

Article

Not peer-reviewed version

Light and Dark Conditions Reveal Distinct Components of the Phosphate Starvation Response in Soybean Roots

[Anamta Shaikh](#) , Izabel Thurber , Nikko R. M. Sacramento , Jennifer Bravo , Lynne Viall , Reemaben Maniyar , [Maria Muhammad Ali](#) , [Jennifer Nguyen](#) , Kristine Tran , [Geronimo Parra](#) , [Kayla Magdaleno](#) , [Trinidad Cruz](#) , [Claudia Uhde-Stone](#) *

Posted Date: 24 December 2025

doi: 10.20944/preprints202512.2167.v1

Keywords: carbon availability; *Glycine max*; local P_i signaling; Nanopore sequencing; phosphate starvation; RNA-seq; sucrose signaling; systemic P_i signaling; transcriptomics



Preprints.org is a free multidisciplinary platform providing preprint service that is dedicated to making early versions of research outputs permanently available and citable. Preprints posted at Preprints.org appear in Web of Science, Crossref, Google Scholar, Scilit, Europe PMC.

Copyright: This open access article is published under a [Creative Commons CC BY 4.0 license](#), which permit the free download, distribution, and reuse, provided that the author and preprint are cited in any reuse.

Disclaimer/Publisher's Note: The statements, opinions, and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions, or products referred to in the content.

Article

Light and Dark Conditions Reveal Distinct Components of the Phosphate Starvation Response in Soybean Roots

Anamta Shaikh, Izabel Thurber, Nikko R. M. Sacramento, Jennifer Bravo, Lynne Viall, Reemaben Maniyar, Maria Muhammad Ali, Jennifer Nguyen, Kristine Tran, Geronimo Parra, Kayla Magdaleno, Trinidad Cruz and Claudia Uhde-Stone *

California State University, East Bay

* Correspondence: Claudia.stone@csueastbay.edu

Abstract

Phosphate (P_i) deficiency causes broad transcriptional changes that enhance P_i uptake and utilization. Because sucrose transport from shoot to root mediates long-distance P_i -deficiency signaling, we asked whether soybean can still respond to $-P$ when sucrose supply is restricted. To limit sucrose availability, we exposed hydroponically grown soybean plants to $+P$ or $-P$ conditions for 30 h under light or darkness, with darkness suppressing photosynthetic carbon production. Root transcriptomes were profiled using Oxford Nanopore cDNA sequencing. Across four biological replicates per treatment, ~90 million reads were generated, with >90% mapping to ~25,300 expressed genes (~54% of annotated coding genes). Principal component analysis showed that light accounted for most of the variance, while P_i status contributed a smaller component. Under light, P_i deficiency activated a strong transcriptional program, including canonical phosphate-starvation markers such as *PHT1*, *PHO1* and *PTEN2α*. In darkness, however, only a small number of genes were induced, including a MYB-domain transcription factor, and none overlapped with the light-responsive set. Together, these findings suggest that light is required for broad transcriptional responses to P_i deficiency, whereas a local P_i -responsive program becomes detectable only when systemic sucrose signaling is suppressed.

Keywords: carbon availability; *Glycine max*; local P_i signaling; Nanopore sequencing; phosphate starvation; RNA-seq; sucrose signaling; systemic P_i signaling; transcriptomics

1. Introduction

Phosphorus (P) is an essential macronutrient for all living organisms. As a component of nucleic acids, phospholipids, and ATP, P supports the structural and energetic foundations of life and plays key roles in energy transfer, membrane stability, and signaling in plant cells [1]. Most P is taken up as inorganic phosphate (P_i), but in many soils P_i is tightly bound to minerals or organic matter, limiting its availability for uptake [1]. To sustain crop productivity, large amounts of P fertilizers are applied, most of it mined from non-renewable geological deposits formed over millions of years. However, intensive mining and fertilizer use have led to declining global reserves and to environmental pollution through runoff and soil erosion [2–4].

Improving plant P acquisition and utilization is therefore critical for sustainable agriculture. Soybean (*Glycine max*), a major source of plant-based protein and oil [5], is particularly relevant because of its global importance and its strong dependence on P to sustain nitrogen fixation and yield. In soybean, progress toward improving P efficiency has come from quantitative trait loci (QTL) analyses [6] and molecular and transcriptomic studies [7–9]. These studies have revealed diverse responses to P deficiency, including changes in root architecture and exudation [10], altered leaf P-allocation, and improved P-use efficiency [8].

Under P deficiency, root system architecture changes, typically showing shorter primary roots and more lateral roots and root hairs, which increase the root surface area exposed to soil P_i [10,11]. Roots also secrete organic acids that dissolve P_i and release phosphatases and nucleases to free P_i from organic compounds [1,10,12]. At the cellular level, high-affinity P_i transporters are upregulated to improve uptake efficiency [13–15], while membrane lipid remodeling conserves P by shifting from phospholipids to P_i -free membrane lipids [16]. Central carbon metabolism also becomes more P_i -efficient by favoring PP_i -dependent reactions that bypass ATP [17,18]. Although P deficiency is defined at the whole-plant level, its transcriptional and metabolic responses are mediated by changes in cellular P_i availability and P_i -dependent signaling pathways.

These metabolic responses are integrated with signaling networks that coordinate P_i perception and redistribution across the plant. SPX-domain proteins and PHR transcription factors form a core regulatory module [19]. Under P_i -sufficient conditions, SPX proteins bind inositol pyrophosphates and repress PHR activity; under P_i deficiency, this repression is relieved, allowing PHRs to activate P_i -responsive genes [20]. Soybean homologs such as GmPHR1, GmSPX1, and GmSPX3 modulate P_i uptake and homeostasis [21], while PHO1 family members export P_i into the xylem and contribute to whole-plant P_i distribution [22].

Long-distance communication between shoots and roots also plays a central role in coordinating P acquisition. Sucrose, the main product of photosynthesis, functions both as a carbon source and as a signal integrating plant carbon status with nutrient responses [23–25]. Under P_i deficiency, increased sucrose flow from leaves to roots stimulates the expression of P_i acquisition genes, including high-affinity transporters and acid phosphatases [26,27]. In soybean, sucrose can trigger rapid transcriptomic shifts within minutes, activating hormone, calcium, and ROS signaling before canonical P_i -starvation genes respond [28]. Other systemic signals, including auxin, ethylene, strigolactones, and microRNAs such as miR399 and miR827, further integrate carbon status with P_i allocation and root development [29–32]. However, the extent to which P_i -responsive transcription depends on photosynthetically derived sucrose, as opposed to sucrose-independent local P_i sensing, remains unclear.

In addition to systemic signaling, root tips and epidermal regions are also able to sense P_i status locally [33,34]. Because sucrose transport from shoots to roots depends on photosynthesis, comparing P_i -deficient plants grown in light versus darkness offers a practical way to probe the influence of carbon availability on P_i -responsive transcription. Darkness strongly reduces photosynthetic carbon production and sucrose transport from shoot to root but also induces broader metabolic and physiological changes. Thus, light–dark comparisons provide an imperfect but useful framework to explore how carbon availability shapes different components of the P_i -starvation response.

In this study, we used Oxford Nanopore cDNA sequencing to analyze root transcriptional responses to 30 hours of P_i deprivation under light and dark conditions in hydroponically grown soybean. Because sucrose transport from shoot to root depends on photosynthesis, comparing P_i -deficient plants grown in light versus darkness offers a practical way to probe the influence of carbon availability on P_i -responsive transcription. Consistent with this rationale, darkness restricts photosynthetic sucrose production and has been shown to reduce sucrose levels in roots [35], although additional metabolic and physiological effects of darkness are expected. Specifically, we sought to i) test sucrose's regulatory role in coordinating the phosphate starvation response (PSR) in soybean and ii) identify sucrose-independent components of the P_i deficiency transcriptome.

2. Results

2.1. Overview of Experimental Design and Data Quality

Soybean plants were grown hydroponically for two weeks and then exposed for 30 h to light or darkness under phosphate-sufficient (+P) or phosphate-deficient (–P) conditions (Figure 1a). Darkness prevents photosynthesis and limits shoot-to-root sucrose transport. Each treatment was performed with four biological replicates to ensure reproducibility across experiments.

