

Transcriptome Profiling of Oil Palm (*Elaeis guineensis* Jacq.) in Phosphorus Deficiency

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ABSTRACT

Phosphorus (P) limitation conditions could be lessened by developing oil palm progenies with increased P absorption efficiency using molecular approaches. The molecular-assisted selection method can be applied to select elite palms with high nutrient-uptake efficiency. The aim of the study was to identify key genes that were differentially expressed under P-deficient conditions. A molecular marker could be developed from the RNA-seq results. RNA-seq was applied for prolific and non-prolific genotypes grown under low- and high- P dosage for 180 days, and their specific morphological characters were also observed. Root length, shoot length, and P-content characteristics

showed significant differences among genotypes under both P dosages. Heat-maps were employed to visualise the RNA sequencing results. In total, 2,905 DEGs involved in P deficiency in oil palm were identified. The GO terms were subsequently divided into 74 sub-categories. Phosphorylation, protein phosphorylation, transferring phosphorus-containing groups, alcohol group as acceptor, and nitrogenous group as acceptor were the five major subcategories related to P deficiency. Five genes were selected based on their response to P deficiency, the up-regulated genes consisted of *NRR1* and *AP4S1*; and the down-regulated consisted of *PTI/STPs*,

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PPD7, and *PPR* genes. These five genes are suggested to be validated using RT-qPCR to determine their expression in the next study. Hence, this research provides a comprehensive understanding of the physiological processes involved in the oil palm seedling response to P deficiency. The results may be used for the development of biomarkers for the rapid selection of candidate palms in oil palm breeding programmes.

Keywords: DEGs; oil palm, P-deficient, RNA-sequencing, transcriptome profiles

INTRODUCTION

Complex NPK is the second fertiliser which the most applied fertiliser in the agricultural industry worldwide. Phosphorus (P) consumption was approximately 12.74 million tons per year (Statista Research Department, 2024). Phosphorus scarcity is a major problem in agricultural lands worldwide, resulting in a decline in crop yields and posing substantial risks to global agriculture (Saputra et al., 2023). In fact, phosphorus is abundant in the lithosphere. Nevertheless, plants can only absorb inorganic orthophosphate (Pi) from soils. This form of P is sluggish and insoluble, dispersed in soils. This situation leads to P's inadequacy in the agricultural industry. Soil microbiota are also sensitive to P availability. They can either compete with plants or engage in mutualistic symbiosis, such as through mycorrhizal associations, to increase plants' phosphorus absorption efficiency (Abobatta et al., 2023). In addition, the application of P fertilisers is often inefficient, with only 15-25% of the applied P being absorbed by plant roots; the remainder is lost through leaching, contributing to soil degradation and water eutrophication (Khan et al., 2023).

P plays an indispensable role in plant physiological processes in improving abiotic stress tolerance, such as heavy metal toxicity, salinity, drought, heat, waterlogging, and elevated CO₂ levels (George et al., 2016). Plants respond to phosphorus availability through specific signalling cascades, modification of root architecture and alteration of stomatal morphology (Shen et al., 2011). Furthermore, by enhancing phosphorus absorption, plants can endure diverse abiotic stresses (Malhotra et al., 2018). Understanding plants' strategies to recognise and respond to phosphorus availability is critical in order to increase productivity and improve crop stress tolerance. Phosphate or phosphate esters are essential for numerous physiological and metabolic processes, including DNA synthesis, phospholipid biosynthesis, cell division, and energy metabolism (Isidra-Arellano et al., 2021). Inadequate phosphorus severely affects fruit production, root development, and vegetative growth, which eventually diminishes yields (Deng et al., 2018).

Development of an oil palm genotype with high phosphorus uptake efficiency using molecular approaches might mitigate the P limitation condition (Faucon et al., 2015). Molecular markers can be used to select elite oil palms with high P uptake efficiency.

However, the molecular mechanism involved in oil palm response to P deficiency is not fully understood. One of the potential approaches that can be used to develop molecular markers for certain characters is through transcriptome profiling by RNA sequencing (Maryanto et al., 2021). This approach has been applied in wheat, rice, and maize. A functional analysis of P deficiency in rice, conducted to understand the biological regulatory systems underlying P-insufficiency tolerance, has been reported (Wissuwa, 2001). In this study, RNA from oil palm seedlings grown under P starvation was sampled and subjected to RNA sequencing, and seedlings grown under adequate P supply were used as the control.

