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TRANSCRIPTIONAL REGULATION OF PHT 1 INVOLVED IN PHOSPHATE DEFICIENCY RESPONSE IN OIL PALM

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Abstract: Phosphate transporter 1 (PHT 1) proteins are responsible for acquisition of phosphate (Pi) by the plants. Pi uptake by the plant needs to across a steep concentration gradient from lower concentration in the soil usually less than 10µM into high Pi concentration inside plant cells which are in the range of 5 to 20mM.Transcription factor that regulates the transcription of PHT1 is phosphate starvation response *1* (PHR 1). In this study, 10 *PHT 1* genes in oil palm were identified which are designated as *EgPHT1*;1 to *EgPHT1*;10 and two *PHR 1* (*EgPHR1;1* and *EgPHR1;2).* The profile expression of different *PHT 1* and *PHR 1* genes under Pi deficiency were determined by quantitative real-time PCR (qPCR). Both genes are highly expressed in root under Pi deficiency condition. Further study needs to be done to identify other proteins involved in PHT 1 regulatory pathway to fully understand the mechanism of Pi uptake in plant.

Keywords**:** expression pattern, PHR 1, PHT 1, Pi-deficiency, oil palm.

INTRODUCTION

Among the three main nutrients, Pi is the most immobilized element (Gu *et al.,* 2010). Pi accessibility is a worldwide constraint for crop growth in most soils. The uptake efficiency of Pi fertilizer is also very poor, where up to 80% of applied Pi cannot be acquired by the crops (Nussaume *et al.,* 2011). Assimilation of Pi with microbes and strong interaction with most of the cations such as Fe, Al and Ca worsen the availability of Pi to the crops (Nussaume *et al.,* 2011; Mohidin *et al*., 2015). Pi transporter, PHT 1 is the membrane protein which acts as the main transporter for the uptake of Pi by the plant (Muchhal *et al*., 1996). The transcription factor that regulates *PHT 1* and most of the phosphate starvation induced (PSI) genes is PHR 1. PHR 1 is the central regulator in Pi regulation pathway (Xue *et al.,* 2017). It activates the transcription of most of the genes required to combat Pi-deficiency. The objectives of this study are to find the homologue of the *PHT 1* and *PHR 1* genes in oil palm and to profile the expression of the genes during Pi-deficiency. Most of the crops already identified their *PHT 1* and *PHR 1* genes such as in rice, bean, wheat, corn and including the other major oil crop besides oil palm, soy bean (Walder *et al.,* 2015; Zhou *et al.,* 2008; Valdes-Lopez *et al*., 2008; Wang *et al*., 2013; Xue *et al.,* 2017). By identifying the *PHT 1* and *PHR 1* genes in oil palm, researchers can understand the Pi regulatory mechanism for enhancing the Pi uptake by oil palm which can increase the growth and

reduce the cost spent for Pi fertilizer. In this study, the *PHT 1* and *PHR 1* genes are identified by using bioinformatics analysis. The genes expression is profiled by using qPCR. *EgPHR1;1*, *EgPHR1;2* and *EgPHT1;4* show increased in expression during Pi-deficiency.

METHODS

The homologues of *PHT 1* and *PHR 1* genes in oil palm were identified by using BLAST in NCBI website (https://blast.ncbi.nlm.nih.gov/Blast.cgi). The *PHT 1* and *PHR 1* nucleotide sequences in *Arabidopsis thaliana* were used as the query sequences for BLAST search.

The oil palm seedlings were grown hydroponically in the Transgenic Greenhouse, Institute of Plantation Studies, Universiti Putra Malaysia. The oil palms seedlings were from the variety Dura X Pisifera provided by Sime Darby Seeds & Agricultural Services Sdn. Bhd. The Cooper nutrient solution was used in this study (Cooper, 1979). The seedlings were grown in different treatments: Pi-sufficient and Pi-deficient condition. The seedlings were acclimatized in Pi-sufficient medium for four weeks, and then followed by two weeks of treatments before harvesting.

The total RNA was extracted from the leaves and roots of the seedlings from each treatment using the technique described by Prescott and Martin (1987) with minor modification. RNA samples were treated with RNase-free DNaseI (Thermo Scientific, USA) to remove the contamination of genomic DNA. The purity and concentration of the RNA samples were quantified by using NanoDrop™ Lite Spectrophotometer (Thermo Scientific, USA). The RNA was converted to cDNA by using the SuperScript® IV First-Strand Synthesis System (Invitrogen, USA) according to the protocol from the supplier. The samples were subjected to qPCR by using QPCR green master mix LRox (Biotechrabbit, Germany) for the expression analysis.

