] (Figure 1). The results strongly suggest that P deficiency could increase accumulations of enzymes functioning in anthocyanin synthesis, and thus enhance anthocyanin accumulations in leaves.

Polyamines are aliphatic amines and play an important role in plant adaptation to biotic and abiotic stresses [81]. Interestingly, several DAPs controlling polyamines biosynthesis were found in maize leaves under P deficiency conditions, such as spermidine synthase (SPDS) and arginine decarboxylase (ADC) [18]. Although the detailed functions of polyamines in plant adaptation to Pi starvation remained fragmentary, identification of DAPs mediating its biosynthesis strongly suggests that polyamines could participate in plant adaption to P deficiency, which merits further study.

Phosphorus deficiency also leads to the elevation of ROS in plant leaves [82]. Therefore, increased accumulations of antioxidation-related proteins were generally observed in plant leaves under P deficiency conditions, including glutathione S-transferase (GST) in maize and barley [18,23], ascorbate peroxidase (APX) in maize [18], 2-cysteine peroxiredoxin B in ramie [26], non-specific lipid-transfer protein 1, and extra-large G protein 3 in tomato [20],
as well as proteins belonging to peroxidase superfamily in Arabidopsis [17]. Increased accumulations of these proteins may help plants against ROS damage under low-P stress.

3.2. Identification of DAPs in Plant Roots

Recently, DAPs controlling root responses to P deficiency have been identified in different plants, such as soybean [25], rape [39], and maize [19]. Functions of the DAPs have been suggested to involve a series of adaptive strategies in plant roots, including changes in root development, regulating organic acid synthesis and secretion, increasing activities of root-associated purple acid phosphatases (PAPs). Furthermore, several studies were conducted to elucidate complex responses of protein modifications to P deficiency in plant roots, especially for protein phosphorylation and succinylation.

3.2.1. Pi Starvation-Responsive Proteins Participated in Root System Remodeling

It is well known that root architecture remodeling involves changes in hormones, such as auxin, ethylene, cytokinin (CK), or jasmonic acid (JA), which react differently but coordinately regulate root growth [83–86]. Thus, a set of DAPs was found to involve hormone synthesis, transport, and distribution in root response to P deficiency [19,31–35] (Figure 2). For example, in several plant species, such as rice and masson pine (Pinus massoniana), comparative proteomic studies were conducted to find that abundance of several ethylene-precursor synthetases, including 1-aminocyclopropane-1-carboxylate oxidase (ACCO) and SAMS, was up-regulated by P deficiency, even though ACCO and SAMS were down-regulated in maize and Arabidopsis, respectively [30,33,34,42]. The results strongly suggest that ethylene participated in regulating root growth under P-deprivation conditions, which was probably attributed to changes in its biosynthesis [87,88]. Consistently, in Arabidopsis, the number of lateral roots was significantly higher in aco1-1 mutant compared to that in wild-type, strongly suggesting that ethylene could regulate lateral root development [89]. In addition to ethylene, auxin is considered to play a key role in mediating the development of lateral root and root hair under Pi-starvation conditions [85,90]. Consistently, a set of DAPs for the auxin signaling pathway was observed to be up-regulated in Arabidopsis and maize, including auxin-response proteins (ARFs), phosphatase 2A (PP2A), and nonspecific phospholipase C4 (NPC4) [16,19,32,41]. Furthermore, overexpression of ZmPP2AA1, encoding a subunit of phosphatase 2A, significantly increased the lateral root density and promoted root growth under P-deficiency conditions in maize, strongly suggesting that auxin could regulate lateral root development [91,92]. Additionally, root growth in response to P deficiency was also found to be mediated by the JA pathway, supported by the evidence that allene oxide synthase (AOS), allene oxide cyclase 1 (AOC1), and jaclin-related lections (e.g., JAL5/23/31) for controlling JA metabolism were up-regulated in Arabidopsis under low-P conditions [17,75]. Interestingly, knockout of atwrky6-1, a mediator in the Pi-signaling network, led to decreases in lateral root formation, but increased accumulations of AOS, strongly suggesting the JA metabolism might participate in regulating Arabidopsis root growth [17,93]. Meanwhile, decreased accumulations of tRNA isopentenyl transferase (tRNA IPT) and beta-glucosidase (BGL) involved CK synthesis were observed in long-term P-deficient maize roots, suggesting that CK could regulate plant root growth [31,32]. Moreover, several studies reported that the application of CK could disturb the expression of PIN genes and prevent the formation of the auxin gradient, which is required for lateral root primordia development. In contrast, the biosynthesis of CK was rapidly suppressed by auxin via the isopenteneyadenosine-50-monophosphate independent pathway [94–96]. Several studies also reported that ethylene could regulate root growth via stimulating auxin biosynthesis and transport in root apex, but DAPs involved in both auxin- and ethylene-signaling pathways were scarcely identified [96–98]. These results suggest that P deficiency-induced changes in root morphology and architecture may be modulated through sophisticated interactions among different phytohormones.

In addition to the identification of phytohormone-related DAPs, a set of DAPs involved in cell cycle, division, and expansion processes was also suggested to regulate
root architecture plasticity in response to low-P stress [99,100] (Figure 2). For example, translationally controlled tumor protein (TCTP), functioning in fundamental biological processes (e.g., mitotic, cell proliferation, cytoprotective, and anti-apoptotic), was observed to be up-regulated by Pi starvation in Arabidopsis [40,101,102]. Further genetic analysis showed that reduced AtTCTP1 expression could result in significant inhibition of lateral root formation [103]. Additionally, accumulations of cell proliferation-related proteins, including GTP-binding nuclear protein RAN-B1 (Ran GTPase), cell division cycle protein 48 (CDC48), and mini-chromosome maintenance protein 6 (MCM6), were increased by Pi starvation in maize [19,31]. Contrastingly, an increased abundance of actin and tubulin was observed in plants in response to low-P stress in plants, including soybean, maize, rice, and barley, while decreased abundance was observed in Arabidopsis [19,23,31,35,36]. Interestingly, a loss of rmd mutant, encoding a rice actin-binding protein, exhibited steeper growth angles for crown roots in rice, strongly suggesting that cell proliferation-related proteins could play a role in root growth [104].

![Figure 2](image-url)

**Figure 2.** A model of integration of adaptive strategies of plant roots to Pi starvation regulated by DAPs. Up or down arrows indicate DAPs exhibiting increased or decreased accumulations; AM, arbuscular mycorrhiza; ACCO, 1-aminocyclopropane-1-carboxylate oxidase; AOC1, allene oxide cyclase 1; AOS, the allene oxide synthase; BGL, beta-glucosidase; CDC48, cell division cycle protein 48; CS, citrate synthase; JALs, jacalin-related lectins; MCM6, mini-chromosome maintenance protein 6; MDH, malate dehydrogenase; PAPs, purple acid phosphatases; PP2A, phosphatase 2A; NPC4, non-phospholipase C4; Ran GTPase, GTP-binding nuclear protein RAN-B1; SAMS, S-adenosyl methionine synthase; TCTP, translationally controlled tumor protein; tRNA IPT, tRNA isopentenyl transferase.