The oil palm response to P deficiency is complex, involving numerous sensing and signalling pathways (Kong et al., 2021). In their research, Kong et al (2021) did not classify between efficient and non-efficient genotypes. Therefore, in this study, the efficient and non-efficient genotypes were used as samples. Previous research found that some phenotypic traits differed significantly among genotypes. This result opens the possibility of improving oil palm P-deficiency tolerance. Progeny A and C were identified as tolerant to P deficiency (prolific), whereas progeny B and D were identified as non-tolerant (non-prolific) based on several genotypic traits (data unpublished). Accordingly, this study aimed to identify P-deficiency-responsive differentially expressed genes (DEGs) in oil palm. The findings may contribute to a clearer understanding of oil palm adaptive responses to P limitation and support oil palm genetic improvement efforts.

METHODS

Plant Materials

Three-month-old Tenera (Dura x Pisifera) seedlings from four oil palm genotypes were used. Genotypes A and C were classified as P-deficiency-tolerant (prolific), and genotypes B and D were classified as P-deficiency-sensitive (non-prolific). The planting materials were classified according to several genotype traits (internal report).

Hydroponic Trial and Morphological Measurement

Seedling which showed uniform plant height were selected and planted in a greenhouse with a culture solution of a hydroponic system. The system had dimensions of 6m × 3m × 1m and comprised six lines with 5cm spacing between them. A total capacity of 120 planting holes, spaced 15cm apart, each with a diameter of 5 cm (Maryanto et al., 2021). The temperature was controlled at 28-30°C. The nutrient solution was prepared following the Hoagland method with several modifications (Hoagland & Arnon, 1950). The seedlings were grown in 1.57% v/v (low-phosphorus/LP), and the control was grown in 14.02% v/v (high-phosphorus/HP). The nutrient solution was replaced biweekly to ensure adequate nutrient availability and was continuously aerated using an air pump.

The experimental design consisted of four oil palm genotypes (A, B, C, and D) x two P-dosages (low- and high-P) x five replicates x 2 repetitions with a total of 80 oil palm seedlings. P treatments were applied for 180 days. Morphological characteristics consisting of root and shoot length were measured biweekly, while P-content was measured at 180 days after transplanting (DAT). The leaf samples for RNA sequencing were collected at 180 DAT and put immediately in RNA Later (Thermo Scientific, Massachusetts, USA).

RNA Extraction, cDNA Library Preparation and Transcriptome Sequencing

Total leaf RNA was isolated by the commercial RNeasy Plant Mini Kit (QIAGEN, Hilden, Germany) based on the protocol. Extracted RNA was quantified using a NanoDrop™ 2000c Spectrophotometer (Thermo Scientific, Massachusetts, USA). RNA that fulfils the requirement for sequencing was preserved by RNAsave (Biomatrix, California, USA) and sent to Novogene Co., Ltd., Beijing, China (Sahara et al., 2023). Total samples for RNAseq were 16 libraries, consisting of two samples for each genotype and for both P dosages.

The RNA was then measured using the 2100 Bioanalyzer Instrument (Agilent Company, USA) for quantifying RNA concentration, rRNA ratio, and RNA integrity number. mRNA was isolated from the total RNA using magnetic beads with Oligo (dT). The mRNA is then fragmented into short fragments and used as a template for cDNA synthesis. Short fragments were purified and resolved with EB buffer for end reparation and poly (A) addition. The short fragments were connected with adapters, and the cDNA was sequenced using the Illumina NextSeq 2000 sequencer platform (Maryanto et al., 2021).

Data Analysis

Raw RNA quantification was analysed using Kallisto software. Expression normalisation was counted based on transcript per million (TPM) (Bray et al., 2016). The DESeq2 was used for DEGs analysis (Love et al., 2014). The Plant Transcriptional Regulatory Map Database was used for gene ontology (GO) enrichment (Tian et al., 2020). The annotation of DEGs for describing biological processes, molecular functions, and cellular components was analysed using Microsoft Excel.

RESULTS AND DISCUSSION

Morphological Characters

P concentration is affected by plant morphology (Figure 1). Seedlings grown in low-P possessed shorter roots and shoots compared to seedlings grown in high-P.

