Deciphering the Role of *WAVH1* in Response to Nutrient Deprivation in *Arabidopsis thaliana*

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INTRODUCTION

The ability to **detect, acquire, and utilize** bioavailable **nitrogen (N) and phosphorus (P)** determines plant productivity[1,2]

Limitations to N and P can result in a **reduction of growth**, leading to **crop yield losses**[2]

The **Ubiquitin–Proteasome System (UPS)** regulates adaptive responses to nutrient stress through the activity of **ubiquitin ligases (E3s)**, which confer substrate specificity by targeting proteins—such as transcription factors—for degradation by the 26S proteasome (Figure 1)

The *Wavy Growth* (*WAV*) family encodes for E3s involved in root gravitropism and the modification of root system architecture, processes essential for foraging nutrients in the rhizosphere[3,4]

WAV family member, ***Wavy-Growth 3 Homolog 1* (*WAVH1*),** is a candidate modulator of responses to **nitrogen (-N) and phosphorus (-P) deprivation stress**[4]

A loss-of-function *wavh1-1* mutant demonstrates differential growth and gene expression under N and P deprivation (–N and –P)[4]

Understanding *WAVH1* function as a modulator of nutrient-deficient stress responses could be leveraged to develop strategies to secure crop productivity amidst decreases in arable landmass[5]