### 3.2.2. Pi Starvation-Responsive Proteins Related to Root Exudates

Plant root exudates mainly include a class of metabolites and proteins with low- or high-molecular weight, such as organic acids (OAs), sugars, flavonoids, and phosphatases [100,105]. It is well known that root exudates play crucial roles in increasing Pi uptake by desorbing immobile P in soils [100,106]. Consistently, differential proteomic analysis led to identifying a set of DAPs controlling root exudates, which contributes to deepening understandings of the molecular mechanisms underlying rhizosphere P mobilization [100,105] (Figure 2). For example, increased OA exudation was found to be
associated with activities of several differential proteins related to OA synthesis, including citrate synthase (CS) in Arabidopsis, maize and soybean, IDH in maize, MDH in rape, soybean, and maize, as well as decreased abundance of aconitase in rice [21,30,32,35,41]. Consistently, overexpression of CS from Daucus carota (DcCS) improved Arabidopsis growth under P deficiency conditions due to enhanced citrate synthesis and exudation from the roots [107]. These results strongly indicate that increased OA synthesis and exudation play an important role for plants when scavenging Pi in soils.

In addition to OA synthesis and exudation, some secreted proteins, such as purple acid phosphatases (PAPs) and ribonucleases, are critical for plants to utilize organic-P pools in soils. Among PAPs, root-associated PAPs were widely found to be involved in the activation and utilization of extracellular organic-P sources, such as ATP, deoxy-ribonucleotide triphosphate (dNTPs), and phytate-P [56,108]. For example, several PAPs were purified and functionally characterized to mediate organic-P utilization, such as SgPAP23 in stylo (stylosanthes guianensis), GmPAP7a/b in soybean, OsPAP10a in rice, and LeSAP1/2 in tomato [25,47,109–111]. Meanwhile, a variety of low P-induced PAPs was identified in plants through proteomic analysis, such as AtPAP12/26 in Arabidopsis, GmPAP1-like, and GmPAP22-like proteins in soybean [25,44]. In Arabidopsis, atpap12/atpap26 mutant exhibited impaired growth coupling the decreases in root secretory APase activity and total Pi concentration in rosettes [112,113]. Overexpressing GmPAP1-like enhanced Arabidopsis growth and P content when dNTP was supplied as the sole external P source [25]. In addition, some DAPs functions in cell wall carbohydrate metabolism were also identified to be up-regulated in Arabidopsis suspension cells or root cells walls under P-deficient conditions, such as polygalacturonase (PG) and xyloglucan endotransglycosylase (XET), which was suggested to release cell wall-bound Pi under low-P conditions [25,112,114]. Additionally, since changes in polysaccharides in cell wall metabolism could influence the rhizosphere size, which was suggested to be positively correlated with P accumulation in plants, DAPs related to cell wall metabolism might affect plant P efficiency partially through changing rhizosphere size [115].

3.2.3. Response of Symbiotic Association to Pi Starvation in Plants

Although it is well known that the formation of a symbiotic association between plants and arbuscular mycorrhiza (AM) is a typical plant strategy in response to P deficiency, no proteomic analysis was conducted to identify DAPs in the symbiotic association at different P levels. However, several studies have highlighted that Pi acquisition efficiency in legume crops could be improved through formation of the symbiotic association between roots and rhizobia, which can directly fix atmosphere N\textsubscript{2} in nodules and supply nitrogen for plant growth. For example, rhizobium inoculation led to enhancing the capability of soybean in utilization of sparingly soluble P forms (e.g., Ca-P, Al-P, Fe-P), as reflected by higher P content and plant biomass [116]. Furthermore, overexpression of two nodule preferring Pi transporters, GmPT7 and GmPT5 promoted soybean nodulation, P content, as well as fresh weight [117,118]. To date, only one study has elucidated differential protein profiles in soybean nodules at two P levels through two-dimensional electrophoresis combined with matrix-assisted laser desorption ionization (MALDI)-time of flight (TOF)/TOF mass spectrometry (MS) analysis. A total of 44 DAPs were identified in soybean nodules, which were mainly involved in stress response and carbon and amino acid metabolism, strongly indicating that a complex but precise module exists in soybean nodules to sustain mutualistic symbiosis to maximize Pi uptake [24]. Among them, GmMDH12 was found to be up-regulated in soybean nodules by Pi starvation, which was consistent with the increased malate concentration in nodules at low-P levels. Furthermore, overexpression of GmMDH12 significantly increased malate concentrations and inhibited nodule size in soybean, strongly suggesting that Pi-starvation increased malate, which might have multiple functions except for the enhancement of malate exudation and the activation of sparingly soluble P in soils [119].
4. Identification of DAPs Exhibiting Post-Transcriptional Modifications

Post-transcriptional modifications (PTMs) play crucial roles in regulating protein activity, longevity, and localization, including phosphorylation, ubiquitination, succinylation, glycosylation, and SUMOylation modification [15,38,120]. Recently, several studies were conducted to investigate changes in protein profiles with PTMs in plant roots at different P levels, such as phosphoproteome in soybean, maize, and rice [15,22,38]. In soybean roots, a total of 427 phosphoproteins were found to be regulated by Pi starvation, including 213 up-regulated and 214 down-regulated [15]. For example, auxin efflux transporter GmPIN2 and ethylene-insensitive protein GmEIN2 were identified to be up-regulated by P deprivation, strongly suggesting participation of auxin and ethylene in modulating root architecture in response to P deficiency [15]. Meanwhile, in rice roots, a total of 554 phosphoproteins were found to exhibit differential accumulations, with 546 down-regulated and 8 up-regulated proteins. Several proteins, including four mitogen-activated protein kinases (MAPKs), five calcium-dependent protein kinases (CDPKs), and OsCK2β3, were observed to be decreased in response to Pi starvation, especially for OsCK2, which was suggested to regulate phosphorylation of OsPT2 and OsPT8 and thus influence their subcellular localization [22,121]. Meanwhile, in maize roots, about 51 phosphoprotein spots were observed to exhibit differential accumulations by low-P treatment; these were involved in a group of cellular and metabolic pathways, such as signal transduction and carbon metabolism [32]. Among them, one auxin receptor, ABP1, was found to be up-regulated in maize roots under low-P conditions [32]. Recently, overexpression of AtABP1 has been found to influence primary root growth, root bending, and lateral root development [122].