Plants launched a series of adaptive morphological, biochemical, and physiological approaches to survive in P deficiency. Alteration of plant architecture and root growth is a well-known morphological response to P-deficiency, including reduction of root and

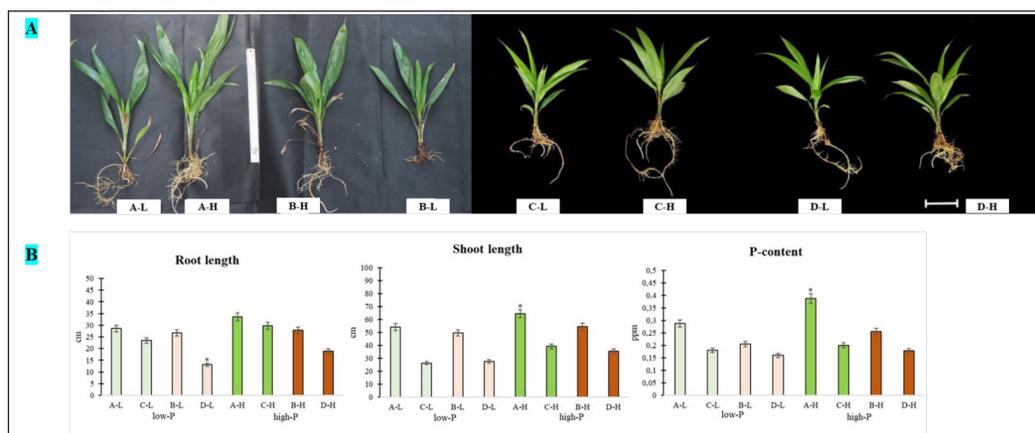


Figure 1. Morphological performance of seedlings at high- and low- P (A) and quantitative data of morphological characters (B). Notes: A-L= prolific progeny in low-P; A-H = prolific progeny in high-P; B-H= commercial prolific progeny in high-P; B-L = commercial prolific progeny in low-P; C-L= non-prolific progeny in low-P; C-H = non-prolific progeny in high-P; D-L= commercial non-prolific progeny in low-P; D-H = commercial non-prolific progeny in high-P; asterisk symbol (*) = significantly different ($p < 0.05$)

shoot length. Moreover, a decay of P content in plants treated with P-deficiency has been reported in another plant. In this study, the prolific genotypes had relatively better growth compared to non-prolific genotypes in low P conditions based on the observations of those three parameters. This might be caused by P homeostasis in prolific areas being better than in non-prolific genotypes. P transfer is arising from older to younger leaves during P-deficiency.

The modification of shoot length observed in this study is a well-documented morphological response to P deficiency. P is an essential element for oil palm growth at both the cellular and whole-plant levels (de Bang et al., 2021), playing a crucial role in cell division and cell enlargement. Consequently, the reduction in shoot length under P-deficient conditions is primarily caused by restricted cell division and limited cell enlargement (Malhotra et al., 2018).

The adaptive response by prolific genotypes under severe P-deficiency is better than that of non-prolific genotypes. The enhancement of root length is a plant endeavour to discover more P in the environment (Fang et al., 2024). Roots function as a sink tissue under P-deficient conditions, promoting root proliferation and more extensive soil exploration. Furthermore, the total phosphorus content in oil palm organs was consistent with the applied treatments: palms receiving low-P doses exhibited reduced tissue P concentrations, whereas those supplied with optimal P levels showed higher P accumulation. This is perhaps caused by P absorption harmonised with the P availability in the cultivation media.

Transcriptome Profile High- and Low- Phosphorus

Transcriptome profiling is addressed to increase the thoughtfulness of the biological regulatory process of P deficiency in oil palm (Maryanto et al., 2021). The Illumina NextSeq 2000 sequencer was used, resulting in a total nucleotide of approximately 18.77 Gb in size. From 151,368,907 total paired final raw reads, approximately 143,904,945 total net reads were obtained (Table 1). The DEGs of seedlings treated in low-P and high-P were shown in the heatmaps (Figure 2 and Figure 3). The heatmap shows two major clusters on the x-axis. The first cluster comprised non-prolific genotypes (B, D), whereas the second was of prolific genotypes (A, C). One group of DEGs showed higher expression in the prolific but lower expression in the non-prolific, while the other group of DEGs showed higher expression in the non-prolific but lower expression in the prolific genotypes. This result exhibited the differences in transcriptome profiles of prolific and non-prolific at the P treatment.