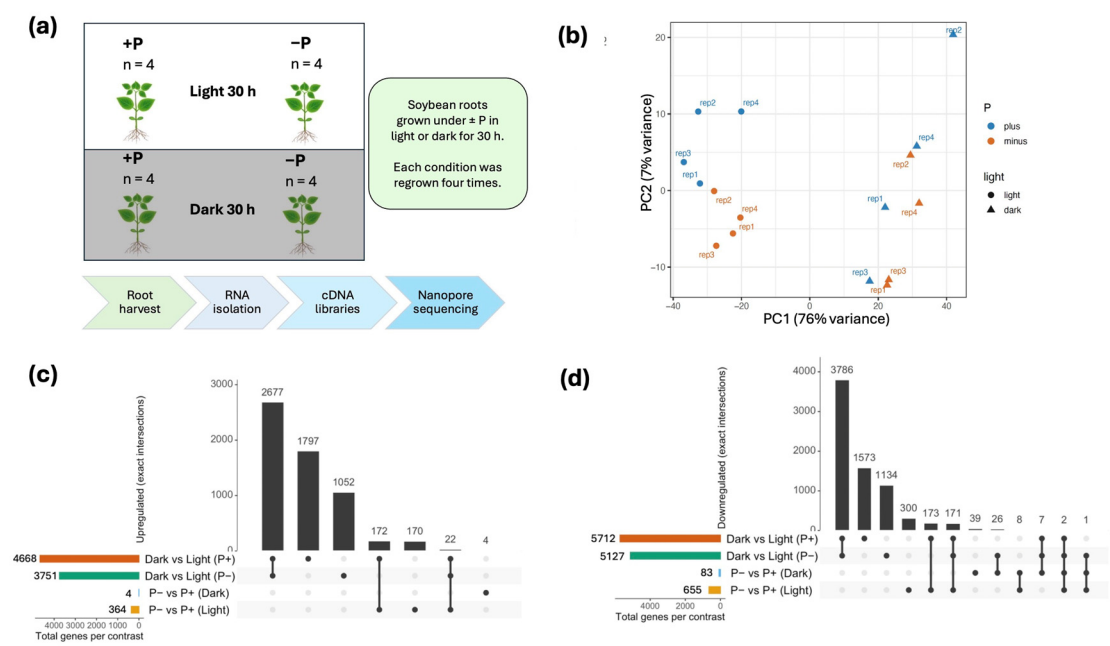


Figure 1. Experimental design and transcriptomic responses of soybean roots to 30 hours of phosphate deficiency (–P) under light and dark conditions. (a) Experimental setup. Fourteen-day-old hydroponically grown soybean plants were exposed for 30 h to light or darkness under phosphate-sufficient (+P) or phosphate-deficient (–P) conditions, with four independent biological replicates per treatment. (b) Principal component analysis (PCA) of variance-stabilized expression data. Samples separated strongly by light condition along PC1 (76% of variance), whereas P_i status contributed secondary variation along PC2 (7%). Each point represents one biological replicate, and replicate identifiers are shown to illustrate clustering within treatments. (c–d) UpSet plots showing differentially expressed genes (DEGs; adjusted p < 0.05, |log₂FC| > 1) that were upregulated (c) or downregulated (d) in the indicated contrasts. The majority of transcriptional differences occurred between light and dark treatments, while P_i availability produced clearer effects under light and only limited changes in darkness.

Oxford Nanopore cDNA sequencing generated 4.8–6.9 × 10⁶ passed reads per sample, with mean quality scores of Q10–11 and N50 values of ~800–1,000 bp (Table 1, see Table S1 for additional sequencing and mapping metrics). Across all samples, sequencing yielded ~90 million passed reads (mean 5.6 million per sample) with >90% mapping efficiency. After filtering out low-abundance genes (≥10 raw reads in ≥3 replicates), 25,348 genes were retained for downstream analyses. These represent ~54% of the 47,068 protein-coding genes annotated in the *Glycine max* v4.0 genome. Together, these results indicate that the sequencing depth and replicate consistency were adequate for capturing major transcriptional differences across light, dark, +P, and –P treatments.

Table 1. Summary of Oxford Nanopore cDNA sequencing and read-mapping metrics for soybean root transcriptomes across light and dark and phosphate treatments.

Condition	Passed reads (×10 ⁶)	Avg. read Quality (Q)	N50 (bp)	Mapped reads (%)
+P Light (n = 4)	6.9 ± 2.5	11 ± 0	1,034 ± 75	96.9 ± 0.5
–P Light (n = 4)	4.8 ± 1.9	10 ± 0	814 ± 96	93.4 ± 2.6

+P Dark (n = 4)	6.7 ± 2.2	10 ± 0	879 ± 113	95.6 ± 1.5
–P Dark (n = 4)	4.9 ± 1.1	11 ± 0	972 ± 103	96.8 ± 0.4

2.2. RNA-Seq Reveals Major Effects of Light and Minor Effects of P_i Deficiency

Principal component analysis (PCA; Figure 1b) showed that light condition was the primary source of transcriptomic variation: PC1 explained 76% of the variance and separated all light-grown samples from dark-grown samples. Phosphate status contributed a smaller component of variation, captured along PC2 (7%). Under light, +P and –P samples separated clearly, whereas these groups overlapped more extensively in darkness, suggesting that transcriptomic responses to P_i deficiency were reduced or less distinct when plants were grown without light.

Treatment-specific differences were examined using UpSet plots (Figure 1c–d). Consistent with the PCA, the number of differentially expressed genes (DEGs) was substantially higher between light and dark conditions than between +P and –P conditions within either light or dark. However, despite the strong light–dark effect, a distinct set of >300 genes was upregulated under –P in light-grown roots. In contrast, only four genes passed significance thresholds for –P upregulation in darkness, with 13 emerging under relaxed criteria (see Section 3.2).

This sharp contrast indicates that P_i-deficiency transcriptional responses are markedly attenuated in darkness. Because darkness restricts photosynthetic carbon supply and also alters broader metabolic and hormonal processes, the reduced –P response likely reflects a combination of limited carbon availability and additional physiological changes associated with dark-grown roots.

2.3. Light Enables Genome-Wide Activation of the Phosphate Starvation Program

To visualize transcriptional responses to P_i deficiency under each light condition, we compared –P and +P samples using MA and volcano plots (Figure 2). MA plots show overall fold-change patterns across expression levels, while volcano plots emphasize genes with larger expression shifts and stronger statistical support. An exploratory threshold of adjusted p < 0.1 was used in MA plots to highlight subtle changes, whereas volcano plots apply a more stringent adjusted p < 0.05 cutoff.

Under light, –P treatment induced extensive transcriptional changes, with hundreds of genes significantly up- or downregulated across a wide dynamic range. This pattern is consistent with activation of the canonical phosphate starvation response (PSR) in roots under light conditions. In darkness, however, only a small number of genes passed significance thresholds, suggesting that transcriptomic responses to –P are markedly reduced under dark conditions.

Because darkness restricts photosynthetic sucrose production and alters broader aspects of root physiology, the attenuated –P response likely reflects a combination of reduced carbon supply and other dark-associated changes. Despite these factors, the strong contrast between light and dark conditions is clear: light-grown roots show robust –P-induced transcriptional reprogramming, whereas dark-grown roots display a much smaller response.

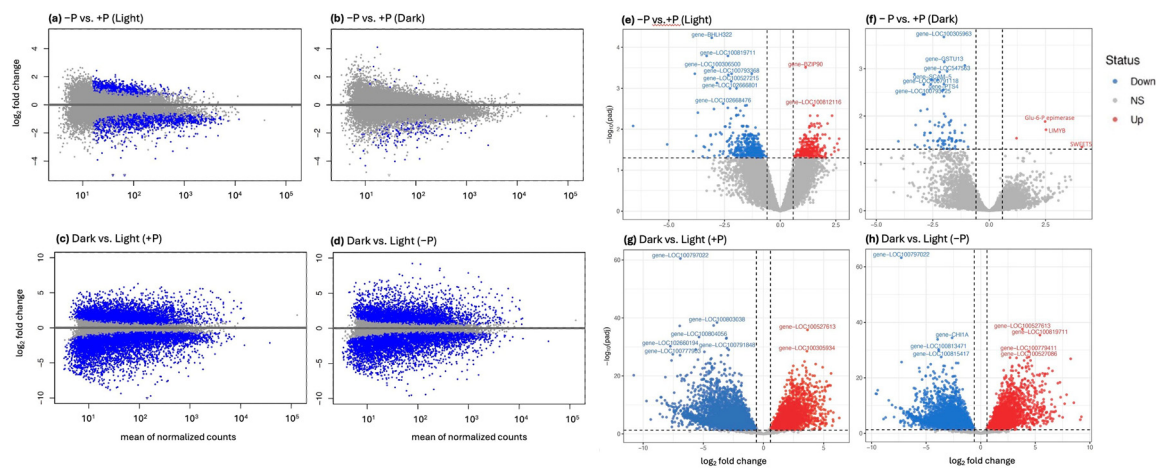


Figure 2. MA and volcano plots illustrating transcriptional responses to Pi deficiency under light and dark conditions. (a–d) MA plots showing the relationship between mean normalized counts and log₂ fold change for each contrast. Blue points indicate differentially expressed genes (DEGs) using an exploratory threshold of adjusted $p < 0.1$ and $|\log_2FC| > 1$. Contrasts shown are: (a) –P vs. +P in light, (b) –P vs. +P in darkness, (c) dark vs. light under +P, and (d) dark vs. light under –P conditions. These plots highlight broad transcriptional responses to –P under light and more limited changes in darkness. (e–h) Volcano plots depicting the magnitude and statistical support of expression changes for the same contrasts, using a more stringent criterion of adjusted $p < 0.05$ and $|\log_2FC| > 1$. Red and blue points represent significantly up- and downregulated genes, respectively, and gray points indicate genes not passing significance thresholds. Together, the MA and volcano plots show strong –P-associated transcriptional changes in light-grown roots, whereas –P responses in dark-grown roots are fewer and generally lower in magnitude.

2.4. Canonical Phosphate Starvation Response Genes Are Induced Only in Light

To examine how transcriptional responses to Pi deficiency differ between light and dark conditions, we compared the genes most strongly affected by –P in each treatment (Figures 3 and 4). Under light, roots showed clear activation of well-established phosphate-starvation markers. Among the top 50 upregulated genes were *PHT1;14* (high-affinity Pi transporter) and two *PTEN2α* (phosphatidylinositol 3,4,5-trisphosphate 3-phosphatase) genes. Additional canonical markers were present within the broader set of ~300 upregulated –P genes, including *PHO1*, *SAP* (secreted acid phosphatase), and *PIP5K* (phosphatidylinositol 4-phosphate 5-kinase). Together, these patterns indicate a coordinated transcriptional response to Pi deficiency under light.