RESULTS AND DISCUSSION

The bioinformatic analysis by using BLAST in NCBI website predicted that there are ten *PHT 1* genes in oil palm. There are named as *EgPHT1;1* to *EgPHT1;10*. The NCBI BLAST also predicted two *PHR 1* genes, *EgPHR1;1* and *EgPHR1;2* in oil palm based on the sequence similiraty with *PHR 1* gene in *A. thaliana*. The qPCR analysis showed that the expression of EgPHR1;1 and EgPHR1;2 increased in the roots under Pi-deficient condition and no significant change of expression was observed in the leaves. This suggests that both of the transcription factors are produced at high levels in the root cells during Pi-deficiency. Induced expression of PHR 1 is important during Pi-deprivation to activate the PSI genes. The high expression of EgPHR1;1 and EgPHR1;2 during Pi-deficiency also show that both transcription factors are responsive to reduced Pi level in the plant. Similarly, the expression of EgPHT1;4 increased in the roots and no significant change in expression was observed in the leaves during Pi-deprivation. This show the importance of EgPHT1;4 to transport more Pi into the plant cells during low Pi level.

Table 1. Summary of *PHT 1* and *PHR 1* genes in oil palm.

Gene name	Accession number	Locus	Length of coding region (bp)	Number of exons
EgPHT1;1	XM_010916297.1	LOC105039959	1557	1
EgPHT1;2	XM_010916307.1	LOC105039964	1278	2
EgPHT1;3	XM_010916319.1	LOC105039971	999	2
EgPHT1;4	XM_010920505.1	LOC105043079	1602	1
EgPHT1;5	XM_010920530.1	LOC105043112	1587	1
EgPHT1;6	XM_010923255.1	LOC105045093	1716	2
EgPHT1;7	XM_010937441.1	LOC105055581	1623	1
EgPHT1;8	XM_010942309.1	LOC105059103	1605	1
EgPHT1;9	XM_010906287.1	LOC105031976	1611	1
EgPHT1;10	XM_010945200.1	LOC105061222	1647	2
EgPHR1;1	XM_010935942.2	LOC105054439	1440	8
EgPHR1;2	XM_010922000.1	LOC105044188	2011	8

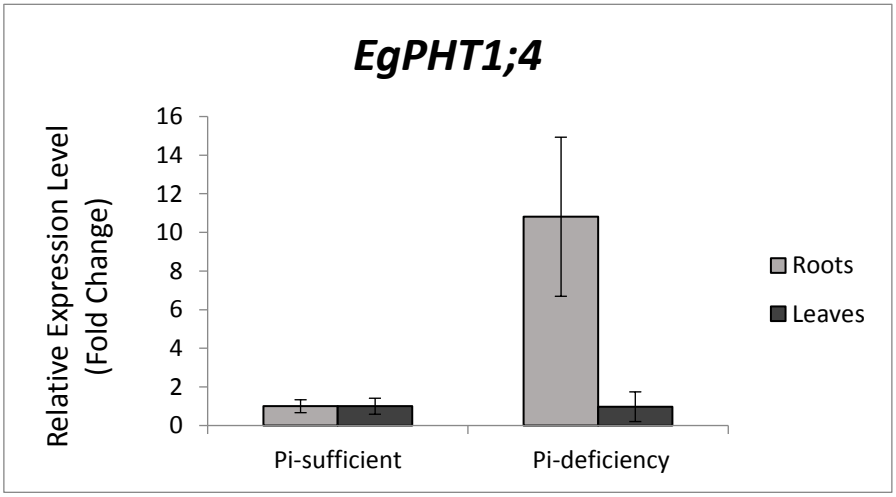

Figure 1. Expression profile of *EgPHT1;4.*

CONCLUSIONS

EgPHT1;4 is a vital Pi transpoter during Pi-deficiency to combat the low Pi level in the oil palm. EgPHT1;4 is a very promising candidate of Pi transporter for the development of low Pi tolerant oil palm variety. Interestingly, transcription factors *EgPHR1;1* and *EgPHR1;2* are both highly expressed during Pi-deficiency specifically in roots, the entry tissue of Pi acquisition. Both of the transcription factors are responsive to Pi deficiency condition and are strongly required for activation of PSI genes. Hence, this study shows the prominent function of EgPHR1;1 and EgPHR1;2 as the key regulators in Pi regulatory pathway in oil palm. Further study needs to be done to find other proteins that are involved during Pi-deprivation to fully understand the Pi regulatory pathway in oil palm.

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