In total, 2,905 DEGs were identified, and their distribution was shown in a volcano plot (Figure 4). The y-axis indicated the significant gene expression level between samples, which was quantified by the p-value, whereas the x-axis denoted the fold change (\log_2 fold change) of the DEGs between the treatment and control. Furthermore, red dot colour indicated upregulated genes, while green dot colour indicated downregulated genes, and black dot colour indicated genes which have similar expression in both samples (Figure 4). Genes located on the outer line with $|\log_2FC| > 1$ and a p-value < 0.05 were assumed to be differentially expressed. The p-value indicates the statistical significance of differentially expressed genes (DEGs) relative to the total transcriptome of all samples, reflecting the reliability of DEG abundance compared with overall transcript levels. Meanwhile, the \log_2 fold change (\log_2FC) represents the magnitude of expression differences of DEGs between samples.

Furthermore, PCA is a technique used to assess relationships among samples and to leverage all collected data. In this study, grouping of samples and correlations among parameters were determined based on transcripts per million (TPM) of the differentially expressed genes (DEGs). PCA clustered the samples into two major groups: the prolific genotype (blue) and the non-prolific genotypes (another colour), based on TPM values. These results indicate that transcriptome profiles of prolific genotypes were more similar to each other and distinct from those of non-prolific genotypes under both low-P and high-P treatments (Figures 5A and B).

Functional Classification Based on Gene Ontology

Three main gene ontologies (GO) are commonly used in gene categorisations, namely, biological process, cellular component, and molecular function. The obtained DEGs were grouped into 74 GOs, comprising 44 GOs in biological processes (59.45%), 28 GOs

Table 1
 Statistics of sequencing data with two P dosage in prolific and non-prolific genotypes

Genotype	P-dosages	Raw reads (paired-end)	Raw nucleotides (bp)	Clean reads (paired-end)	Clean nucleotides (bp)	Percentage of high-quality reads (%)	mapped reads	Percentages of mapped (%)
A (prolific)	LP	21,478,971	6,017,660,359	20,448,575	5,537,984,536	95.20	17,027,891	83.27
C (prolific)	LP	16,441,596	4,600,215,676	15,692,765	4,246,713,393	95.45	14,453,806	92.10
B (non-prolific)	LP	20,330,623	5,576,442,686	19,396,304	5,147,507,641	95.40	15,277,804	78.77
D (non-prolific)	LP	16,718,461	4,599,918,591	15,941,377	4,249,937,494	95.35	13,787,189	86.49
A (prolific)	HP	17,602,780	4,700,069,354	16,652,153	4,292,824,438	94.60	12,082,653	72.56
C (prolific)	HP	17,842,632	5,012,887,986	17,000,235	4,614,693,227	95.28	15,193,341	89.37
B (non-prolific)	HP	23,742,360	6,590,035,757	22,540,702	6,039,166,363	94.94	18,751,400	83.19
D (non-prolific)	HP	17,211,484	4,700,006,818	16,232,834	4,324,293,845	94.31	14,229,163	87.66
Total		151,368,907	41,797,237,227	143,904,945	38,453,120,937	-	120,803,246	-

Note. LP = 1.57 % v/v (low-P); HP = 14.02% v/v (high-P)

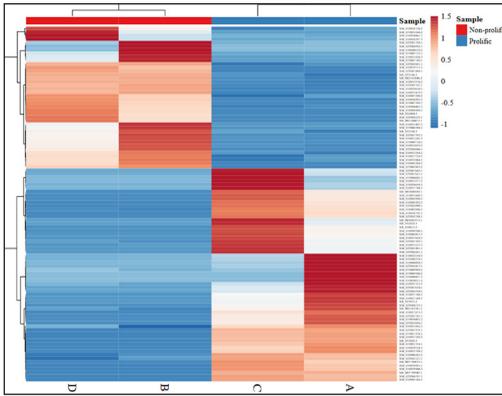


Figure 2. Heat-map of transcriptome profile of prolific and non-prolific samples at 1.57 % v/v (LP) dosage, notes: A, C = prolific genotypes; B, D = non-prolific genotypes; blue-colour = low-expression; red-colour = high-expression