To assess whether the lack of –P induction in darkness reflected biological suppression or low expression, we examined normalized counts for select canonical PSR genes (Table 2). Canonical PSR markers such as *PHT1*, *PHO1*, and *PTEN2α* showed robust induction under –P under light, but only small or no changes under –P in darkness. Importantly, all canonical PSR genes examined in Table 2 exceeded the ≥10-read threshold in at least 3 replicates, including dark-grown roots. For example, *PHO1* showed 24 normalized counts in +P dark roots and 32 under –P dark, while *PHT1* showed 944 and 807 normalized counts, respectively. These values demonstrate that canonical PSR genes are expressed at substantial levels even in darkness.

Table 2. Normalized expression of canonical Pi-responsive genes under light and dark conditions. DESeq2-normalized counts (mean of four biological replicates) are shown for representative phosphate-starvation response (PSR) markers. Canonical PSR genes show strong induction under -P in the light but little or no induction under -P in the dark, despite moderate to high expression levels under all conditions.

Canonical PSR markers*	Light (+P)	Light (-P)	Dark (+P)	Dark (-P)
<i>PHO1</i>	28.1	68.7	23.6	31.7
<i>PHT1</i>	537.5	1111.0	943.6	806.5
<i>PTEN2α-1</i>	38.6	84.0	111.8	97.5
<i>PTEN2α-2</i>	76.9	155.0	172.2	184.9
<i>SAP</i>	8.9	24.3	53.9	71.4

**PHO1*, phosphate transporter 1; *PHT1*, high-affinity phosphate transporter; *PTEN2α-1* and *PTEN2α-2*, paralog phosphatidylinositol triphosphate phosphatases; *SAP*, secreted acid phosphatase.

Only four genes passed significance thresholds for upregulation in -P in the dark, including an L10-interacting MYB domain-containing transcription factor, while numerous metabolic and stress-associated genes were downregulated. This reduced response is consistent with a general attenuation of Pi-deficiency transcriptional changes in dark-grown roots.

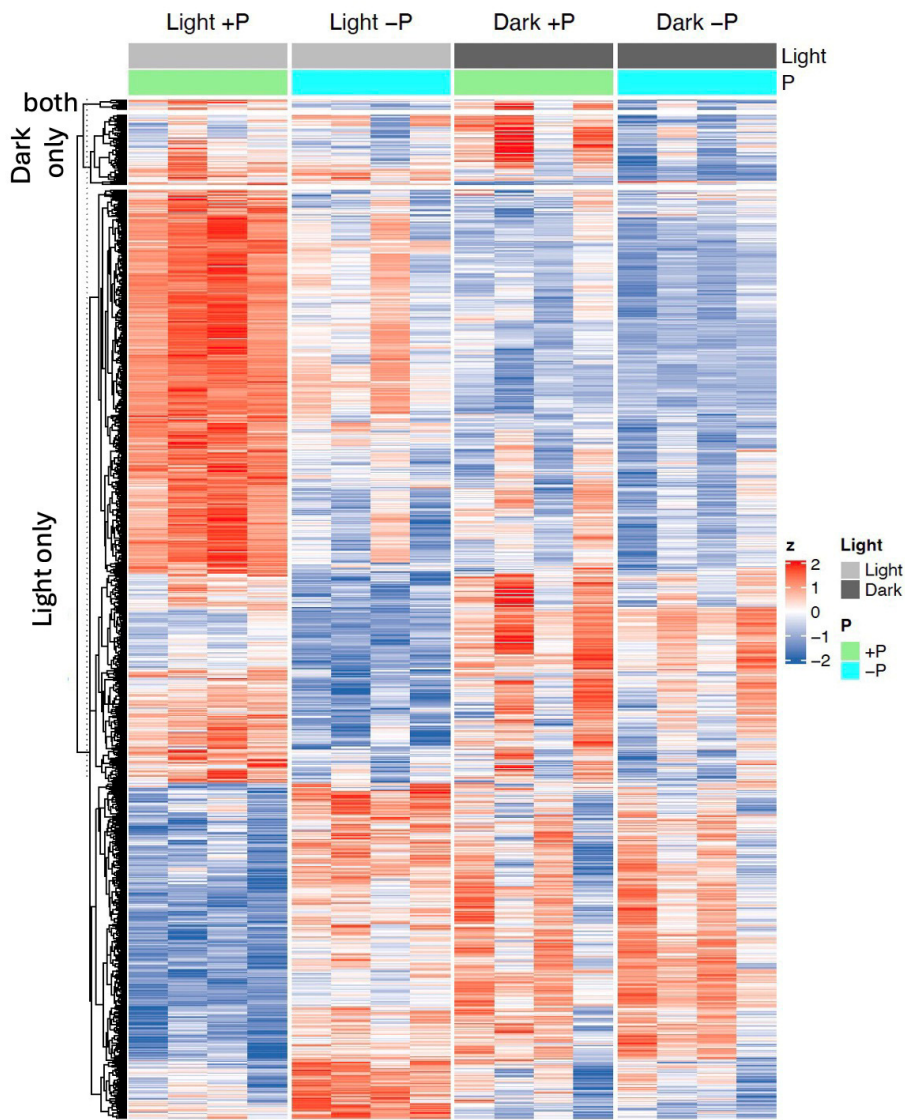


Figure 3. Heatmap of genes differentially expressed under Pi deficiency in light and dark conditions. Heatmap showing genes differentially expressed (adjusted $p < 0.05$, $|\log_2FC| > 1$) in comparisons of $-P$ versus $+P$ samples under light or dark conditions. Rows correspond to individual differentially expressed genes (DEGs), and columns correspond to individual biological replicates. Expression values were z-score normalized across samples, with blue and red indicating lower and higher relative expression, respectively. Column annotations indicate phosphate status ($+P$ or $-P$) and light or dark treatments. The heatmap highlights extensive transcriptional reprogramming under $-P$ in light-grown roots, whereas only a limited subset of DEGs is detected under $-P$ in darkness.

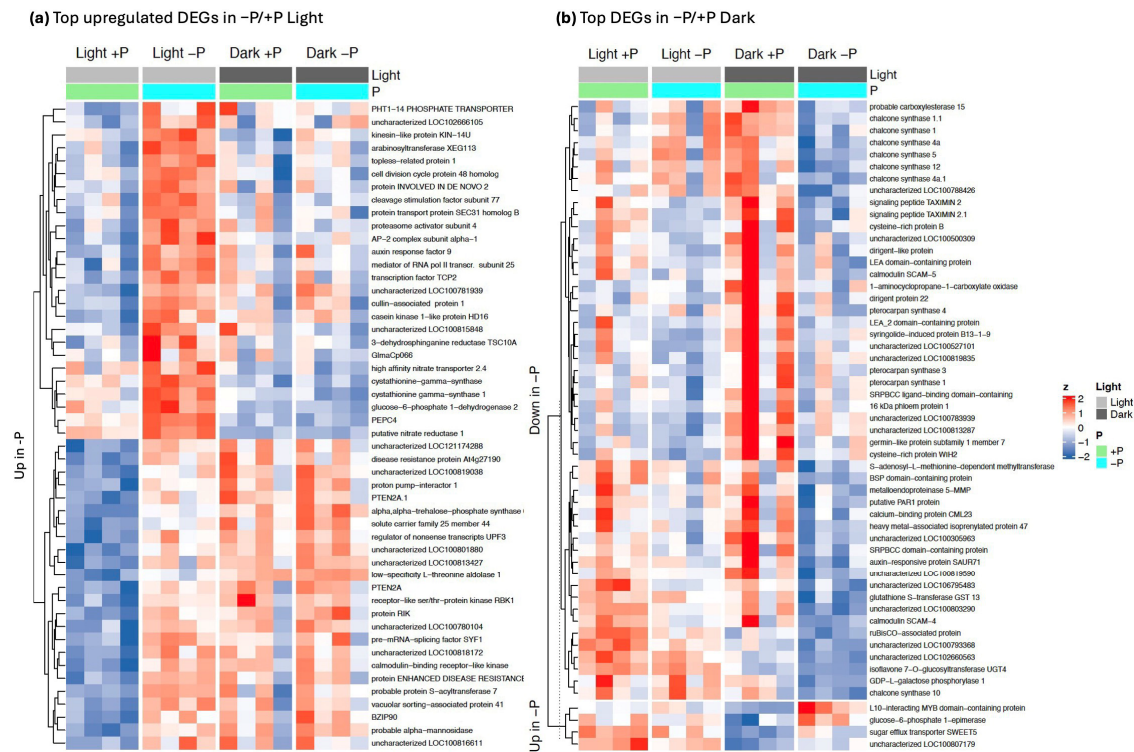


Figure 4. Contrasting transcriptional responses to P_i deficiency under light and dark conditions. (a) Heatmap of the top upregulated genes identified in the $-P/+P$ comparison under light conditions. Several well-characterized phosphate starvation response (PSR) genes are among the most strongly induced transcripts, including high-affinity P_i transporters and components associated with signaling and lipid remodeling. (b) Heatmap of the top differentially expressed genes identified in the $-P/+P$ comparison under dark conditions. In contrast to light-grown plants, fewer genes exhibit strong induction or repression in darkness, consistent with the more limited $-P$ -associated transcriptional responses observed under dark conditions. Rows represent individual genes and columns represent biological replicates. Expression values are shown as z-score-normalized counts (blue, lower relative expression; red, higher relative expression). Column annotations indicate phosphate status ($+P$ or $-P$) and light or dark treatment.