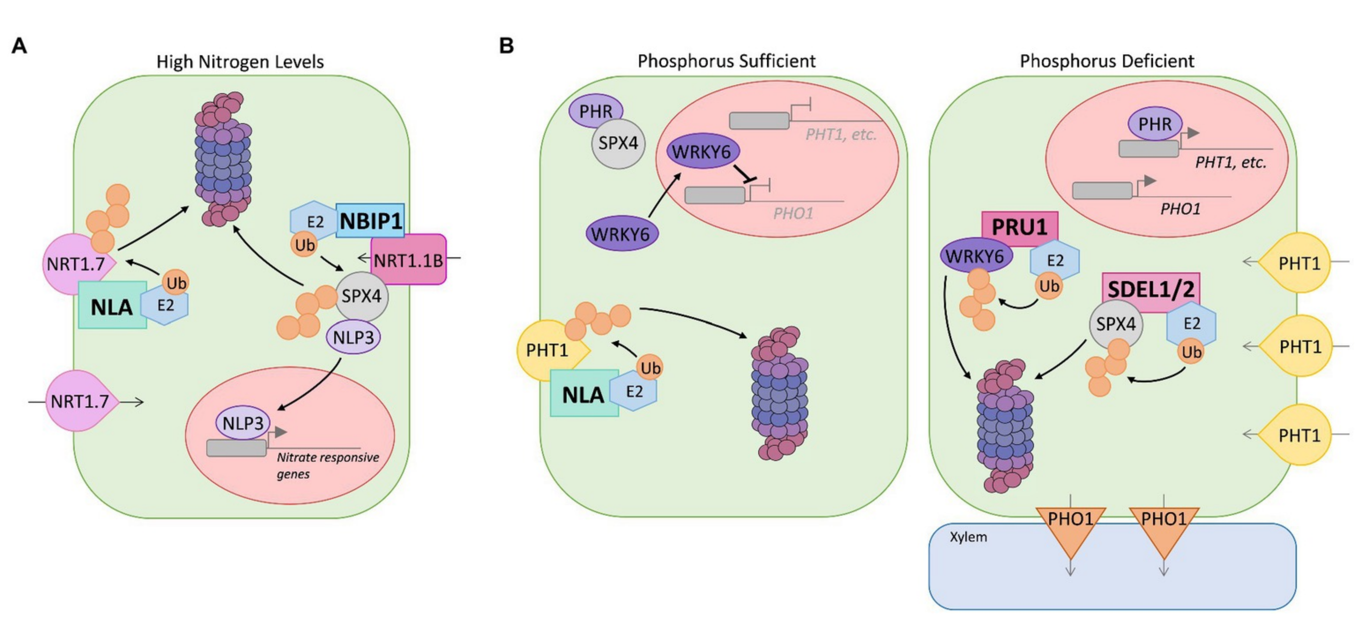


Figure 1. Simplified representation of the role of select E3s from Arabidopsis and *Oryza Sativa* (rice) in regulating nutrient uptake. (A) Under high N, the E3 NLA mediate ubiquitin-dependent degradation of NRT1.7 nitrate transporter to avoid N overaccumulation. The trans receptor NRT1.1B recruits the E3 NBIP1, which ubiquitinates SPX4, allowing the transcription factor NLP3 to enter the nucleus and promote expression of N-responsive genes. **(B)** Under Pi-replete conditions, E3 NLA ubiquitinates PHT1 inorganic phosphate transporters, facilitating degradation by the 26S proteasome to reduce uptake and prevent Pi overaccumulation. Under P-limiting stress conditions, E3s SDEL1 and SDEL2 mediate the degradation of SPX4, which allows the transcription factor PHR1/2 to activate the expression of *PSI* genes such as *PHT1*. Also, the E3 PRU1 mediates degradation of the repressor WRKY6, which relieves inhibition of *PHO1* transcription. Increase in PHO1 transporter abundance promotes loading of Pi into the root xylem.[4]

RESEARCH QUESTION

Does WAVH1 facilitate responses to nutrient limitation stress?

- Hypothesis: Loss of *WAVH1* function will alter plant growth and gene expression under nitrogen and phosphorus deficiency

METHODS

1. Nutrient stress **growth** assay

WT seedlings control (+N+P)	*wavh1-1* seedlings control (+N+P)	WT seedlings phosphorus deficient (-P)	*wavh1-1* seedlings phosphorus deficient (-P)	WT seedlings nitrogen deficient (-N)	*wavh1-1* seedlings nitrogen deficient (-N)

2. (A) Phenotypic assays → **Fresh weight**; **Primary root length**

2. (B) RNA extraction → **RNA sequencing**

2. (C) RNA extraction → **RT-PCR** and **qPCR** verification

RESULTS

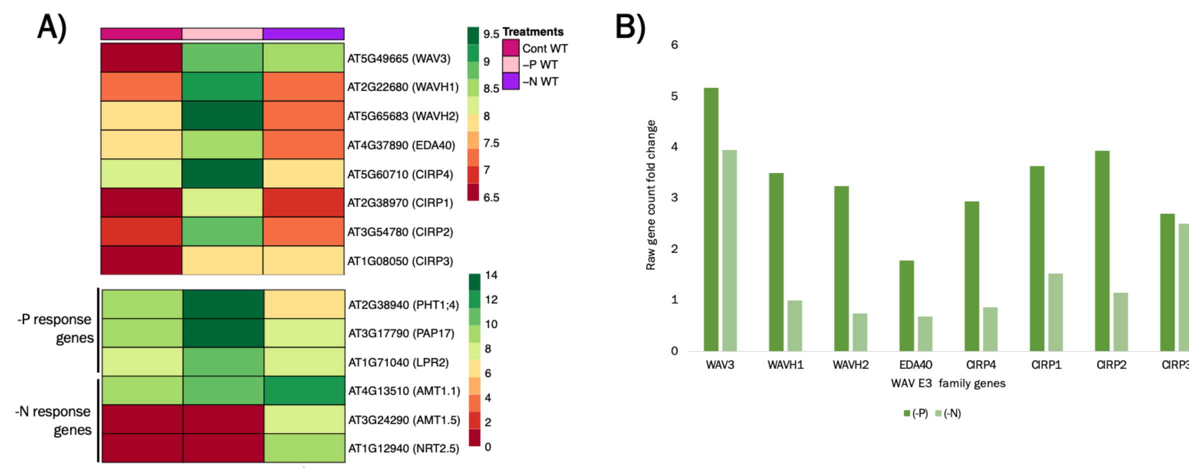


Figure 2. Increase in *WAV* family gene expression under nutrient-deficient conditions. (A) A heatmap displaying Log_2-transformed RNA sequencing data from 2 biological replicates of *WAV* family gene expression in WT seedlings grown under control (+N+P; N(10.31mM), P(0.625mM)), P-limiting (-P; 10μM), and N-limiting (-N; 20μM) conditions. Expression of some known phosphorus and nitrogen deficiency-responsive genes is also shown. **(B)** Fold change in *WAV* family gene expression in WT seedlings grown under –P and –N conditions compared to control.