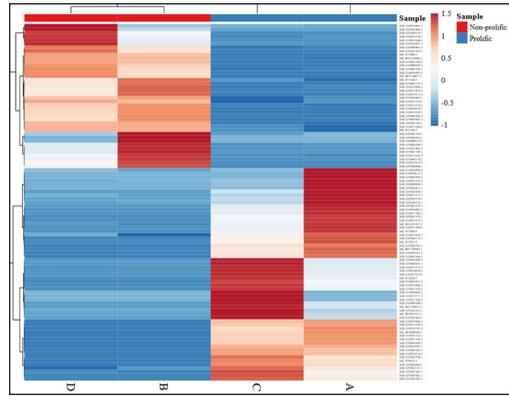


Figure 3. Heat-map of transcriptome profile of prolific and non-prolific samples at 14.02% v/v (HP) dosage, notes: A, C = prolific genotypes; B, D = non-prolific genotypes; blue-colour = low-expression; red-colour = high-expression

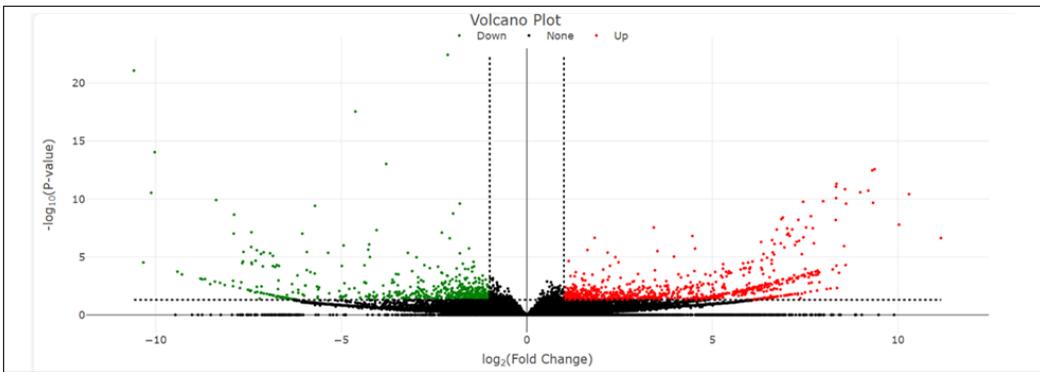


Figure 4. Volcano plots of DEGs, notes: red dot colour = upregulated; green dot colour = downregulated; and black dot colour = genes with similar expression in both samples

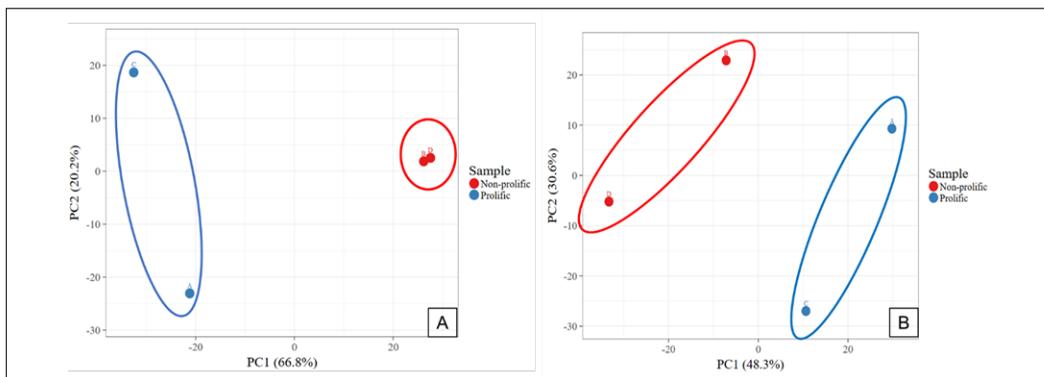


Figure 5. PCA of prolific and non-prolific genotypes based on the transcript per million (TPM) in low-P (A) and high-P (B), prolific (blue) and non-prolific (red)

in molecular functions (37.83%), and 2 GOs in cellular components (2.70%) (Figure 6). The highest gene numbers in biological process, molecular function and cellular component were response to stimulus, binding, and integral component of plasma membrane, respectively. Furthermore, the top five GO classes associated with P deficiency consisted of phosphorylation, protein phosphorylation, transferring P-containing groups, phosphotransferase activity, alcohol group as acceptor, and phosphotransferase activity-nitrogenous group as acceptor. These results indicated that the appearance of these five classes is in accordance with P as a limitation factor during hydroponic trials in oil palm seedlings at the main nursery stages.