In addition to phosphorylation analysis, succinylated-proteomic analysis was also conducted to identify differential proteins with succinylation in barley roots under P-deficiency conditions [38]. A total of 120 succinylation sites were identified across 83 proteins, including 79 increased and 4 decreased succinylated proteins at 48 h of Pi-starvation treatment, respectively [38]. Moreover, these differentially succinylated proteins were enriched in ribosome pathways, glycolysis, and RNA degradation pathways. For example, 60S/50/40S/30 ribosomal protein was suggested to involve ribosome pathways, while TPI, GAPDH, and FBP aldolase were predicted to participate in glycolysis pathways [38]. However, functions for most of the identified proteins with succinylation remain unknown, which merits further study.

5. Perspectives

In recent decades, with the aid of proteomic analysis, identification and functional characterization of DAPs have opened the way to elucidate molecular mechanisms underlying plant adaptation to Pi starvation. For example, the increased abundances of SPX1, PHT1;4, and PHF1 proteins were observed in Arabidopsis roots, and other proteins, such as TaPHT1;9-4B, TaPHT1;3-5B, and TaPHT1;6-5B, were also observed in wheat roots, acting as the main proteins mediating Pi homeostasis [16,37]. However, the functions of most of the DAPs remain largely unknown and require furthered investigation via reverse and forward genetic analysis. For example, a histone chaperone (nap1;2) was found to be up-regulated under Pi starvation via proteomic analysis in Arabidopsis, and its functions in maintaining Pi homeostasis were clarified via analysis of triple nap1;1 nap1;2 nap1;3 mutant lines [13]. Meanwhile, although the method of differential proteomics based on mass spectrometry was established and successfully used, there is still a lack of a more effective method for protein separation and identification in order to qualitatively analyze plant proteins. In order to solve these problems, multiple technologies should be combined to develop a more effective method for protein extraction and separation. Meanwhile, in addition to commonly used quantitative techniques, such as label-free quantitation [123], isobaric tags for relative and absolute quantitation (iTRAQ) [124], tandem mass tags (TMT) [125], and stable isotope labeling by amino acids in cell culture (SILAC) [126], application of a relatively new technique, termed sequential window acquisition of all theoretical mass spectra (SWATH) [127], together with data-independent acquisition (DIA) [128], might be
very helpful to identify more DAPs. Furthermore, the vigorous development and wide application of new technologies (e.g., microfluidic chip, reverse micelles, magnetic nanoparticles) will break the bottleneck of protein separation, enrichment, and detection in single cells and different organelles [129].

Although many questions remain to be solved, we believe that the application of proteomics integrating multiple omics, as well as genetic analysis, will help us to elucidate molecular mechanisms underlying plant adaptation to P deficiency and develop cultivars with high P efficiency in the future.

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References
1. Cashikar, A.G.; Kumaresan, R.; Rao, N.M. Biochemical characterization and subcellular localization of the red kidney bean purple acid phosphatase. *Plant Physiol.* 1997, 114, 907–915. [CrossRef]
2. Raghothama, K.G. Phosphate acquisition. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 1999, 50, 665–693. [CrossRef] [PubMed]
3. Plaxton, W.C.; Shane, M.W. The role of post-translational enzyme modifications in the metabolic adaptations of phosphorus-deprived plants. *Annu. Rev. Plant Biol.* 2015, 48, 99–123.
4. Oldroyd, G.; Leyser, O. A plant’s diet, surviving in a variable nutrient environment. *Science* 2020, 368, eaba0196. [CrossRef] [PubMed]
5. Ham, B.K.; Chen, J.; Yan, Y.; Lucas, W.J. Insights into plant phosphate sensing and signaling. *Curr. Opin. Biotechnol.* 2018, 49, 1–9. [CrossRef]
6. Hinsinger, P. Bioavailability of soil inorganic P in the rhizosphere as affected by root-induced chemical changes: A review. *Plant Soil* 2001, 237, 173–195. [CrossRef]
7. Medici, A.; Szponarski, W.; Dangeville, P.; Safi, A.; Dissanayake, I.M.; Saenchai, C.; Emanuel, A.; Rubio, V.; Lacombe, B.; Ruffel, S.; et al. Identification of molecular integrators shows that nitrogen actively controls the phosphate starvation response in plants. *Plant Cell* 2019, 31, 1171–1184. [CrossRef] [PubMed]
8. Cho, H.; Bouain, N.; Zheng, L.; Rouached, H. Plant resilience to phosphate limitation: Current knowledge and future challenges. *Crit. Rev. Biotechnol.* 2021, 41, 63–71. [CrossRef]
9. Lambers, H.; Finnegan, P.M.; Jost, R.; Plaxton, W.C.; Shane, M.W.; Stitt, M. Phosphorus nutrition in Proteaceae and beyond. *Nat. Plants* 2015, 1, 15109. [CrossRef] [PubMed]
10. Hallama, M.; Pekrun, C.; Lambers, H.; Kandel, E. Hidden miners-the roles of cover crops and soil microorganisms in phosphorus cycling through agroecosystems. *Plant Soil* 2019, 434, 7–45. [CrossRef]
11. Abdelrahman, M.; El-Sayed, M.A.; Hashem, A.; Abd, A.E.; Alqarawi, A.A.; Burritt, D.J.; Tran, L.P. Metabolomics and transcriptomics in legumes under phosphate deficiency in relation to nitrogen fixation by root nodules. *Front. Plant Sci.* 2018, 9, 922. [CrossRef]
12. Deng, Q.; Luo, X.; Chen, Y.; Zhou, Y.; Zhang, F.; Hu, B.; Xie, K. Transcriptome analysis of phosphorus stress responsiveness in the seedlings of Dongxiang wild rice (*Oryza rufipogon* Griff.). *Biol. Res.* 2018, 51, 7. [CrossRef] [PubMed]
13. Iglesias, J.; Trigueros, M.; Rojas-Triana, M.; Fernández, M.; Albar, J.P.; Bustos, R.; Paz-Ares, J.; Rubio, V. Proteomics identifies ubiquitin-proteasome targets and new roles for chromatin-remodeling in the *Arabidopsis* response to phosphate starvation. *J. Proteom.* 2013, 94, 1–22. [CrossRef]
14. Watanabe, T.; Urayama, M.; Shinano, T.; Okada, R.; Osaki, M. Application of ionomics to plant and soil in fields under long-term fertilizer trials. *Springerplus* 2015, 4, 781. [CrossRef] [PubMed]
15. Jiang, W.; He, P.; Zhou, M.; Lu, X.; Chen, K.; Liang, C.; Tian, J. Soybean responds to phosphate starvation through reversible protein phosphorylation. *Plant Physiol. Biochem.* 2021, 167, 222–234. [CrossRef] [PubMed]
16. Lan, P.; Li, W.; Schmidt, W. Complementary proteome and transcriptome profiling in phosphate-deficient Arabidopsis roots reveals multiple levels of gene regulation. *Mol. Cell. Proteom.* 2012, 11, 1156–1166. [CrossRef] [PubMed]

17. Wang, Z.Q.; Zhou, X.; Dong, L.; Guo, J.; Chen, Y.; Zhang, Y.; Wu, L.; Xu, M. iTRAQ-based analysis of the Arabidopsis proteome reveals insights into the potential mechanisms of anthocyanin accumulation regulation in response to phosphate deficiency. *J. Proteom.* 2018, 184, 39–53. [CrossRef] [PubMed]

18. Zhang, K.; Liu, H.; Tao, P.; Chen, H. Comparative proteomic analyses provide new insights into low phosphorus stress responses in maize leaves. *PLoS ONE* 2014, 9, e98215.