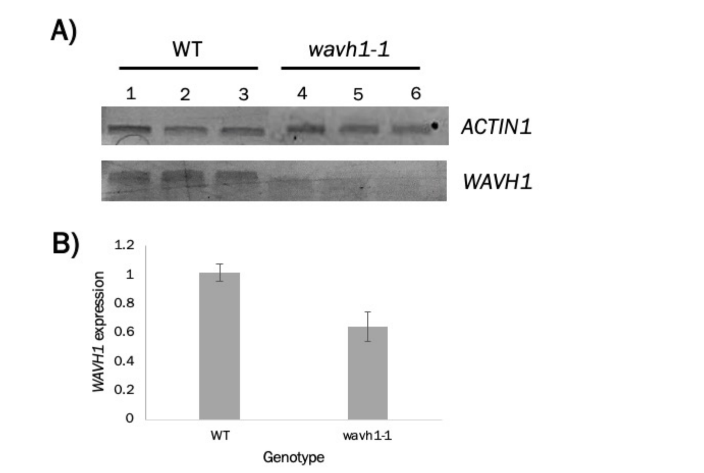
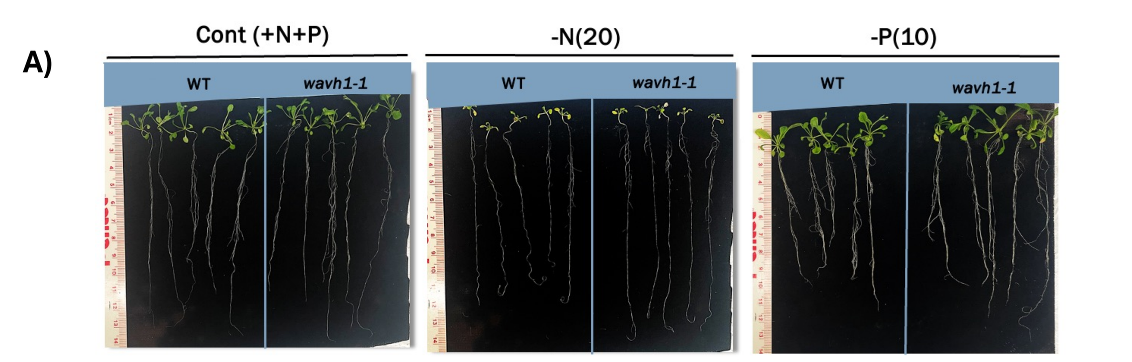


Figure 3. Reduced *WAVH1* transcript levels in *wavh1-1* mutants. (A) RT-PCR showing reduced *WAVH1* gene expression in 15-day-old loss-of-function *wavh1-1* mutant compared to WT seedlings grown under control conditions. Expression of *ACTIN1* housekeeping gene is shown as a control. **(B)** Graph showing *WAVH1* expression from the RT-PCR standardized to *ACTIN1*. Error bars represent ±SE from 3 technical replicates.