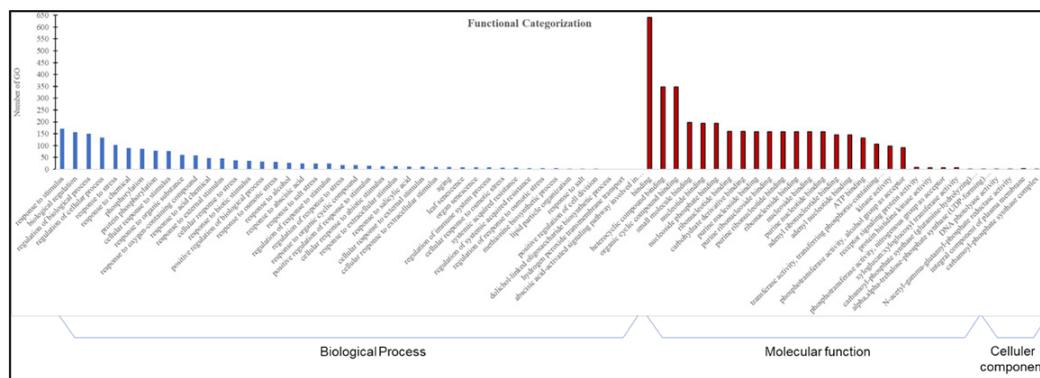


Figure 6. Functional categorisation related P deficiency in oil palm seedlings

Differentially Expressed Gene of High- and Low- Phosphorus

The number of genes involved in a particular treatment is usually shown by a Venn diagram. According to the Venn diagram, the DEGs involved in low-P, high-P and both P dosages were 1,348, 1,465 and 92 DEGs, respectively (Figure 7A). Furthermore, among 92 DEGs involved in both treatments, 45 were upregulated, and 47 were downregulated. Then, particular DEGs were selected for further analysis based on a p-value of less than 0.05 and a q-value of less than 1.00, as well as sorted by highest to lowest log2FC. The highest transcript of log2FC was observed in protein MLN51 homolog (XM_029262920) (Figure 7B). The function of this gene is to stimulate both ATPase and RNA helicase activities. While the lowest transcript was uncharacterised LOC105042954 (Figure 7B). The function of this gene in response to P stress in other crops is unclear (Meng et al., 2024).

The 92 DEGs were then screened based on the highest p-value and q-value, and continued with sorting by the highest to lowest log2FC value. From this selection criteria, 15 DEGs were selected (Table 2). Finally, five genes were chosen based on their physiological response to P condition, consisting of *NRR repressor homolog 1* (XM_010909200, ID-04), *AP-4 complex subunit sigma* (XM_010916464, ID-07),

serine/threonine protein phosphatase (XM_010909466, ID-10), *psbP domain-containing protein 7* (XM_010940137, ID-11), and *pentatricopeptide repeat-containing protein* (XM_010907539, ID-14) (Table 2).

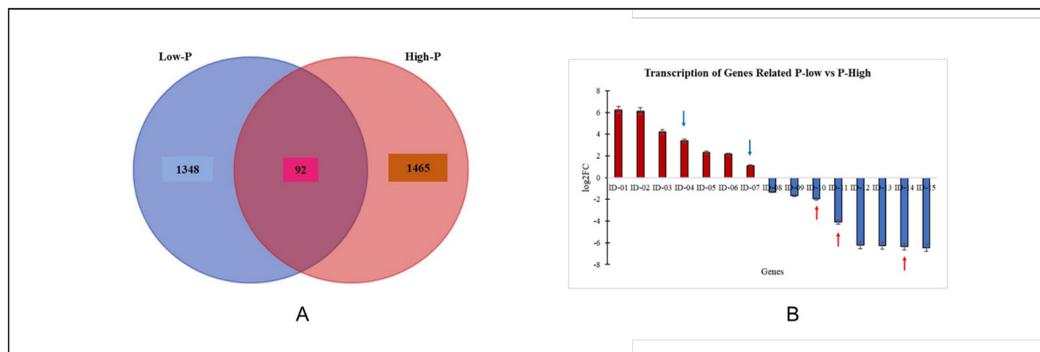


Figure 7. The number of genes involved in particular treatment of oil palm seedlings (A) and transcription level of genes responding to P deficiency (B), notes: red colour= up-regulated; blue colour= down-regulated; ID 01, 02, 03...etc. = geneID