19. Li, K.; Xu, C.; Li, Z.; Zhang, K.; Yang, A.; Zhang, J. Comparative proteome analyses of phosphorus responses in maize (Zea mays L.) roots of wild-type and a low-P-tolerant mutant reveal root characteristics associated with phosphorus efficiency. *Plant J.* 2008, 55, 927–939. [CrossRef] [PubMed]

20. Muneer, S.; Jeong, B.R. Proteomic analysis provides new insights in phosphorus homeostasis subjected to Pi (inorganic phosphate) deficiency. *Int. J. Mol. Sci.* 2012, 13, 2925–2939. [CrossRef] [PubMed]

21. Sepideh, T.; Matthias, W.; Manzar, H.; Mohammad-Reza, N.; Gilany, K.; Mohammad-Reza, H.; Mansoor, O.; Yazdi-Samadi, B.; Abdelbagi, M.I. A comparative proteome approach to decipher the mechanism of rice adaptation to phosphorous deficiency. *Proteomics* 2009, 9, 159–170.

22. Yang, J.; Xie, M.; Yang, X.; Liu, B.; Lin, H. Phosphoproteomic profiling reveals the importance of CK2, MAPKs and CDPKs in response to phosphate starvation in rice. *Plant Cell Physiol.* 2019, 60, 2785–2796. [CrossRef] [PubMed]

23. Nadira, U.A.; Ahmed, I.M.; Zeng, J.; Wu, F.; Zhang, G. Identification of the differentially accumulated proteins associated with low phosphorus tolerance in a Tibetan wild barley accession. *J. Plant Physiol.* 2016, 198, 10–22. [CrossRef] [PubMed]

24. Chen, Z.; Cui, Q.; Liang, C.; Sun, L.; Tian, J.; Liao, H. Identification of differentially expressed proteins in soybean nodules under phosphorus deficiency through proteomic analysis. *Proteomics* 2011, 11, 4648–4659. [CrossRef]

25. Wu, W.; Lin, Y.; Liu, P.; Chen, Q.; Tian, J.; Liang, C. Association of extracellular dNTP utilization with a GmPAP1-like protein identified in cell wall proteomic analysis of soybean roots. *J. Exp. Bot.* 2018, 69, 603–617. [CrossRef]

26. Deng, G.; Liu, L.J.; Zhong, X.Y.; Lao, C.Y.; Wang, H.Y.; Wang, B.; Zhu, C.; Shah, F.; Peng, D.X. Comparative proteome analysis of the response of ramei under N, P and K deficiency. *Planta* 2014, 239, 1175–1186. [CrossRef] [PubMed]

27. Zhang, K.; Liu, H.; Song, J.; Wu, W.; Li, K.; Zhang, J. Physiological and comparative proteome analyses reveal low-phosphate tolerance and enhanced photosynthesis in a maize mutant owing to reinforced inorganic phosphate recycling. *BMC Plant Biol.* 2016, 16, 129. [CrossRef] [PubMed]

28. Chu, S.; Li, H.; Zhang, X.; Yu, K.; Chao, M.; Han, S.; Zhang, D. Physiological and proteomics analyses reveal low-phosphorus stress affected the regulation of photosynthesis in soybean. *Int. J. Mol. Sci.* 2018, 19, 1688. [CrossRef]

29. Cheng, L.; Min, W.; Li, M.; Zhou, L.; Hsu, C.; Yang, X.; Jiang, X.; Ruan, Z.; Zhong, Y.; Wang, Z.; et al. Quantitative proteomics reveals that GmENO2 proteins are involved in response to phosphate starvation in the leaves of Glycine max L. *Int. J. Mol. Sci.* 2021, 22, 920. [CrossRef]

30. Chevalier, F.; Rossignol, M. Proteomic analysis of Arabidopsis thaliana ecotypes with contrasted root architecture in response to phosphate deficiency. *J. Plant Physiol.* 2011, 168, 1885–1890. [CrossRef]

31. Li, K.; Xu, C.; Zhang, K.; Yang, A.; Zhang, J. Proteomic analysis of roots growth and metabolic changes under phosphorus deficit in maize (Zea mays L.). *Proteomics* 2007, 7, 1501–1512. [CrossRef]

32. Li, K.; Xu, C.; Fan, W.; Zhang, H.; Hou, J.; Yang, A.; Zhang, K. Phosphoproteome and proteome analyses reveal low-phosphate mediated plasticity of root development and metabolic regulation in maize (Zea mays L.). *Plant Physiol. Biochem.* 2014, 83, 232–242. [CrossRef]

33. Jiang, H.; Zhang, J.; Han, Z.; Yang, J.; Ge, C.; Wu, Q. Revealing new insights into different phosphorus-starving responses between two maize (Zea mays) inbred lines by transcriptomic and proteomic studies. *Sci. Rep.* 2017, 7, 44294. [CrossRef]

34. Kim, S.G.; Wang, Y.; Lee, C.H.; Mun, B.G.; Kim, P.J.; Lee, S.Y.; Kim, Y.C.; Kang, K.Y.; Rakwal, R.; Agrawal, G.K.; et al. A comparative proteomics survey of proteins responsive to phosphorusrstarvation in roots of hydroponically-grown rice seedlings. *J. Korean Soc. Appl. Biol. Chem.* 2011, 54, 667–677. [CrossRef]

35. Vengavasi, K.; Pandey, R.; Abraham, G.; Yadav, R. Comparative analysis of soybean root proteome reveals molecular basis of differential carbohydrate efflux under low phosphorus stress. *Genes* 2017, 8, 341. [CrossRef] [PubMed]

36. Zhao, H.; Yang, A.; Kong, L.; Xie, F.; Wang, H.; Ao, X. Proteome characterization of two contrasting soybean genotypes in response to different phosphorus treatments. *AoS Plants* 2021, 13, plab019. [CrossRef] [PubMed]

37. Wang, P.; Li, G.; Li, G.; Yuan, S.; Wang, C.; Xie, Y.; Guo, T.; Kang, G.; Wang, D. TaPHT1;9-4B and its transcriptional regulator TaMYB4-7D contribute to phosphate uptake and plant growth in bread wheat. *New Phytol.* 2021, 231, 1968–1983. [CrossRef] [PubMed]