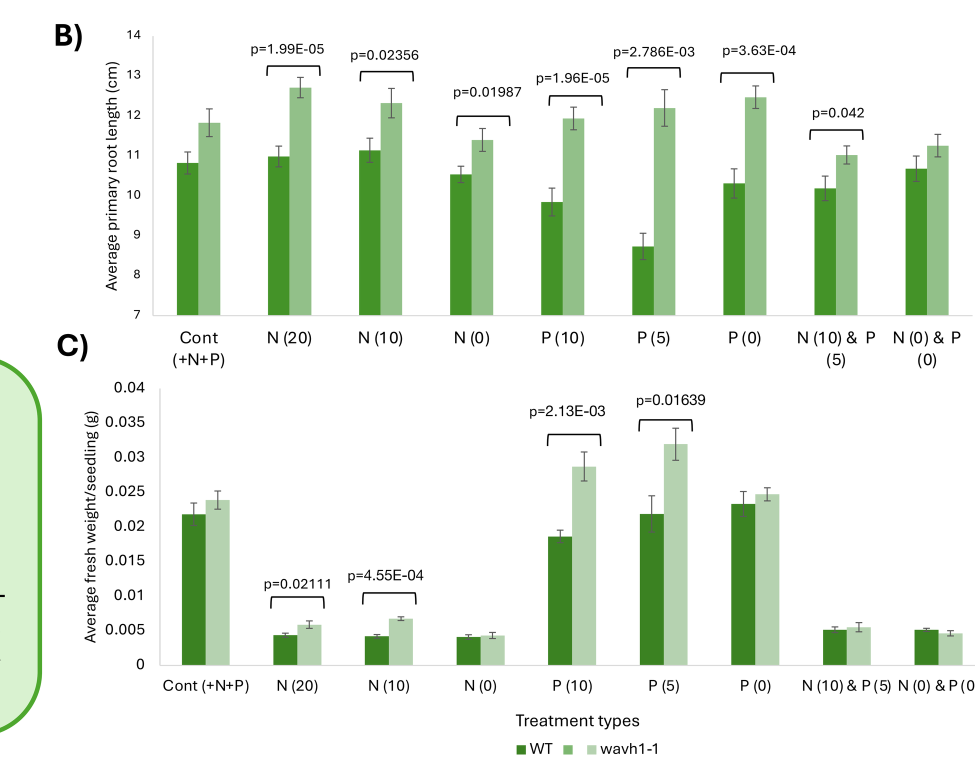
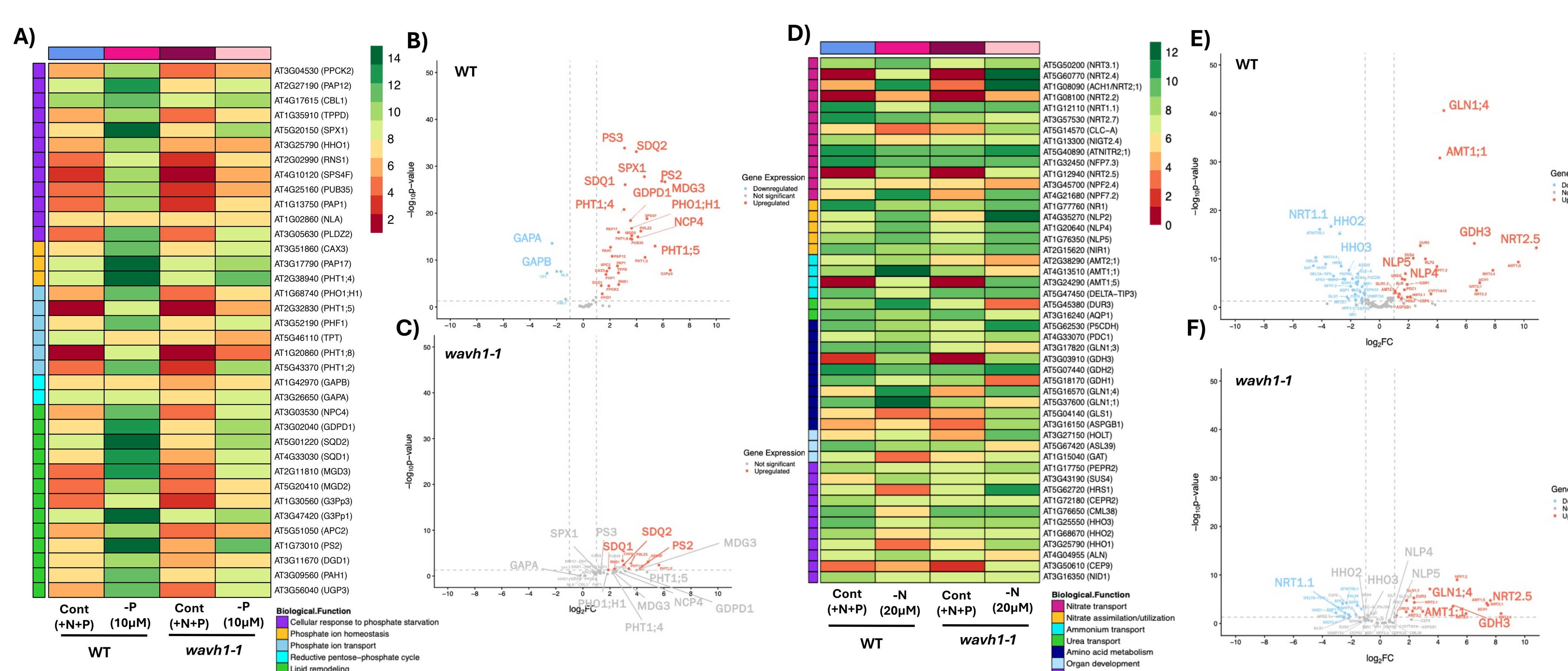