2.5. Sensitivity Analysis Reveals a Small Set of P_i -Responsive Genes in Darkness

To ensure that the minimal P_i response observed in darkness was not an artifact of stringent statistical filtering, we reanalyzed the dark dataset using relaxed criteria ($|\log_2FC| > 0.5$, adjusted $p < 0.1$). This analysis identified 13 upregulated genes (including the original four), among them *PHR11*, two L10-interacting MYB domain-containing transcription factors, and several genes related to sugar metabolism (Table 3). These subtle changes support the interpretation that local or basal sensing mechanisms remain active in darkness, whereas systemic activation of the phosphate-starvation program requires light-derived carbon.

Table 3. Upregulated genes in the –P/+P comparison under darkness. Four genes passed standard significance criteria ($\log_2FC > 1$, adjusted $p < 0.05$). Relaxed thresholds ($\log_2FC > 1$, adjusted $p < 0.1$) identified nine additional candidate genes.

Gene_ID	Description	–P/+P dark log2FC	–P/+P dark padj	–P/+P light log2FC	–P/+P light padj
SWEET5	sugar efflux transporter SWEET5	4.1	0.045	-0.9	0.215
LOC100787217	glucose-6-phosphate 1-epimerase	2.5	0.013	-0.4	0.612
LOC100820117	L10-interacting MYB domain- containing protein	2.5	0.019	0.1	NA
LOC100807179	uncharacterized LOC100807179	1.2	0.029	-0.7	0.072
LOC100818214	WEB family protein	2.7	0.061	0.6	NA
LOC100776105	arabinogalactan protein 9	2.0	0.091	-0.7	0.351
PHR11	PSR 11	1.8	0.061	-0.2	0.738
LOC100775446	aspartyl protease AED3	1.7	0.055	-0.1	0.878
LOC113000488	L10-interacting MYB domain- containing protein	1.5	0.077	0.6	NA
LOC100808302	cyclin-dependent kinase B2-2	1.5	0.078	-0.6	0.352
LOC100785581	kinesin-like protein KIN-14U	1.5	0.089	1.7	0.006
LOC100804267	uncharacterized	1.5	0.091	0.2	0.766
LOC100802287	glucan endo-1,3-beta-glucosidase 7	1.3	0.077	-0.3	0.588

2.6. Functional Enrichment Highlights Light-Dependent Transcriptional Reprogramming During *Pi* Deficiency

To characterize the biological processes underlying the observed transcriptional patterns, we performed functional enrichment analyses using KEGG annotations (Figure 5). As expected from the large number of –P-responsive genes under light conditions, multiple pathways were significantly enriched among genes upregulated under –P in the light (Figure 5a). These included signaling and regulatory categories such as MAPK and plant hormone signaling, as well as several lipid- and inositol-related processes. In contrast, enrichment among –P-upregulated genes in darkness was highly limited: only glycolysis/gluconeogenesis passed the threshold for significance (Figure 5b).

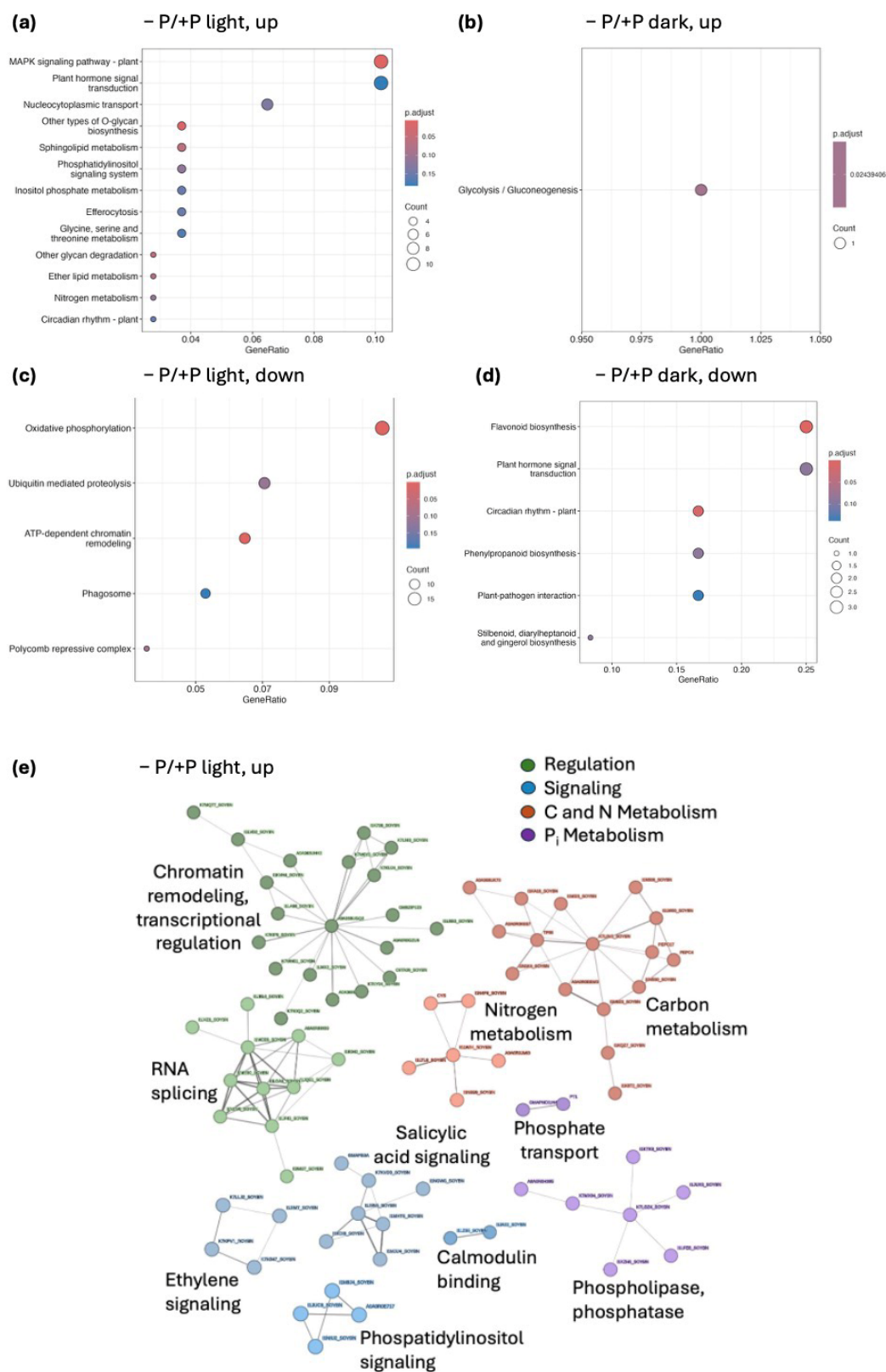


Figure 5. KEGG enrichment and STRING network analysis of genes differentially expressed under phosphate deficiency (-P) in light and darkness. (a–d) KEGG pathway enrichment for genes differentially expressed between -P and +P roots under light and dark conditions. (a, b) Pathways enriched among genes upregulated under -P in light or darkness. (c, d) Pathways enriched among genes downregulated under the same contrasts. Under light, several signaling- and metabolism-related pathways were enriched among -P-responsive genes, although some categories contained relatively few genes. Under darkness, enrichment was highly limited, with glycolysis/gluconeogenesis as the only pathway passing padj (adjusted p-value) correction

in the upregulated gene set (panel **b**). **(e)** STRING interaction network of genes upregulated under $-P$ in light. Nodes are grouped into broad functional categories: Regulation (green), Signaling (blue), Carbon and Nitrogen Metabolism (brown), and P_i Metabolism (purple). STRING integrates predicted and experimentally supported interactions from multiple species; therefore, these clusters indicate general functional associations rather than confirmed physical interactions in soybean. All clusters shown correspond to significantly enriched interaction networks ($p < 1 \times 10^{-4}$ to $p < 1 \times 10^{-16}$).

Among downregulated genes, $-P$ in light showed enrichment in categories such as oxidative phosphorylation, ubiquitin-mediated proteolysis, and ATP-dependent chromatin remodeling (Figure 5c), consistent with downregulation of energetically demanding or housekeeping pathways during P_i stress. $-P$ in dark displayed a different pattern, with downregulated genes enriched in flavonoid biosynthesis, plant hormone signal transduction, and plant-pathogen interaction (Figure 5d).

Analyses of the light-dark comparisons (Supplementary Figures S1–S2) revealed similar global trends: photosynthesis- and energy-related pathways were enriched under light but reduced under darkness, reinforcing the dominant effect of light on root transcriptome structure.

2.7. Protein-Protein Interaction Network Identifies Core Response Modules

To examine potential functional relationships among genes upregulated under $-P$ in the light, we constructed a protein-protein interaction network using STRING (Figure 5e). The network showed significant enrichment for known interactions ($p < 1 \times 10^{-4}$ to $p < 1 \times 10^{-16}$), indicating that these genes form coordinated functional modules rather than independent responses.