There are two model responses of genes to nutrient deprivation. The first are early responsive genes, which respond very quickly and are often non-specific, and the second are slow responsive genes, influenced by long-term stress, which are considered to be more specific for nutrient deficiency. The classification of the five selected genes was not yet known. Stress-responsive genes can be classified into two groups: early responsive genes, which react rapidly and are often non-specific, and slow responsive genes, which are influenced by long-term stress and are considered more specific to nutrient deficiencies. The classification of the five selected genes in this study has not yet been determined. The first selected gene, *NRR* repressor homolog 1, is known to play a key role in plant defence responses against pathogens and various stresses. In rice, *NRR* undergoes alternative splicing, producing two 5'-coterminal transcripts, *NRRa* and *NRRb*. The expression of *NRR* in rice roots is notably affected by macronutrient deficiencies, particularly nitrogen and phosphorus. These transcripts, *NRRa* and *NRRb*, function as regulatory elements controlling root architecture in response to nitrogen or phosphorus deficiency (Zhang et al., 2012). The *NRRa* embraces an extra CCT domain at the C-terminus. Knockdown of both *NRR* by RNAi enhanced rice root growth. By contrast, over-expression of *NRRa* in rice revealed significantly stunted root growth. These results show that *NRR* played negative functions in rice root growth (Zhang et al., 2012). In this research, the expression of *NRR* was upregulated in P deficiency trials (Table 2). High expression of *NRR* would be possible and would exhibit significantly retarded root growth in oil palm. The finding corresponded with root length observations. The roots of seedlings grown in low-P were shorter than the roots of seedlings grown in high-P (Figure 1B).

Table 2
List of selected differential expression genes based on the highest to lowest of *p*-value, *q*-value and *log2FC*

Code	ID_Gene	Description	pvalue	qvalue	log2FC	Genes	Function
ID-01	XM_029262920	protein MLN51 homolog	0,044	1	6,23	MLN51	MLN51 stimulates both ATPase and RNA helicase activity
ID-02	XR_003801506	uncharacterised LOC105049634	0,049	1	6,11	uncharacterised LOC105049634	not characterised
ID-03	XM_010915242	kinesin-like protein KIN-4C	0,046	1	4,22	KIN4C	Microtubule-dependent motor protein plays a role in the control of the oriented deposition of cellulose microfibrils
ID-04	XM_010909200	NRR repressor homolog 1	0,038	1	3,40	NRR1	Play a role in a plant's defence response to biotic and abiotic stresses
ID-05	XM_010935203	NAC domain-containing protein 71	0,046	1	2,33	NAC071	plays a role in the tissue reunion of wounded inflorescence stems
ID-06	XM_010924311	ferritin-4	0,041	1	2,15	FER4	involved in iron homeostasis and mitochondrial iron trafficking
ID-07	XM_010916464	AP-4 complex subunit sigma	0,046	1	1,10	AP4S1	involved in plant immunity and vacuolar protein sorting
ID-08	XM_019852988	histidine protein methyltransferase 1 homolog	0,040	1	-1,31	METTL18	controlling the biosynthesis of other amino acids, growth and development of plant embryos, chelation transport of metal ions.
ID-09	XM_010933404	protein DEHYDRATION-INDUCED 19 homolog 2	0,042	1	-1,66	DI19-3	a nuclear transcriptional activator that helps plants tolerate drought stress

Table 2 (continued)

Code	ID_Gene	Description	pvalue	qvalue	log ₂ FC	Genes	Function
ID-10	XM_010909466	serine/threonine protein phosphatase	0,036	1	-1,95	PTI/STPs	enzymes that remove phosphate from threonine and serine residues in proteins
ID-11	XM_010940137	psbP domain-containing protein 7	0,035	1	-4,10	PPD7	involved in photosynthesis, defence responses, growth and development of plant
ID-12	XM_010910002	uncharacterised LOC105034740	0,048	1	-6,22	uncharacterised LOC105034740	not characterised
ID-13	XM_010926490	uncharacterised LOC105047534	0,047	1	-6,26	uncharacterised LOC105047534	not characterised
ID-14	XM_010907539	pentatricopeptide repeat-containing protein At2g13600	0,041	1	-6,36	PPR	plant growth and development
ID-15	XR_003800558	uncharacterised LOC105042954	0,043	1	-6,47	uncharacterised LOC105042954	not characterised

The second gene, which is upregulated, is *AP4SI*. *AP* complexes are important regulators of intracellular protein transport. *AP* complexes also accomplish essential tasks in controlling various physiological processes and protein sorting in plants. *AP* also engaged in plant immunity and vacuole protein sorting in response to environmental stress. *AP-1* activity leads to hindered growth, male and female sterility, and cytokinesis imperfections. *AP* demonstrated significantly retarded growth of *Arabidopsis* in P deficiency condition (Wang et al., 2023). The expression of *AP4SI* corresponded with the observed root and shoot phenotypes. In this study, seedlings grown under low-P conditions exhibited shorter roots and shoots compared with those grown under high-P conditions (Figure 1B).