38. Wang, J.; Ma, Z.; Li, C.; Ren, P.; Yao, L.; Li, B.; Meng, Y.; Ma, X.; Si, E.; Yang, K.; et al. Dynamic responses of barley root succinyl-proteome to short-term phosphate starvation and recovery. *Front. Plant Sci.* 2021, 12, 649147. [CrossRef] [PubMed]

39. Chen, S.; Luo, Y.; Ding, G.; Xu, F. Comparative analysis of Brassica napus plasma membrane proteins under phosphorus deficiency using label-free and MaxQuant-based proteomics approaches. *J. Proteom.* 2016, 133, 144–152. [CrossRef] [PubMed]

40. Li, L.; Huang, L.; Pan, G.; Liu, L.; Wang, X.; Lu, L. Identifying the genes regulated by AtWRKY6 using comparative transcript and proteome analysis under phosphorus deficiency. *Int. J. Mol. Sci.* 2017, 18, 1046. [CrossRef]
41. Yao, Y.; Sun, H.; Xu, F.; Zhang, X.; Liu, S. Comparative proteome analysis of metabolic changes by low phosphorus stress in two *Brassica napus* genotypes. *Planta* 2011, 233, 523–537. [CrossRef]

42. Fan, F.H.; Ding, G.J.; Wen, X.P. Proteomic analyses provide new insights into the responses of *Pinus massoniana* seedlings to phosphorus deficiency. *Proteomics* 2016, 16, 504–515. [CrossRef]

43. Tran, H.T.; Plaxton, W.C. Proteomic analysis of alterations in the secretome of *Arabidopsis thaliana* suspension cells subjected to nutritional phosphorus deficiency. *Proteomics* 2008, 8, 4317–4326. [CrossRef]

44. Mehta, D.; Ghahremani, M.; Pérez Fernández, M.; Tan, M.; Schläpfer, P.; Plaxton, W.C.; Uhrig, R.G. Phosphate and phosphate have a differential impact on the proteome and phosphoproteome of *Arabidopsis thaliana* suspension cell cultures. *Plant J.* 2021, 105, 924–941. [CrossRef]

45. Uzokwe, V.N.E.; Asafo-Adjei, B.; Fawole, I.; Abaidoo, R.; Odeh, I.O.A.; Ojo, D.K.; Dashiell, K.; Sanginga, N. Generation mean analysis of phosphorus-use efficiency in freely nodulating soybean crosses grown in low-phosphorus soil. *Plant Breed.* 2017, 136, 139–146. [CrossRef]

46. Sims, L.; Pastor, J.; Lee, T.; Dewey, B. Nitrogen, phosphorus and light effects on growth and allocation of biomass and nutrients in wild rice. *Oecologia* 2012, 170, 65–76. [CrossRef] [PubMed]

47. Tian, J.; Wang, C.; Zhang, Q.; He, X.; Whelan, J.; Shou, H. Overexpression of *OsPAP10a*, a root-associated acid phosphatase, increased extracellular organic phosphorus utilization in rice. *J. Integr. Plant Biol.* 2012, 54, 631–639. [CrossRef]

48. Liang, C.; Wang, J.; Zhao, J.; Tian, J.; Liao, H. Control of phosphate homeostasis through gene regulation in crops. *Curr. Opin. Plant Biol.* 2014, 21, 59–66. [CrossRef] [PubMed]

49. Dissanayaka, D.M.S.B.; Nishida, S.; Tawaraya, K.; Wasaki, J. Organ-specific allocation pattern of acquired phosphorus and dry matter in two rice genotypes with contrasting tolerance to phosphorus deficiency. *Soil Sci. Plant Nutr.* 2018, 64, 282–290. [CrossRef]

50. Lopez-Bucio, J.; Cruz-Ramirez, A.; Herrera-Estrella, L. The role of nutrient availability in regulating root architecture. *Curr. Opin. Plant Biol.* 2003, 6, 280–287. [CrossRef] [PubMed]

51. Peret, B.; Desnos, T.; Jost, R.; Kanno, S.; Berkowitz, O.; Nussaume, L. Root architecture responses: In search of phosphate. *Plant Physiol.* 2014, 166, 1713–1723. [CrossRef]

52. Horsham, V. Proteoid root morphology and function in *lupinus albus*. *Plant Soil* 1981, 60, 143–147.

53. Neumann, G.; Massonneau, A.; Langlade, N.; Dinkelaker, B.; Hengeler, C.; Roemheld, V.; Martinoia, E. Physiological aspects of cluster root function and development in phosphorus-deficient white lupin (*Lupinus albus* L.). *Ann. Bot.* 2000, 85, 909–919. [CrossRef]

54. Lambers, H.; Finnegan, P.M.; Laliberte, E.; Pearse, S.J.; Ryan, M.H.; Shane, M.W.; Veneklaas, E.J. Update on phosphorus nutrition of proteaceae in severely phosphorus-impoverished soils: Are there lessons to be learned for future crops? *Plant Physiol.* 2011, 156, 1058–1066. [CrossRef] [PubMed]

55. Chen, Z.C.; Liao, H. Organic acid anions: An effective defensive weapon for plants against aluminum toxicity and phosphorus deficiency in acidic soils. *J. Genet. Genom.* 2016, 43, 631–638. [CrossRef] [PubMed]

56. Dissanayaka, D.; Plaxton, W.C.; Lambers, H.; Siebers, M.; Marambe, B.; Wasaki, J. Molecular mechanisms underpinning phosphorus-use efficiency in rice. *Plant Cell Environ.* 2018, 41, 1483–1496. [CrossRef] [PubMed]

57. Taraafdar, J.C.; Marchner, H. Efficiency of VAM hyphae in utilisation of organic phosphorus by wheat plants. *Soil Sci. Plant Nutr.* 1994, 40, 593–600. [CrossRef]

58. Sa, T.M.; Israel, D.W. Energy status and functioning of phosphorus-deficient soybean nodules. *Plant Physiol.* 1991, 97, 928–935. [CrossRef]

59. Drevon, J.; Hartwig, U.A. Phosphorus deficiency increases the argon-induced decline of nodule nitrogenase activity in soybean and alfalfa. *Planta* 1997, 201, 463–469. [CrossRef]

60. Hodge, A.; Berta, G.; Doussan, C.; Merchan, F.; Crespi, M. Plant root growth, architecture and function. *Plant Soil* 2009, 321, 153–187. [CrossRef]

61. Hiruma, K.; Gerlach, N.; Sacristan, S.; Nakano, R.T.; Hacquard, S.; Kracher, B.; Neumann, U.; Ramirez, D.; Bucher, M.; O’Connell, R.J.; et al. Root endophyte *colletotrichum tofieldiae* confers plant fitness benefits that are phosphate status dependent. *Cell* 2016, 165, 464–474. [CrossRef]

62. Umehara, M.; Hanada, A.; Magome, H.; Takeda-Kamiya, N.; Yamaguchi, S. Contribution of strigolactones to the inhibition of tiller bud outgrowth under phosphorus deficiency in rice. *Plant Cell Physiol.* 2010, 51, 1118–1126. [CrossRef]

63. Mghase, J.J.; Shiwachi, H.; Takahashi, H.; Irie, K. Nutrient deficiencies and their symptoms in upland rice. *J. ISSAAS* 2011, 17, 59–67.