Clustering using the Markov Cluster Algorithm (MCL) resolved several groups organized by annotated biological functions. The largest clusters were associated with chromatin remodeling and transcriptional regulation, containing multiple transcription factors and chromatin-associated proteins, features consistent with broad regulatory control of the P_i response. A second major cluster corresponded to carbon and nitrogen metabolism, encompassing enzymes from glycolytic and amino acid pathways, in line with the metabolic reprogramming revealed by KEGG enrichment. Additional clusters were linked to signaling processes, including salicylic acid, ethylene, and phosphatidylinositol signaling, as well as calmodulin binding, highlighting crosstalk between P_i signaling and hormonal or calcium-dependent regulation. Finally, a distinct P_i -metabolism cluster contained phosphate transporters and phosphatases, representing direct components of P_i uptake and turnover.

Overall, the protein-protein interaction network highlights that the $-P$ -responsive genes induced under light form several coordinated functional groups spanning regulation, signaling, and metabolism.

2.8. qRT-PCR Assessment of Selected P_i -Responsive Genes

Expression patterns of selected genes were evaluated by qRT-PCR (quantitative Reverse-Transcription PCR), including one gene upregulated under $-P$ in light-grown roots (*PHO1*), two genes showing $-P$ -associated induction in darkness in the RNA-seq dataset (*L10-interacting MYB* and *SWEET5*), and *PHR1*, which showed a slight but non-significant increase under $-P$ in darkness. qRT-PCR confirmed that *PHO1* was significantly upregulated under $-P$ in the light, whereas expression in darkness remained low, consistent with the RNA-seq results. The *L10-interacting MYB* gene also showed detectable induction under $-P$ in darkness, but not in light.

For *PHR1*, qRT-PCR reproduced the small increase observed in the RNA-seq dataset, but this change was not statistically significant in either assay. Such low-amplitude shifts should be interpreted cautiously, as they may fall near the limit of detection for both methods. *SWEET5* expression in darkness could not be reliably quantified ($C_t > 32$), preventing validation; this is consistent with its very low expression in the RNA-seq data and illustrates the challenge of validating

genes with extremely low transcript abundance. Overall, qRT-PCR results were broadly consistent with the major expression patterns identified by RNA-seq.

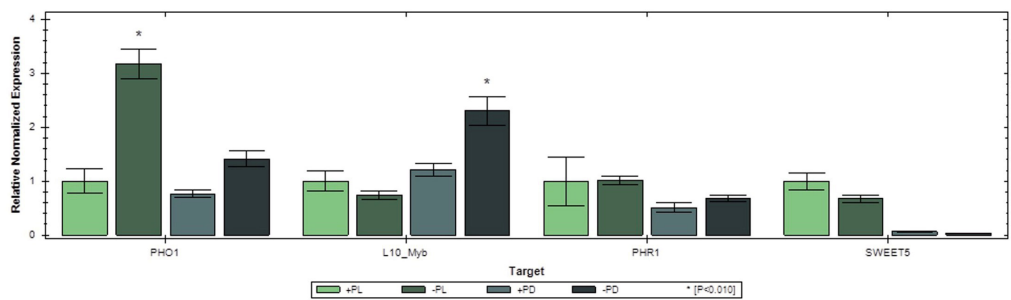


Figure 6. qRT-PCR assessment of selected P_i -responsive genes. Relative transcript levels of *PHO1*, *L10-interacting MYB*, *PHR1*, and *SWEET5* were measured under phosphate-sufficient (+P) and phosphate-deficient (–P) conditions in light (L) and dark (D). Bars represent the mean $\pm$ SE of three technical replicates of a representative biological replicate. Expression values were normalized to a methyltransferase reference gene that showed stable expression across treatments in the RNA-seq dataset. Asterisks indicate significant differences between +P and –P within each light condition ($p < 0.01$). *SWEET5* expression in darkness was near the detection limit ($Ct > 32$), and corresponding qRT-PCR values should be interpreted with caution.

3. Discussion

3.1. Systemic P_i Signaling Enabled by Light

P_i deficiency triggered a broad transcriptional response in soybean roots under light, consistent with activation of the canonical Phosphate Starvation Response (PSR). Genes encoding P_i transporters such as *PHT1* and *PHO1* were strongly induced, in agreement with their established roles in high-affinity uptake and xylem loading, and with the known relief of *PHO2*-mediated repression under P_i limitation [36]. The induction of *PTEN2 α* , phospholipases, as well as KEGG enrichment for lipid metabolism, is consistent with the shift from phospholipids to P-free membrane lipids also characteristic of the PSR [21,37]. In total, 364 genes were upregulated under –P in light, spanning transport, signaling, transcriptional regulation, and metabolism. This broad transcriptional activation under light corresponds to the “canonical” component of the P_i starvation response illustrated in Figure 7.

While sucrose-mediated regulation of individual P_i -responsive genes has been well documented in multiple plant species, most extensively in Arabidopsis [23], our results highlight the extent to which sucrose availability enables genome-wide transcriptional reprogramming during P_i deficiency. Light-dependent sucrose production is likely a major contributor to this effect, as photosynthesis supports both carbon availability and long-distance sucrose signaling from shoot to root.

Dark treatment has been widely used as an experimental approach to reduce endogenous sucrose levels in studies investigating sucrose-mediated signaling in plants [24,35,38,39]. Consistent with this rationale, 24 hour dark treatment has been shown to significantly reduce sucrose levels in Arabidopsis roots [35]. Together with these prior observations, our results support the interpretation that reduced sucrose availability limits activation of the canonical PSR in the dark.

In Arabidopsis, sucrose accumulation has been shown to be required not only for P_i -deficiency signaling but also for activation of Fe-deficiency responses, with darkness suppressing *FRO2* and *IRT1* induction by limiting sucrose delivery to the root [35]. These studies established sucrose as a central systemic signal linking shoot carbon status to root nutrient responses. In soybean, sucrose-feeding experiments similarly demonstrate that exogenous sucrose enhances root growth and induces metabolic signatures resembling P_i starvation [40]. Together with our observations, these findings suggest that abundant shoot-derived sucrose under light enables engagement of the

canonical PSR, supporting broad transcriptional activation across multiple functional categories in soybean roots.

3.2. *P_i Signaling in Darkness: A Small Sucrose-Independent Response That Includes a MYB Transcription Factor*

In contrast to the broad response seen under light, *P_i*-deficient roots in darkness showed very limited transcriptional activation, with only four genes passing standard significance thresholds. Notably, among this small set, an *L10*-interacting MYB-domain transcription factor met stringent differential expression criteria ($|\log_2FC| > 1$; $p_{adj} < 0.05$), and its upregulation was independently confirmed by qRT-PCR.

When relaxing statistical stringency ($|\log_2FC| > 1$; $p_{adj} < 0.1$), a total of 13 upregulated genes emerged, including two additional MYB-domain transcription factors: *PHR11*, a homolog of the central PSR regulator *PHR1* [41], and another *L10*-interacting MYBs. *L10*-interacting MYB factors have been linked to nutrient and ribosomal stress [42], and their activation in darkness suggests they may participate in a sucrose-independent *P_i* deficiency response.

Conservative statistical thresholds using adjusted *p*-values (p_{adj}) can reduce the number of detected DEGs, but even relaxed criteria revealed only a small set of $-P$ responsive genes in darkness. This indicates that the minimal PSR observed in dark-grown roots likely reflects true biological attenuation rather than an artifact of stringent statistical filtering.

Local *P_i* sensing is known to occur in the root apex, involving *LPR1/LPR2*-dependent Fe redox chemistry, auxin transport, ROS gradients, and ethylene or strigolactone signaling [36,43]. These mechanisms typically involve only small cell populations that would be diluted in bulk RNA-seq and thus may not have been detected in our study.

Additional insight comes from 11 genes consistently downregulated under $-P$ in both light and dark. Because sucrose-dependent systemic signaling is largely inactive in darkness, these genes likely represent a carbon-independent component of the $-P$ response. These include proteins associated with metabolism (e.g., RuBisCO-associated protein, phosphoglycerate mutase-like protein), signaling (*TAXIMIN2*, a VQ-motif protein), and secondary metabolism (dirigent protein 22).

Taken together, our data support a two-component view of the soybean *P_i* response. Light enables a broad, carbon-supported PSR, whereas darkness reveals a much smaller, sucrose-independent transcriptional subset (Figure 7).

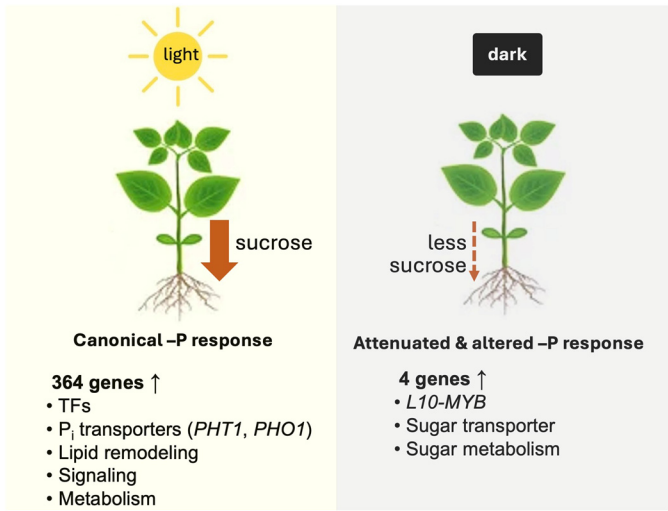


Figure 7. Light enables the canonical PSR, whereas darkness reveals an attenuated sucrose-independent response. Under light, sucrose transport from shoot to root supports activation of the canonical phosphate-starvation program, including strong induction of *PHT1* and *PHO1*, lipid-remodeling enzymes, signaling components, and metabolic pathways. In total, 364 genes were upregulated under $-P$ in light, spanning transcription factors, *P_i* transporters, lipid remodeling, signaling, and metabolism. In darkness, reduced sucrose

flow limits transcriptional activation. Only four genes showed significant upregulation including an L10-interacting MYB transcription factor as well as a sugar transporter and sugar-metabolism genes. Targeted sucrose-feeding experiments under dark conditions will be an important next step to test whether restoration of sucrose supply is sufficient to re-activate the canonical phosphate starvation response in the absence of light.