Figure 4. *wavh1-1* seedlings are more tolerant of nutrient-deficient growth conditions compared to WT. WT and *wavh1-1* seedlings were grown on control media (+N+P; N(10.31mM), P(0.625mM)) for 5 days and then transferred to growth media containing varying concentrations of P and N for 10 days. P deficiency treatments consisted of 10μM (P(10)), 5μM (P(5)), and 0μM (P(0)) KH_2PO_4. N deficiency treatments were 20μM (N(20)), 10μM (N(10)), and 0μM (N(0)) NH_4NO_3. **(A)** Pictures show representative WT and *wavh1-1* seedlings grown under control, nitrogen-deficient (-N(20 μM)), and phosphorus-deficient (-P(10 μM)) conditions. **(B and C)** Average primary root length (B) and fresh weight (C) of 15-day-old WT and *wavh1-1* seedlings following growth on N and P deficient media. One-way ANOVA was used for statistical analysis. Error bars indicate ±SE over 2 trials (N > 30).



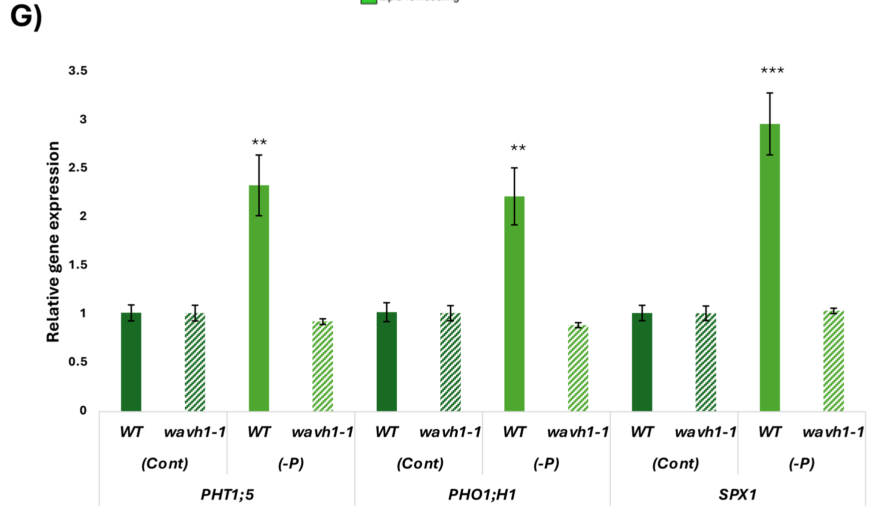
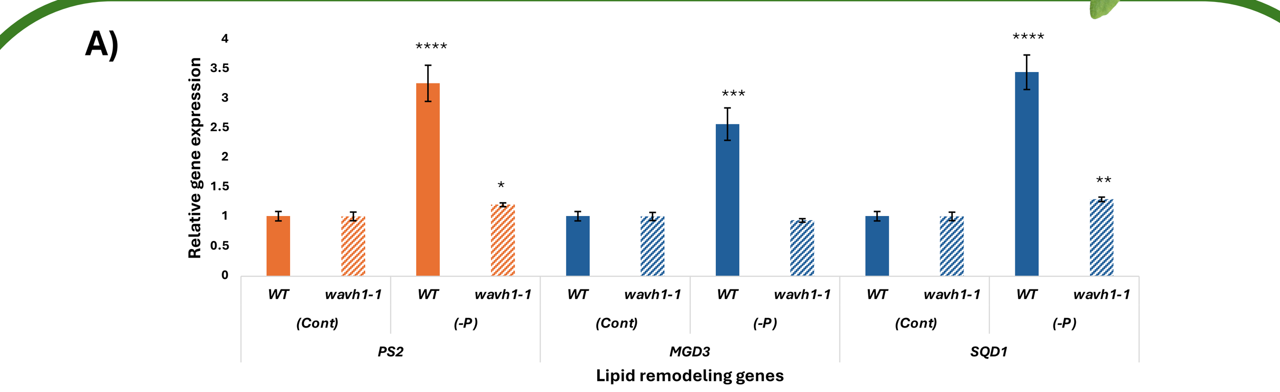