64. Ruan, W.; Guo, M.; Xu, L.; Wang, X.; Zhao, H.; Wang, J.; Yi, K. An SPX-RLI1 module regulates leaf inclination in response to phosphate availability in rice. *Plant Cell* 2018, 30, 853–870. [CrossRef]

65. Mo, X.; Zhang, M.; Zhang, Z.; Lu, X.; Liang, C.; Tian, J. Phosphate (Pi) starvation up-regulated *GmCSN5A/B* participates in anthocyanin synthesis in Soybean (*Glycine max*) dependent on Pi availability. *Int. J. Mol. Sci.* 2021, 22, 12348. [CrossRef] [PubMed]

66. Ticconi, C.A.; Abel, S. Short on phosphate: Plant surveillance and countermeasures. *Trends Plant Sci.* 2004, 9, 548–555. [CrossRef] [PubMed]

67. Yuan, H.; Liu, D. Signaling components involved in plant responses to phosphate starvation. *J. Integr. Plant Biol.* 2008, 50, 849–859. [CrossRef]
68. Liang, C.; Tian, J.; Liao, H. Proteomics dissection of plant responses to mineral nutrient deficiency. *Proteomics* 2013, 13, 624–636. [CrossRef] [PubMed]

69. Motte, H.; Vanneste, S.; Beeckman, T. Molecular and environmental regulation of root development. *Annu. Rev. Plant Biol.* 2019, 70, 465–488. [CrossRef]

70. Noctor, G.; Foyer, C.H. Homeostasis of adenylate status during photosynthesis in a fluctuating environment. *J. Exp. Bot.* 2000, 51, 347–356. [CrossRef]

71. Rochaix, J.D. Role of thylakoid protein kinases in photosynthetic acclimation. *FEBS Lett.* 2007, 581, 2768–2775. [CrossRef]

72. Carmo-Silva, E.; Scales, J.C.; Madgwick, P.J.; Parry, M.A. Optimizing Rubisco and its regulation for greater resource use efficiency. *Plant Cell Environ.* 2015, 38, 1817–1832. [CrossRef] [PubMed]

73. Nakamura, Y. Phosphate starvation and membrane lipid remodeling in seed plants. *Prog. Lipid Res.* 2013, 52, 43–50. [CrossRef] [PubMed]

74. Preiss, J. Bacterial glycogen synthesis and its regulation. *Annu. Rev. Microbiol.* 1984, 38, 419–458. [CrossRef] [PubMed]

75. Carrera, D.N.I.; Oddsson, S.; Grossmann, J.; Trachsel, C.; Streb, S. Comparative proteomic analysis of plant acclimation to six different long-term environmental changes. *Plant Cell Physiol.* 2018, 59, 510–526. [CrossRef] [PubMed]

76. Jiang, C.; Gao, X.; Liao, L.; Harberd, N.P.; Fu, X. Phosphate starvation root architecture and anthocyanin accumulation responses are modulated by the Gibberellin-DELLA signaling pathway in Arabidopsis. *Plant Physiol.* 2007, 145, 1460–1470. [CrossRef]

77. Yin, Y.; Borges, G.; Sakuta, M.; Crozier, A.; Ashihara, H. Effect of phosphate deficiency on the content and biosynthesis of anthocyanins and the expression of related genes in suspension-cultured grape (*Vitis* sp.) cells. *Plant Physiol. Biochem.* 2012, 55, 77–84. [CrossRef]

78. Manna, M.; Islam, T.; Kaul, T.; Reddy, C.S.; Fartyal, D.; James, D.; Reddy, M.K. A comparative study of effects of increasing concentrations of phosphate and phosphite on rice seedlings. *Acta Physiol. Plant* 2015, 37, 258. [CrossRef]

79. Tominaga-Wada, R.; Masakane, A.; Wada, T. Effect of phosphate deficiency-induced anthocyanin accumulation on the expression of *Solanum lycopersicum* GLABRA3 (GL3) in tomato. *Plant Signal. Behav.* 2016, 13, e1477907. [CrossRef]

80. Sakuta, M. Diversity in plant red pigments: Anthocyanins and betacyanins. *Plant Biotechnol. Rep.* 2014, 8, 37–48. [CrossRef]

81. Lutts, S.; Hausman, J.F.; Quinet, M.; Lett, J.F.; Vavasseur, A.; Pariza, M.; Prasad, M.N.V., Eds.; Springer: New York, NY, USA, 2013; pp. 315–353.

82. Niu, Y.F.; Chai, R.S.; Jin, G.L.; Wang, H.; Tang, C.X.; Zhang, Y.S. Responses of root architecture development to low phosphorus availability: A review. *Ann. Bot.* 2011, 112, 391–408. [CrossRef] [PubMed]

83. Borch, K.; Poulsen, M.; Häussler, I. Polyamines and their roles in the alleviation of ion toxicities in Plants. In *Ecophysiology and Responses of Plants under Salt Stress*; Parvaiz, A., Azooz, M.M., Prasad, M.N.V., Eds.; Springer: New York, NY, USA, 2013; pp. 315–353.

84. Zhang, Y.J.; Lynch, J.P.; Brown, K.M. Ethylene and phosphorus availability have interacting yet distinct effects on root hair development. *J. Exp. Bot.* 2003, 54, 2351–2361. [CrossRef] [PubMed]

85. Talboys, P.J.; Healey, J.R.; Withers, P.J.; Jones, D.L. Phosphate depletion modulates auxin transport in *Triticum aestivum* leading to altered root branching. *J. Exp. Bot.* 2014, 65, 5023–5032. [CrossRef] [PubMed]

86. Khan, G.A.; Vogiatzaki, E.; Glauser, G.; Poirier, Y. Phosphate depletion stress interacts with the auxin and jasmonate pathways to regulate root development. *Plant Signal. Behav.* 2014, 9, 632–644. [CrossRef] [PubMed]

87. Song, L.; Yu, H.; Dong, J.; Che, X.; Jiao, Y.; Liu, D. The molecular mechanism of ethylene-mediated root hair development induced by phosphate starvation. *PloS Genet.* 2012, 12, e1006194. [CrossRef] [PubMed]