3.3. Limitations and Future Directions

Although sucrose availability was not directly quantified, our experimental design relied on well-established effects of darkness on photosynthetic sucrose export. Dark treatment has been widely used to reduce endogenous sucrose levels in studies of sucrose-mediated signaling, and has been shown to significantly decrease sucrose concentrations in roots after extended periods of darkness [35]. Using darkness to suppress sucrose delivery therefore provides a tractable way to probe carbon dependencies, but it also introduces additional metabolic and hormonal changes. Future experiments that supplement sucrose under darkness, or selectively disrupt shoot-root carbon transport (e.g., by girdling), will help clarify the extent to which sucrose regulates the transcriptional response to P_i deficiency.

While Nanopore cDNA sequencing provided sufficient depth to capture robust genome-wide responses under light, additional weakly responsive genes may remain below detection thresholds, particularly if expressed at low levels or restricted to specific cell types. Approaches with increased sensitivity or spatial resolution, such as cell type-resolved or spatial transcriptomic methods, will be valuable to determine whether the sucrose-independent gene set marks discrete P_i -sensing domains within the root apex.

Despite these limitations, our study reveals a clear contrast between the extensive P_i -responsive transcriptome activated under light and the minimal sucrose-independent subset detectable in darkness. Understanding how carbon availability modulates P_i uptake, membrane remodeling, and stress acclimation has practical implications for improving P_i -use efficiency, particularly under field conditions where shading or environmental stress may restrict carbon allocation to the root system.

4. Materials and Methods

4.1. Overview

This study investigated transcriptomic responses of soybean (*G. max*) roots to phosphate (P) availability under light and dark conditions using Oxford Nanopore long-read sequencing. Plants were grown hydroponically under controlled conditions and exposed to P deficiency for 30 hours under light or dark treatments. Root RNA was extracted for full-length cDNA library preparation, sequencing was performed on MinION flow cells, and reads were processed through a reproducible bioinformatics workflow.

4.2. Plant Growth and Treatment

Glycine max cv. Williams 82 seeds (a friendly gift from John Harada and Julie Marie Pelletier, University of California, Davis) were germinated for 3–4 days and transferred to hydroponic containers containing 8 L of half-strength Hoagland solution. The nutrient solution was replenished every 3–4 days. Plants were grown in a temperature-controlled chamber maintained at 27 °C with a 12 h light/12 h dark photoperiod.

After 10 days, cotyledons were removed to deplete stored P_i reserves. At 14 days, plants were subjected to one of four conditions: phosphate-sufficient (+P) light, phosphate-deficient (–P) light, +P dark, and –P dark, each maintained for 30 h. Each treatment was independently regrown four times to generate biological replicates, with each replicate representing a separate hydroponic experiment performed on different days. At harvest, ~5 cm segments of the main root axes, including the root tip region, were excised, immediately flash-frozen in liquid nitrogen, and stored at –80 °C until RNA extraction.

4.3. RNA Isolation and Quality Assessment

Total RNA was isolated from frozen root tissue using the RNeasy Plant Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol for "Purification of Total RNA from Plant Cells and Tissues, and Filamentous Fungi." RNA concentration and integrity were evaluated using a Qubit 4 Fluorometer (Thermo Fisher Scientific, Waltham, MA, USA) with the Qubit RNA High Sensitivity and RNA IQ assays. The RNA IQ assay measures the proportion of large or structured RNA relative to small RNA to assess sample integrity. Only samples with RNA IQ ≥ 7 were used for sequencing; most samples had RNA IQ > 8 .

4.4. cDNA Library Preparation and Quantification

For each sample, 500 ng of total RNA was used to synthesize cDNA using the cDNA-PCR Sequencing Kit V14 with Barcoding (Oxford Nanopore Technologies, Oxford, UK), following the manufacturer's instructions. cDNA library concentration and quality were verified with an Agilent TapeStation (Agilent Technologies, Santa Clara, CA, USA). For each biological replicate, four barcoded cDNA libraries were pooled to a final amount of 50 fmol.

4.5. Nanopore Sequencing

Sequencing was performed using the Oxford Nanopore Technologies cDNA-PCR Sequencing V14 – Barcoding (SQK-PCB114.24) protocol with R10.4.1 flow cells (FLO-MIN114). Flow cells were primed and loaded with pooled cDNA according to the manufacturer's instructions. Each MinION sequencing run was conducted for 72 h.

4.6. Nanopore Sequencing Analysis

Raw reads were processed in MinKNOW, retaining only passed reads ($Q > 7$) and demultiplexed during sequencing. Dorado basecalling was tested for comparison, but MinKNOW produced higher read counts and was used for all downstream analyses. Sequencing data were transferred to the Expanse high-performance computing cluster (San Diego Supercomputer Center, USA).

Read orientation and trimming: Full-length cDNA reads were identified and oriented using *PyChopper v2.7* (RRID:SCR_018966; <https://github.com/epi2me-labs/pychopper>) with the -m edlib setting.

Read alignment: Reads were aligned to the *G. max* reference genome (GCF_000004515.6 v4.0; downloaded 1 Oct 2025) using *Minimap2 v2.30-r1287* [44]. Alignments were sorted and indexed with *Samtools v1.20* [45].

Gene quantification: Gene counts were obtained using *featureCounts v2.0.6* [46] in long-read mode (-L) with single-end, stranded settings (-s 1). The resulting count matrix and summary tables were exported for downstream analysis in R.

Quality control: Mapping statistics were evaluated using *samtools flagstat* and *featureCounts* summary reports.

4.7. Differential Expression Analysis

Differential expression analyses were performed in R (v4.4.0) within RStudio. Raw count matrices from *featureCounts* were imported into the DESeq2 (v1.42.0) package [47] for normalization and differential expression analysis. Before differential expression analysis, genes were prefiltered by requiring at least 10 raw counts in at least 3 biological replicates within any one of the four treatment groups. This removed low-abundance genes prior to DESeq2 dispersion estimation. Normalized counts were obtained using the variance-stabilizing transformation (VST) in DESeq2. Genes with adjusted $p < 0.05$ and $|\log_2 \text{fold change}| \geq 1$ were considered significantly differentially expressed. Genes used to exemplify read depth (Table 2) were selected based on their well-established functions in P_i uptake, transport, or transcriptional signaling, ensuring that representative and biologically relevant PSR components were included.

Principal component analysis (PCA) was performed on variance-stabilizing transformed (VST) counts using DESeq2. The PCA was calculated using the top 500 most variable genes, and the first two principal components were visualized with *ggplot2*. Replicate identifiers were added to the plot to assess clustering within and across treatments.

UpSet plots: Overlaps among DEG sets were visualized using UpSetR v1.4.0 [48] with separate plots for up- and downregulated genes.

Heatmaps: Expression patterns were visualized using ComplexHeatmap v2.20.0 [49], with per-gene z-score scaling of VST counts.

GO enrichment: Gene Ontology (GO) enrichment was performed with **g:Profiler** (organism = “gmax”) on up- and downregulated DEG sets (FDR < 0.05). The background set consisted of all expressed genes. Results were summarized by Biological Process terms with gentle term collapsing and flattened list columns before export.

KEGG enrichment: KEGG pathway analysis was performed using clusterProfiler v4.10.0 [50] mapping Glyma IDs to KEGG Orthology (KO) terms via KEGGREST. Enrichment was tested using DEGs as the query set and all expressed genes as background.

STRING network analysis: Protein–protein interaction (PPI) networks were analyzed using STRING v12.0 [51] with *G. max* as the reference organism. Upregulated genes under –P light were uploaded to identify functional clusters using the Markov Cluster Algorithm (MCL, default inflation). Significant network enrichment was defined by STRING-calculated *p*-values ranging from < 1 × 10^{–4} to < 1 × 10^{–16}.

4.8. Validation by qRT-PCR

For each sample, 2000 ng of total RNA was treated with RNase-free DNase to remove genomic DNA, then reverse-transcribed using the iScript™ gDNA Clear cDNA Synthesis Kit (Bio-Rad, Hercules, CA, USA). A methyltransferase (LOC100819876) gene was selected as reference based on stable expression across all conditions. Primers were designed in Primer-BLAST, preferentially spanning exon junctions or framing introns, with amplicon sizes of 70–220 bp and minimal 3' complementarity to prevent primer–dimer formation.

Primer sequences:

Methyltransferase 17 (LOC100819876): F TTGAGCCCTCACGAGGATTC;
R CTTATGTACACCCGGCGCA;
PHO1: F ACAGAGCCAGCTTATATGGACAT;
R CAATGCTGTGCTTGGTGAGA;
L10-interacting MYB-domain protein (LOC100820117): F TCTCAACTGAGCGGAAGTGC; R
TCATTTTGCAACCTCCTGCG;
PHR11: F CCAGAATCAGATGACTGAAAGCTTG;
R GTTCAGCTTTTGCAAGTTCCATA;
SWEET5: F AGGACTACTACATCGCTCTACCA;
R TCCTCAATAGTTATCTCAGTCACAG

qPCR was performed using SYBR Green Supermix (Bio-Rad) on a CFX96 instrument under the following program: 95 °C for 30 s; 40 cycles of 95 °C for 15 s and 60 °C for 30 s. A melt curve analysis followed from 65 °C to 95 °C with 0.5 °C increments every 5 s. CFX Maestro Software (Bio-Rad) was used to obtain Cq values and calculate relative expression using the ΔΔCq method.