Figure 5. *WAVH1* mutants do not show the expected increase in expression of nutrient deficiency-responsive genes. (A-F) RNA sequencing analysis of phosphorus (A-C) and nitrogen (D-F) deficiency-responsive gene expression from WT and *wavh1-1* seedlings. 15-day-old seedlings transferred at 5 days from control plates, grown under control (+N+P (N(10.31mM), P(0.625mM)), phosphorus-deficient (-P (10μM) and nitrogen-deficient (-N (20μM)) conditions for 10 days. The heatmap displays log2-transformed data for phosphorus-deficient (A) and nitrogen-deficient (D) treatment types along the columns and biological functions along the rows. Volcano plots for phosphorus (B and C) and nitrogen (E and F) deficiency-responsive genes, displaying log_2 fold change and $-log_{10}$ of p-values (|LFC|> 1.0, p-value≤0.05). **(G)** Validation of RNAseq data by qPCR analysis. Relative gene expression of select phosphorus deficiency-responsive genes standardized to two housekeeping genes (*ERF-a* and GAP2C). One-way ANOVA was used for statistical analysis (P≤0.05=*, P ≤0.01=**, P ≤0.001=***, P ≤0.0001=****). Error bars indicate ±SE from 6 technical replicates, which consisted of 2 biological replicates (N = 6).