88. Zhu, X.F.; Zhu, C.Q.; Zhao, X.S.; Zheng, S.J.; Shen, R.F. Ethylene is involved in root phosphorus remobilization in rice (*Orzya sativa*) by regulating cell-wall pectin and enhancing phosphate translocation to shoots. *Ann. Bot.* 2016, 118, 645–653. [CrossRef]

89. Park, C.H.; Roh, J.; Youn, J.; Son, S.; Park, J.H.; Kim, S.Y.; Kim, T.; Kim, S. Arabidopsis ACC oxidase 1 coordinated by multiple signals mediates ethylene biosynthesis and is involved in root development. *Mol. Cells* 2018, 41, 923–932. [PubMed]

90. Bhoraskar, V.; Giri, J.; Pandey, B.K.; Giehl, R.F.H.; Hartmann, A.; Truskina, J.; Leftley, N.; Hanlon, M.; Swarup, K.; et al. Auxin regulation of cytokinin biosynthesis in Arabidopsis *thaliana*: A factor of potential importance for auxin-cytokinin-regulated development. *Proc. Natl. Acad. Sci. USA* 2004, 101, 8039–8044. [CrossRef] [PubMed]

91. Su, Y.; Li, M.; Guo, L.; Wang, X. Different effects of phospholipase Dc2 and non-specific phospholipase C4 on lipid remodeling and root hair growth in Arabidopsis thaliana to phosphate deficiency. *Plant J.* 2018, 94, 315–326. [CrossRef] [PubMed]

92. Wang, J.; Pei, L.; Jin, Z.; Zhang, K.; Zhang, J. Overexpression of the protein phosphatase 2A regulatory subunit a gene ZmPP2AA1 improves low phosphate tolerance by remodeling the root system architecture of maize. *PloS ONE* 2017, 12, e0176538. [CrossRef]

93. Chen, Y.F.; Li, L.Q.; Xu, Q.; Kong, Y.H.; Wang, H.; Wu, W.H. The WRKY6 transcription factor modulates PHOSPHATE1 expression in response to low Pi stress in Arabidopsis. *Plant Cell* 2009, 21, 3554–3566. [CrossRef]

94. Nordstrom, A.; Tarkowski, P.; Tarkowska, D.; Norbaek, R.; Astot, C.; Dolezal, K.; Sandberg, G. Auxin regulation of cytokinin biosynthesis in Arabidopsis *thaliana*: A factor of potential importance for auxin-cytokinin-regulated development. *Proc. Natl. Acad. Sci. USA* 2004, 101, 8039–8044. [CrossRef] [PubMed]

95. Laplaze, L.; Benkova, E.; Casimiro, I.; Maes, L.; Vanneste, S.; Swarup, R.; Weijers, D.; Calvo, V.; Parizot, B.; Herrera-Rodriguez, M.B.; et al. Cytokins act directly on lateral root founder cells to inhibit root initiation. *Plant Cell* 2007, 19, 3889–3900. [CrossRef] [PubMed]
96. Ruzicka, K.; Ljung, K.; Vanneste, S.; Podhorska, R.; Beeckman, T.; Friml, J.; Benkova, E. Ethylene regulates root growth through effects on auxin biosynthesis and transport-dependent auxin distribution. *Plant Cell* 2007, 19, 2197–2212. [CrossRef]

97. Swarup, R.; Perry, P.; Hagenbeek, D.; Streeten, D.V.D.; Beemster, G.T.S.; Sandberg, G.; Bhalarao, R.; Ljung, K.; Bennett, M.J. Ethylene upregulates auxin biosynthesis in *Arabidopsis* seedlings to enhance inhibition of root cell elongation. *Plant Cell* 2007, 19, 2186–2196. [CrossRef] [PubMed]

98. Stepanova, A.N.; Yun, J.; Likhacheva, A.V.; Alonso, J.M. Multilevel interactions between ethylene and auxin in *Arabidopsis* roots. *Plant Cell* 2007, 19, 2169–2185. [CrossRef] [PubMed]

99. Guo, W.; Zhao, J.; Li, X.; Qin, L.; Yan, X.; Liao, H. A soybean beta-expansin gene GmEXPB2 intrinsically involved in root system architecture responses to abiotic stresses. *Plant J.* 2011, 66, 541–552. [CrossRef]

100. Lopez-Arredondo, D.L.; Leyva-Gonzalez, M.A.; Gonzalez-Morales, S.L.; Lopez-Bucio, J.; Herrera-Estrella, L. Phosphate nutrition: Improving low-phosphate tolerance in crops. *Annu. Rev. Plant Biol.* 2014, 65, 95–123. [CrossRef] [PubMed]

101. Bommer, U.A.; Thiele, B.J. The translationally controlled tumour protein (TCTP). *Int. J. Biochem. Cell Biol.* 2004, 36, 379–385. [CrossRef]

102. Tao, J.J.; Cao, Y.R.; Chen, H.W.; Wei, W.; Li, Q.T.; Ma, B.; Zhang, W.K.; Chen, S.Y.; Zhang, J.S. Tobacco translationally controlled tumour protein interacts with ethylene receptor tobacco histidine kinase1 and enhances plant growth through promotion of cell proliferation. *Plant Physiol.* 2015, 169, 96–114. [CrossRef] [PubMed]

103. Branco, R.; Masle, J. Systemic signalling through translationally controlled tumour protein controls lateral root formation in *Arabidopsis*. *J. Exp. Bot.* 2019, 70, 3927–3940. [CrossRef] [PubMed]

104. Huang, G.; Liang, W.; Sturrock, C.J.; Pandey, B.K.; Giri, J.; Mairhofer, S.; Wang, D.; Muller, L.; Tang, H.; York, L.M.; et al. Rice actin binding protein RMD controls crown root angle in response to external phosphate. *Nat. Commun.* 2018, 9, 2346. [CrossRef]

105. Chai, Y.N.; Schachtman, D.P. Root exudates impact plant performance under abiotic stress. *Trends Plant Sci.* 2021, 27, 80–91. [CrossRef]

106. Tian, J.; Wang, X.; Tong, Y.; Chen, X.; Liao, H. Bioengineering and management for efficient phosphorus utilization in crops and pastures. *Curr. Opin. Biotechnol.* 2012, 23, 866–871. [CrossRef]

107. Koyama, H.; Kawamura, A.; Kihara, T.; Hara, T.; Takita, E.; Shibata, D. Overexpression of mitochondrial citrate synthase in *Arabidopsis thaliana* improved growth on a phosphorus-limited soil. *Plant Cell Physiol.* 2000, 41, 1030–1037. [CrossRef] [PubMed]

108. Wang, L.; Liu, D. Functions and regulation of phosphate starvation-induced secreted acid phosphatases in higher plants. *Plant Sci.* 2018, 281, 108–116. [CrossRef]