The third selected gene, *PTI/STPs*, displayed a downregulated expression pattern. Serine/threonine protein phosphatases (*STPs*) are enzymes found in eukaryotes that play key roles in signal transduction and regulation of various cellular processes (Bajsa et al., 2011). *STPs* are important enzymes in plant dephosphorylation, a process that removes phosphate from serine and threonine residues on proteins. These phosphatases play crucial roles in different plant processes, including stress responses, development, and hormonal signalling. In *Arabidopsis*, *STPs* may eliminate phosphate from threonine and serine residues in proteins as a response to P deficiency. The serine/threonine protein phosphatase enzyme is controlled by *PTI/STPs* (Pais et al., 2009). The expression of *PTI/STPs* was in line with the plants' P-content. *STPs* would eradicate phosphate from threonine and serine that might be used in other metabolic reactions, thus triggering low P-content in leaves of the oil palm seedlings (Figure 1B). The expression of *PTI/STPs* may be responsive to P availability, as it is involved in plant signalling processes.

The fourth selected gene, *PPD7*, encodes a *PsbP* domain-containing protein, which is a key component of the oxygen-evolving complex (OEC) in chloroplasts and plays a crucial role in photosynthesis. Studies in *Arabidopsis* have shown that *PsbP* homologs have distinct and essential functions in maintaining photosynthetic electron transfer. In this study, *PPD7* was downregulated under P-deficient conditions (Table 2). A low transcript of *PPD7* indicated that its activity is affected by P deficiency. *PPD7* activity is influenced by the photosynthesis rate. A low transcript of the gene would diminish the rate of photosynthesis, thus inhibiting plant growth (Ishihara et al., 2007). P is known to have an essential function in photosynthesis and carbohydrate production, even though the effect of P on crops' photosynthesis is species-specific (Thuynsma et al., 2016). The expression of *PPD7* in this study corresponded with the observed morphological traits, as roots and shoots of seedlings grown under low-P conditions were shorter than those of seedlings grown under high-P conditions (Figure 1B). This suggests that *PPD7* may be particularly responsive to P availability.

The fifth selected gene, *PPR*, encodes a pentatricopeptide repeat-containing protein, which is involved in transcriptional, post-transcriptional, and translational regulation within

organelles (Meng et al., 2024). Proteins containing the PHD finger motif, including some PPR proteins, have been reported to participate in responses to abiotic stresses as well as in plant growth and development, as observed in black cottonwood (Quan et al., 2023). The expression of PPR may therefore be specifically responsive to P availability. Hence, this result suggested that five genes need to be validated as marker candidates related to P deficiency in oil palm using the RT-qPCR approach in the next study.

CONCLUSION

Understanding the basic molecular mechanisms involved in P regulations is important for developing P-use-efficient genotypes. Specific morphology characters consisted of root length, shoot length, and P-content parameters, which indicated a significant difference under the two conditions. Transcriptome profiling can be applied to identify key genes participating in the P deficiency response of oil palm seedlings. The total identified DEGs were 2,905. A total of 1,348, 1,465, and 92 DEGs were identified in low-P, high-P, and both conditions, respectively. Forty-five DEGs (49%) out of 92 DEGs identified in both treatments were upregulated, and 47 (51%) DEGs were downregulated in P-deficient conditions. Five genes were selected for further analysis based on their function in P regulation in other plants, including nutrition response and root growth (*NRR*) repressor homolog 1 (XM_010909200), *AP-4 complex subunit sigma* (XM_010916464), *serine/threonine protein phosphatase* (XM_010909466), *psbp domain-containing protein 7* (XM_010940137) and *pentatricopeptide repeat-containing protein* (XM_010907539). Five genes need to be analysed using the RT-qPCR approach for validation as marker candidates related to P deficiency in oil palm in the next study.

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