4.9. Statistical Analysis

All statistical analyses were conducted in R (v4.4.0). For RNA-seq data, differential expression was evaluated with the Wald test in DESeq2, using Benjamini–Hochberg FDR correction (*p*_{adj} < 0.05, |log₂FC| ≥ 1). Multivariate relationships among samples were examined by PCA. For enrichment analyses, overrepresentation of GO terms and KEGG pathways was assessed in clusterProfiler with adjusted *p*-value < 0.05 as the significance threshold.

5. Conclusions

Soybean roots require photosynthetically derived sucrose to mount the canonical phosphate starvation response (PSR). Under P deficiency in light, abundant sucrose transport from shoot to root supports coordinated induction of P_i transporters, signaling components, lipid-remodeling enzymes, and metabolic pathways. In contrast, darkness, which greatly reduces photosynthetic sucrose transport, suppresses these responses and reveals only a small, sucrose-independent subset that includes a MYB transcription factor and genes linked to sugar metabolism. Together, these findings highlight the importance of light in shaping early P_i-deficiency responses in soybean roots.

Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org. **Table S1:** Detailed Nanopore cDNA sequencing metrics for soybean roots under light and dark ±P conditions. **Figure S1:** GO enrichment of differentially expressed genes under phosphate deficiency (–P) and phosphate sufficiency (+P) in light and dark conditions.; **Figure S2:** KEGG enrichment of differentially expressed genes under phosphate-sufficient (+P) and phosphate-deficient (–P) conditions in light and dark.

Author Contributions: Conceptualization, all authors; methodology, all authors; software, not applicable; validation, all authors; formal analysis, C.U.-S.; investigation, all authors; resources, C.U.-S.; data curation, all authors; writing—original draft preparation, A.S., I.T., N.S., J.B., L.V., R.M., M.A., J.N., K.T., G.P., K.M., and T.C.; writing—review and editing, all authors; visualization, all authors; supervision, C.U.-S.; project administration, C.U.-S.; funding acquisition, C.U.-S. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by an internal RSCA grant 2024–2025, California State University, East Bay and by the National Science Foundation Plant Genome Research Program (USA) under grant number 2329893. The use of supercomputers (San Diego Supercomputer Expanse) was supported by an ACCESS grant SEE210001 (2025-2026).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Nanopore sequencing data has been deposited in the NCBI Gene Expression Omnibus (GEO) under accession number GSE312797. Additional data supporting this study are available from the corresponding author upon reasonable request.

Acknowledgments: The authors used ChatGPT (OpenAI, GPT-5.1) for language editing and technical guidance related to RNA-seq data processing on the Expanse supercomputer and figure preparation in R. All AI-generated output was reviewed and edited by the authors, who take full responsibility for the final content.

Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript:

DEG	Differentially Expressed Genes
GO	Gene Ontology
KEGG	Kyoto Encyclopedia of Genes and Genomes
MA-plot	Mean–average plot (log ₂ fold change vs mean expression)
p _{adj}	Adjusted p-value (Benjamini–Hochberg correction)
P _i	inorganic phosphate
PCA	Principal Component Analysis
PP _i	Pyrophosphate
PSR	Phosphate starvation response
qRT-PCR	Quantitative reverse transcription PCR
RNA-seq	RNA sequencing

References

- Vance, C.P.; Uhde-Stone, C.; Allan, D.L. Phosphorus acquisition and use: critical adaptations by plants for securing a nonrenewable resource. *New Phytologist* **2003**, *157*, 423-447.
- Cordell, D.; Drangert, J.; White, S. The story of phosphorus: Global food security and food for thought. *Global Environmental Change* **2009**, *19*, 292-305.
- Geissler, B.; Mew, M.C.; Matschullat, J.; Steiner, G. Innovation potential along the phosphorus supply chain: A micro and macro perspective on the mining phase. *Sci Total Environ* **2020**, *714*, 136701, doi:10.1016/j.scitotenv.2020.136701.
- Alewell, C.; Ringeval, B.; Ballabio, C.; Robinson, D.A.; Panagos, P.; Borrelli, P. Global phosphorus shortage will be aggravated by soil erosion. *Nature communications* **2020**, *11*, 4546, doi:10.1038/s41467-020-18326-7.
- Volkova, E.; Smolyaninova, N. Analysis of world trends in soybean production. In Proceedings of the BIO Web of Conferences, 2024; p. 01026.
- Cai, Z.; Cheng, Y.; Xian, P.; Ma, Q.; Wen, K.; Xia, Q.; Zhang, G.; Nian, H. Acid phosphatase gene GmHAD1 linked to low phosphorus tolerance in soybean, through fine mapping. *TAG. Theoretical and applied genetics. Theoretische und angewandte Genetik* **2018**, *131*, 1715-1728, doi:10.1007/s00122-018-3109-3.
- O'Rourke, J.A.; Graham, M.A. Gene Expression Responses to Sequential Nutrient Deficiency Stresses in Soybean. *International journal of molecular sciences* **2021**, *22*, doi:10.3390/ijms22031252.
- Chen, G.; Ran, Q.-X.; Wang, C.; Pang, J.; Ren, M.-J.; Wang, Z.-Y.; He, J.; Lambers, H. Enhancing photosynthetic phosphorus use efficiency through coordination of leaf phosphorus fractions, allocation, and anatomy during soybean domestication. *Journal of Experimental Botany* **2025**, *76*, 1446-1457, doi:doi:10.1093/jxb/erae009.
- Fang, Y.; Lu, L.; Chen, K.; Wang, X. Tradeoffs among root functional traits for phosphorus acquisition in 13 soybean genotypes contrasting in mycorrhizal colonization. *Annals of Botany* **2024**, *134*, 179-190.
- Tantriani; Cheng, W.; Oikawa, A.; Tawaraya, K. Phosphorus deficiency alters root length, acid phosphatase activity, organic acids, and metabolites in root exudates of soybean cultivars. *Physiol Plant* **2023**, *175*, e14107, doi:10.1111/ppl.14107.
- Péret, B.; Clément, M.; Nussaume, L.; Desnos, T. Root developmental adaptation to phosphate starvation: better safe than sorry. *Trends in plant science* **2011**, doi:S1360-1385(11)00104-X [pii]10.1016/j.tplants.2011.05.006.
- Xu, H.; Zhang, H.; Fan, Y.; Wang, R.; Cui, R.; Liu, X.; Chu, S.; Jiao, Y.; Zhang, X.; Zhang, D. The purple acid phosphatase GmPAP17 predominantly enhances phosphorus use efficiency in soybean. *Plant Sci* **2022**, *320*, 111283, doi:10.1016/j.plantsci.2022.111283.
- Li, F.; Mai, C.; Liu, Y.; Deng, Y.; Wu, L.; Zheng, X.; He, H.; Huang, Y.; Luo, Z.; Wang, J. Soybean PHR1-regulated low phosphorus-responsive GmRALF22 promotes phosphate uptake by stimulating the expression of GmPTs. *Plant Sci* **2024**, *348*, 112211, doi:10.1016/j.plantsci.2024.112211.
- Qin, L.; Guo, Y.; Chen, L.; Liang, R.; Gu, M.; Xu, G.; Zhao, J.; Walk, T.; Liao, H. Functional characterization of 14 Pht1 family genes in yeast and their expressions in response to nutrient starvation in soybean. *PloS one* **2012**, *7*, e47726, doi:10.1371/journal.pone.0047726.
- 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 nodulation in soybean. *Plant physiology* **2012**, *159*, 1634-1643, doi:10.1104/pp.112.199786.
- Miao, N.; Zhou, J.; Li, M.; Zhang, J.; Hu, Y.; Guo, J.; Zhang, T.; Shi, L. Remodeling and protecting the membrane system to resist phosphorus deficiency in wild soybean (*Glycine soja*) seedling leaves. *Planta* **2022**, *255*, 53, doi:10.1007/s00425-022-03834-1.
- Yao, Y.; Yuan, H.; Liu, D.; Cheng, L. Response of soybean root exudates and related metabolic pathways to low phosphorus stress. *PloS one* **2024**, *19*, e0314256, doi:10.1371/journal.pone.0314256.
- Plaxton, W.C.; Tran, H.T. Metabolic adaptations of phosphate-starved plants. *Plant physiology* **2011**, *156*, 1006-1015, doi:pp.111.175281 [pii] 10.1104/pp.111.175281.
- Ried, M.K.; Wild, R.; Zhu, J.; Pipercevic, J.; Sturm, K.; Broger, L.; Harmel, R.K.; Abriata, L.A.; Hothorn, L.A.; Fiedler, D.; et al. Inositol pyrophosphates promote the interaction of SPX domains with the coiled-coil motif