109. Liu, P.; Cai, Z.; Chen, Z.; Mo, X.; Ding, X.; Liang, C.; Liu, G.; Tian, J. A root-associated purple acid phosphatase, SgPAP23, mediates extracellular phytate-P utilization in *Stylosanthes guianensis*. *Plant Cell Environ.* 2018, 41, 2821–2834. [CrossRef]

110. Zhu, S.; Chen, M.; Liang, C.; Xue, Y.; Lin, S.; Tian, J. Characterization of purple acid phosphatase family and functional analysis of GmPAP7a/b involved in extracellular ATP utilization in soybean. *Front. Plant Sci.* 2020, 11, 661. [CrossRef] [PubMed]

111. Bozzo, G.G.; Dunn, E.L.; Plaxton, W.C. Differential synthesis of phosphate-starvation inducible purple acid phosphatase isozymes in tomato (*Lycopersicon esculentum*) suspension cells and seedlings. *Plant Cell Environ.* 2006, 29, 303–313. [CrossRef] [PubMed]

112. Tran, H.T.; Qian, W.; Hurley, B.A.; She, Y.M.; Wang, D.; Plaxton, W.C. Biochemical and molecular characterization of AtPAP12 and AtPAP26: The predominant purple acid phosphatase isozymes secreted by phosphate-starved *Arabidopsis thaliana*. *Plant Cell Environ.* 2010, 33, 1789–1803. [CrossRef] [PubMed]

113. Robinson, W.D.; Park, J.; Tran, H.T.; Del, Y.H.; Ying, S.; Zins, J.L.; Patel, K.; McKnight, T.D.; Plaxton, W.C. The secreted purple acid phosphatase isozymes AtPAP12 and AtPAP26 play a pivotal role in extracellular phosphate-scavenging by *Arabidopsis thaliana*. *J. Exp. Bot.* 2012, 63, 6531–6542. [CrossRef]

114. Wu, W.; Zhu, S.; Chen, Q.; Lin, Y.; Tian, J.; Liang, C. Cell wall proteins play critical roles in plant adaptation to phosphorus deficiency. *Int. J. Mol. Sci.* 2019, 20, 5259. [CrossRef]

115. Brown, L.K.; George, T.S.; Thompson, J.A.; Wright, G.; Lyon, J.; Dupuy, L.; Hubbard, S.F.; White, P.J. What are the implications of variation in root hair length on tolerance to phosphorus deficiency in combination with water stress in barley (*Hordeum vulgare*)? *Ann. Bot.* 2012, 110, 319–328. [CrossRef] [PubMed]

116. Qin, L.; Jiang, H.; Tian, J.; Zhao, J.; Liao, H. Rhizobia enhance acquisition of phosphorus from different sources by soybean plants. *Plant Soil* 2011, 349, 25–36. [CrossRef]

117. Chen, L.; Qin, L.; Zhou, L.; Li, X.; Chen, Z.; Sun, L.; Wang, W.; Lin, Z.; Zhao, J.; Yamaji, N.; et al. A node-localized phosphate transporter *GmPT7* plays an important role in enhancing symbiotic N2 fixation and yield in soybean. *New Phytol.* 2018, 221, 2013–2025. [CrossRef] [PubMed]

118. Qin, L.; Zhao, J.; Tian, J.; Chen, L.; Sun, Z.; Guo, Y.; Lu, X.; Gu, M.; Xu, G.; Liao, H. The high-affinity phosphate transporter *GmPT5* regulates phosphate transport to nodules and nodule formation in soybean. *Plant Physiol.* 2012, 159, 1634–1643. [CrossRef]

119. Zhu, S.; Chen, Z.; Xie, B.; Guo, Q.; Chen, M.; Liang, C.; Bai, Z.; Wang, X.; Wang, H.; Liao, H.; et al. A phosphate starvation responsive malate dehydrogenase, GmMDH12 mediates malate synthesis and nodule size in soybean (*Glycine max*). *Environ. Exp. Bot.* 2021, 189, 105600. [CrossRef]

120. Pan, W.; Wu, Y.; Xie, Q. Regulation of ubiquitination is central to the phosphate starvation response. *Trends Plant Sci.* 2019, 24, 755–769. [CrossRef]

121. Chen, J.; Wang, Y.; Wang, F.; Yang, J.; Gao, M.; Li, C.; Liu, Y.; Liu, Y.; Yamaji, N.; Ma, J.F.; et al. The rice CK2 kinase regulates trafficking of phosphate transporters in response to phosphate levels. *Plant Cell* 2015, 27, 711–723. [CrossRef]
122. Gelova, Z.; Gallei, M.; Pernisova, M.; Brunoud, G.; Zhang, X.; Glanc, M.; Li, L.; Michalko, J.; Pavlovicova, Z.; Verstraeten, I.; et al. Developmental roles of auxin binding protein 1 in Arabidopsis thaliana. Plant Sci. 2021, 303, 110750. [CrossRef] [PubMed]

123. Washburn, M.; Wolters, D.; Yates, J. Large-scale analysis of the yeast proteome by multidimensional protein identification technology. Nat. Biotechnol. 2001, 19, 242–247. [CrossRef]

124. Unwin, R.; Pierce, A.; Watson, R.; Sternberg, D.; Whetton, A. Quantitative proteomic analysis using isobaric protein tags enables rapid comparison of changes in transcript and protein levels in transformed cells. Mol. Cell. Proteom. 2005, 4, 924–935. [CrossRef]

125. Thompson, A.; Schafer, J.; Kuhn, K.; Kienle, S.; Schwarz, J.; Schmidt, G.; Neumann, T.; Hamon, C. Tandem mass tags: A novel quantification strategy for comparative analysis of complex protein mixtures by MS/MS. Anal. Chem. 2003, 75, 1895–1904. [CrossRef]

126. Ong, S.; Blagoev, B.; Kratchmarova, I.; Kristensen, D.; Steen, H.; Pandey, A.; Mann, M. Stable isotope labeling by amino acids in cell culture, SILAC, as a simple and accurate approach to expression proteomics. Mol. Cell. Proteom. 2002, 1, 376–386. [CrossRef] [PubMed]

127. Messner, C.; Demichev, V.; Bloomfield, N.; Yu, J.; White, M.; Kreidl, M.; Egger, A.; Freiwald, A.; Ivosev, G.; Wasim, F.; et al. Ultra-fast proteomics with scanning SWATH. Nat. Biotechnol. 2021, 39, 846–854. [CrossRef] [PubMed]

128. Zhang, H.; Liu, P.; Guo, T.; Zhao, H.; Bensaddek, D.; Aebersold, R.; Xiong, L. Arabidopsis proteome and the mass spectral assay library. Sci. Data 2019, 6, 278. [CrossRef]

129. Liu, S.; Li, Z.; Yu, B.; Wang, S.; Shen, Y.; Cong, H. Recent advances on protein separation and purification methods. Adv. Colloid Interface Sci. 2020, 284, 102254. [CrossRef] [PubMed]