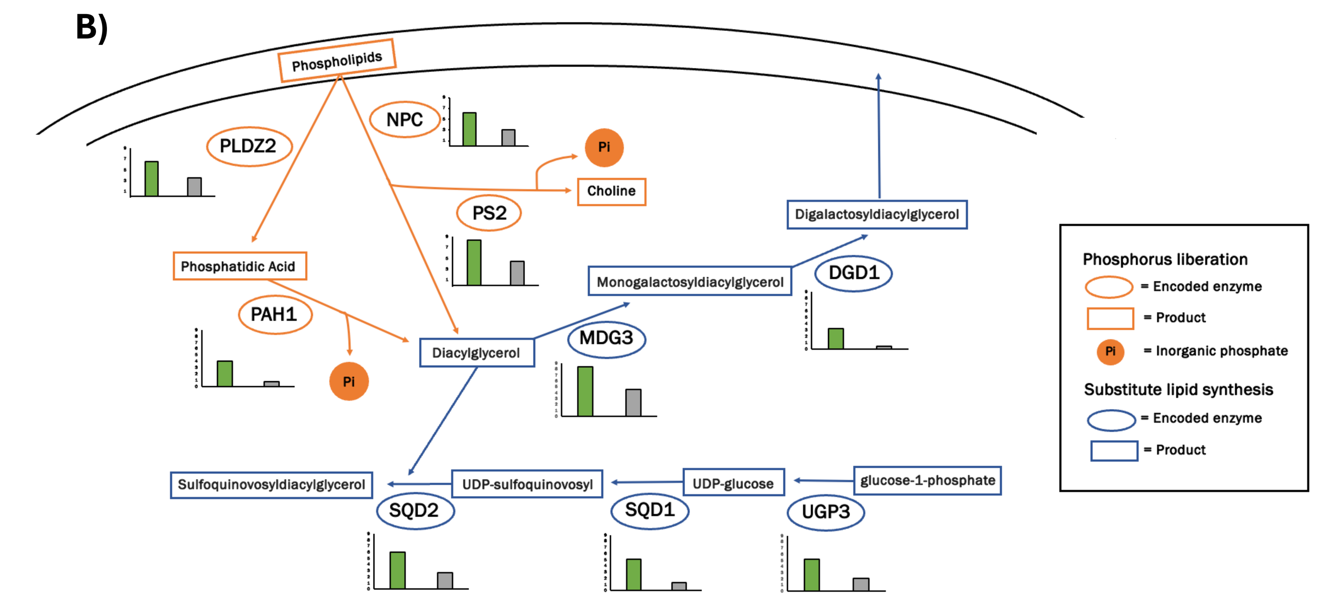


Figure 6. Select aspects of the lipid remodelling pathway that occur under phosphorus deficiency, demonstrating how gene expression varies with a reduction in *WAVH1* expression. (A) qPCR data of relative gene expression of select phosphorus deficiency-responsive lipid remodelling genes standardized to two housekeeping genes (*ERF-a* and GAP2C). One-way ANOVA was used for statistical analysis (P≤0.05=*, P ≤0.01=**, P ≤0.001=***, P ≤0.0001=****). Error bars indicate ±SE from 6 technical replicates, which consisted of 2 biological replicates (N = 6). **(B)** A simplified diagram of encoded enzymes and products of the lipid remodelling pathway, showing phosphorus liberation in orange and substitute lipid synthesis in blue. Green (WT) and grey (*wavh1-1*) bar graphs show Log_2FC of RNA sequencing data of 2 biological replicates of 15-day-old seedlings grown under –P conditions.

DISCUSSION

All **WAV family genes** show an **increase in transcription under phosphorus-deficient conditions**, suggesting roles for the E3s in modulating a tolerance to nutrient deficiency stress.

Reduction in *WAVH1* expression resulted in a significant increase in tolerance to phosphorus-deficient conditions. Slight increase in tolerance to nitrogen deficiency was also observed for *wavh1-1* mutant seedlings.

Gene expression analysis (RNAseq and qPCR) shows that the expected increase in nutrient deficiency-responsive genes was attenuated in *wavh1-1* seedlings. The ***WAVH1* mutation had a greater impact on the upregulation of phosphorus deficiency-responsive genes**, compared to nitrogen deficiency-responsive genes. Genes encoding enzymes involved in **phosphorus liberation from the bilayer** and **substitute lipid synthesis are reduced when *WAVH1* function is lost** under phosphorus deficiency.

The impact **of *WAVH1* loss-of-function mutation on nitrogen deficiency stress tolerance may be due to crosstalk** in stress signalling during the response to nitrogen and phosphorus-deficient conditions.

CONCLUSIONS

1. ***WAVH1* expression** is potentially needed for **sensing nutrients in the rhizosphere.**
2. **WAVH1 is a positive regulator** required to mount a proper response to **phosphorus deficiency,** by possibly **regulating the abundance of a transcriptional repressor.**
3. ***WAVH1*** appears to play a role in **lipid remodelling** when phosphorus is scarce.

CITATIONS

1. Choi SJ, Lee Z, Jeong E, Kim S, Seo JS, Um T, Shim JS. 2023. Signaling pathways underlying nitrogen transport and metabolism in plants. BMB Reports. 56(2):56–64. doi:https://doi.org/10.5483/bmbrep.2022-0178. https://pmc.ncbi.nlm.nih.gov/articles/PMC9978367/.
2. Razaq M, Zhang P, Shen H, Salahuddin N. 2017. Influence of nitrogen and phosphorous on the growth and root morphology of Acer mono. PLoS ONE. 12(2):e0171321–e0171321. doi:https://doi.org/10.1371/journal. pone.0171321. https://pmc.ncbi.nlm.nih.gov/articles/PMC5325205/.
3. Sakai, Tatsuya, et al. 2012. "The WAVY GROWTH 3 E3 Ligase Family Controls the Gravitropic Response in Arabidopsis Roots." *The Plant Journal*, vol. 70, no. 2, pp. 303–314, onlinelibrary.wiley.com/doi/10.1111/j.1365-313X.2011.04870.x, https://doi.org/10.1111/j.1365-313x.2011.04870.x.
4. Mackinnon E. (2024). Identifying RING-type E3s involved in nutrient stress responses in Arabidopsis thaliana.
5. Safavi-Rizi, V., Uhlig, T., Lutter, F., Safavi-Rizi, H., Krajinski-Barth, F., Sasso, S. 2024. Reciprocal modulation of responses to nitrate starvation and hypoxia in roots and leaves of *Arabidopsis thaliana*. Plant Signaling & Behavior. 19(1). doi:https://doi.org/10.1080/15592324.2023.2300228.