- of PHR transcription factors to regulate plant phosphate homeostasis. *Nature communications* **2021**, *12*, 384, doi:10.1038/s41467-020-20681-4.
20. Secco, D.; Wang, C.; Arpat, B.A.; Wang, Z.; Poirier, Y.; Tyerman, S.D.; Wu, P.; Shou, H.; Whelan, J. The emerging importance of the SPX domain-containing proteins in phosphate homeostasis. *New Phytologist* **2012**, *193*, 842-851.
 21. Mo, X.; Liu, G.; Zhang, Z.; Lu, X.; Liang, C.; Tian, J. Mechanisms Underlying Soybean Response to Phosphorus Deficiency through Integration of Omics Analysis. *International journal of molecular sciences* **2022**, *23*, doi:10.3390/ijms23094592.
 22. Hamburger, D.; Rezzonico, E.; MacDonald-Comber Petetot, J.; Somerville, C.; Poirier, Y. Identification and characterization of the Arabidopsis PHO1 gene involved in phosphate loading to the xylem. *The Plant cell* **2002**, *14*, 889-902, doi:10.1105/tpc.000745.
 23. Hammond, J.; White, P. Sucrose transport in the phloem: integrating root responses to phosphorus starvation. *J Exp Bot* **2008**, *59*, 93-109.
 24. Karthikeyan, A.S.; Varadarajan, D.K.; Jain, A.; Held, M.A.; Carpita, N.C.; Raghothama, K.G. Phosphate starvation responses are mediated by sugar signaling in Arabidopsis. *Planta* **2007**, *225*, 907-918, doi:10.1007/s00425-006-0408-8.
 25. Ruffel, S. Nutrient-Related Long-Distance Signals: Common Players and Possible Cross-Talk. *Plant Cell Physiol* **2018**, *59*, 1723-1732, doi:10.1093/pcp/pcy152.
 26. Hammond, J.P.; White, P.J. Sugar signaling in root responses to low phosphorus availability. *Plant physiology* **2011**, *156*, 1033-1040, doi:pp.111.175380 [pii] 10.1104/pp.111.175380.
 27. Lei, M.; Liu, Y.; Zhang, B.; Zhao, Y.; Wang, X.; Zhou, Y.; Raghothama, K.G.; Liu, D. Genetic and genomic evidence that sucrose is a global regulator of plant responses to phosphate starvation in Arabidopsis. *Plant physiology* **2011**, *156*, 1116-1130, doi:10.1104/pp.110.171736.
 28. Nidumolu, L.C.M.; Lorilla, K.M.; Chakravarty, I.; Uhde-Stone, C. Soybean Root Transcriptomics: Insights into Sucrose Signaling at the Crossroads of Nutrient Deficiency and Biotic Stress Responses. *Plants (Basel)* **2023**, *12*, doi:10.3390/plants12112117.
 29. Franco-Zorrilla, J.; González, E.; Bustos, R.; Linhares, F.; Leyva, A.; Paz-Ares, J. The transcriptional control of plant responses to phosphate limitation. *J Exp Bot* **2004**, *55*, 285-293, doi:erh009 [pii] 10.1093/jxb/erh009.
 30. Chien, P.S.; Chiang, C.P.; Leong, S.J.; Chiou, T.J. Sensing and Signaling of Phosphate Starvation - from Local to Long Distance. *Plant Cell Physiol* **2018**, doi:10.1093/pcp/pcy148.
 31. Lin, W.-Y.; Huang, T.-K.; Leong, S.J.; Chiou, T.-J. Long-distance call from phosphate: systemic regulation of phosphate starvation responses. *Journal of experimental botany* **2014**, *65*, 1817-1827.
 32. Marro, N.; Lidoy, J.; Chico, M.A.; Rial, C.; Garcia, J.; Varela, R.M.; Macias, F.A.; Pozo, M.J.; Janouskova, M.; Lopez-Raez, J.A. Strigolactones: New players in the nitrogen-phosphorus signalling interplay. *Plant Cell Environ* **2022**, *45*, 512-527, doi:10.1111/pce.14212.
 33. Poirier, Y.; Jaskolowski, A.; Clua, J. Phosphate acquisition and metabolism in plants. *Current biology : CB* **2022**, *32*, R623-R629, doi:10.1016/j.cub.2022.03.073.
 34. Rouached, H.; Arpat, A.B.; Poirier, Y. Regulation of phosphate starvation responses in plants: signaling players and cross-talks. *Molecular plant* **2010**, *3*, 288-299, doi:10.1093/mp/ssp120.
 35. Lin, X.Y.; Ye, Y.Q.; Fan, S.K.; Jin, C.W.; Zheng, S.J. Increased Sucrose Accumulation Regulates Iron-Deficiency Responses by Promoting Auxin Signaling in Arabidopsis Plants. *Plant physiology* **2016**, *170*, 907-920, doi:10.1104/pp.15.01598.
 36. Madison, I.; Gillan, L.; Peace, J.; Gabrieli, F.; Van den Broeck, L.; Jones, J.L.; Sozzani, R. Phosphate starvation: response mechanisms and solutions. *J Exp Bot* **2023**, *74*, 6417-6430, doi:10.1093/jxb/erad326.
 37. Pribat, A.; Sormani, R.; Rousseau-Gueutin, M.; Julkowska, M.M.; Testerink, C.; Joubes, J.; Castroviejo, M.; Laguerre, M.; Meyer, C.; Germain, V.; et al. A novel class of PTEN protein in Arabidopsis displays unusual phosphoinositide phosphatase activity and efficiently binds phosphatidic acid. *Biochem J* **2012**, *441*, 161-171, doi:10.1042/BJ20110776.
 38. Price, J.; Laxmi, A.; St Martin, S.; Jang, J. Global transcription profiling reveals multiple sugar signal transduction mechanisms in Arabidopsis. *The Plant cell* **2004**, *16*, 2128-2150.

39. Liu, J.; Samac, D.; Bucciarelli, B.; Allan, D.; Vance, C. Signaling of phosphorus deficiency-induced gene expression in white lupin requires sugar and phloem transport. *The Plant journal : for cell and molecular biology* 2005, **41**, 257-268.
40. Yang, A.; Kong, L.; Wang, H.; Yao, X.; Xie, F.; Wang, H.; Ao, X. Response of Soybean Root to Phosphorus Deficiency under Sucrose Feeding: Insight from Morphological and Metabolome Characterizations. *BioMed research international* 2020, **2020**, 2148032, doi:10.1155/2020/2148032.
41. Rubio, V.; Linhares, F.; Solano, R.; Martín, A.; Iglesias, J.; Leyva, A.; Paz-Ares, J. A conserved MYB transcription factor involved in phosphate starvation signaling both in vascular plants and in unicellular algae. *Genes & development* 2001, **15**, 2122-2133, doi:10.1101/gad.204401.
42. Ferreira, M.A.; Teixeira, R.M.; Brustolini, O.J.B.; Saia, T.F.F.; Jean-Baptiste, J.; Ribeiro, N.G.A.; Breves, S.S.; Sampaio, F.R.; Santos, E.G.D.; Leon, B.A.; et al. The immune NIK1/RPL10/LIMYB signaling module regulates photosynthesis and translation under biotic and abiotic stresses. *Nature communications* 2025, **16**, 4433, doi:10.1038/s41467-025-59571-y.
43. Puga, M.I.; Poza-Carrion, C.; Martinez-Hevia, I.; Perez-Liens, L.; Paz-Ares, J. Recent advances in research on phosphate starvation signaling in plants. *J Plant Res* 2024, **137**, 315-330, doi:10.1007/s10265-024-01545-0.
44. Li, H. Minimap2: pairwise alignment for nucleotide sequences. *Bioinformatics* 2018, **34**, 3094-3100, doi:10.1093/bioinformatics/bty191.
45. Li, H.; Handsaker, B.; Wysoker, A.; Fennell, T.; Ruan, J.; Homer, N.; Marth, G.; Abecasis, G.; Durbin, R.; Genome Project Data Processing, S. The Sequence Alignment/Map format and SAMtools. *Bioinformatics* 2009, **25**, 2078-2079, doi:10.1093/bioinformatics/btp352.
46. Liao, Y.; Smyth, G.K.; Shi, W. featureCounts: an efficient general purpose program for assigning sequence reads to genomic features. *Bioinformatics* 2014, **30**, 923-930, doi:10.1093/bioinformatics/btt656.
47. Love, M.I.; Huber, W.; Anders, S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol* 2014, **15**, 550, doi:10.1186/s13059-014-0550-8.
48. Conway, J.R.; Lex, A.; Gehlenborg, N. UpSetR: an R package for the visualization of intersecting sets and their properties. *Bioinformatics* 2017, **33**, 2938-2940, doi:10.1093/bioinformatics/btx364.
49. Gu, Z.; Hubschmann, D. Make Interactive Complex Heatmaps in R. *Bioinformatics* 2022, **38**, 1460-1462, doi:10.1093/bioinformatics/btab806.
50. Wu, T.; Hu, E.; Xu, S.; Chen, M.; Guo, P.; Dai, Z.; Feng, T.; Zhou, L.; Tang, W.; Zhan, L.; et al. clusterProfiler 4.0: A universal enrichment tool for interpreting omics data. *Innovation (Camb)* 2021, **2**, 100141, doi:10.1016/j.xinn.2021.100141.
51. Szklarczyk, D.; Nastou, K.; Koutrouli, M.; Kirsch, R.; Mehryary, F.; Hachilif, R.; Hu, D.; Peluso, M.E.; Huang, Q.; Fang, T.; et al. The STRING database in 2025: protein networks with directionality of regulation. *Nucleic acids research* 2025, **53**, D730-D737, doi:10.1093/nar/gkae1113.

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.