Posted on 23 Nov 2022 — The copyright holder is the author/funder. All rights reserved. No reuse without permission. — https://doi.org/10.22541/au.166920214.45888392/v1 — This a preprint and has not been peer reviewed. Data may be preliminary.

Physiological and transcriptomic analysis reveal the response mechanisms to nutrient deficiencies in aquatic plant Spirodela polyrhiza

Hongwei Hou[1], Xuyao Zhao[1], Xiaozhe Li[1], Zuoliang Sun[1], Gaojie Li[1], Wenjun Guo[1], Yan Chen[1], Manli Xia[1], Yimeng Chen[1], Xiaoyu Wang[1], Yixian Li[1], Kangsheng Luo[1], and Jingjing Yang[1]

[1]Chinese Academy of Sciences State Key Laboratory of Freshwater Ecology and Biotechnology

November 23, 2022

Abstract

Macrophytes are critical primary producers in freshwater ecosystem and provide potential crop output to feed the expanding human population, they also have been used to mitigate eutrophication and upgrade the water quality. Aquatic plants adapt themselves to the more complicated, changeable and unstable conditions compared to terrestrial plants, especially the fluctuated nutrient environments. Nitrogen (N) and phosphorus (P) are the key nutrient elements for plants, and their cycles have been massively altered by anthropogenic activities in diverse ecosystems. However, there is still a lack of comprehensive understanding about the adapt mechanisms of N and P stress in aquatic plants. Therefore, we investigated the response mechanisms at the molecular, physiological, and morphological levels in the macrophyte *Spirodela polyrhiza* under N deficiency, P deficiency, combined N and P deficiency, and total nutrient deficiency using RNA-seq, physiological, and biochemical measurements in this study. We found that the similar response mechanisms are shared between terrestrial plants and this tiny aquatic plant, such as nutrient deficiency-induced root system architecture (RSA) changes and photosynthetic inhibition, interacting of N/P signaling networks and uptake, and the consistent changes of gene expression profiles at transcriptional level. Encouragingly, novel findings have been found in *S. polyrhiza*. The dramatic accumulation of starch or protein without significantly growth inhibition under nutrient deficiencies, improve the crop output of *S. polyrhiza*. It has a more complex P-signaling network, which is made up of miR399, PHO2, PHT1 and lncRNAs, and miR399 should be a dual-function regulator in Pi homeostasis of *S. polyrhiza*. The N assimilation process explained the prioritizing usage of ammonium (NH_4^+)-N in duckweed, enhancing its application to phytoremediation of NH_4^+ waste water.

Physiological and transcriptomic analysis reveal the response mechanisms to nutrient deficiencies in aquatic plant *Spirodela polyrhiza*

Xuyao Zhao[1], Xiaozhe Li[1], Zuoliang Sun[1,2], Gaojie Li[1], Wenjun Guo[1,2], Yan Chen[1,2], Manli Xia[1,2], Yimeng Chen[1,2], Xiaoyu Wang[1,2,3], Yixian Li[1,2], Kangsheng Luo[1], Jingjing Yang[1*], Hongwei Hou[1*]

1 The State Key Laboratory of Freshwater Ecology and Biotechnology, The Key Laboratory of Aquatic Biodiversity and Conservation of Chinese Academy of Sciences, Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan 430072, Hubei, China

2 University of Chinese Academy of Sciences, Beijing 100049, China

3 College of Fisheries and Life Science, Dalian Ocean University, Dalian 116023, Liaoning, China

* Corresponding Author: Tel: (+8627) 68788691; Fax: (+8627) 68780123; Email: houhw@ihb.ac.cn

Posted on 23 Nov 2022 — The copyright holder is the author/funder. All rights reserved. No reuse without permission. — https://doi.org/10.22541/au.166920214.45888392/v1 — This a preprint and has not been peer reviewed. Data may be preliminary.

ABSTRACT

Macrophytes are critical primary producers in freshwater ecosystem and provide potential crop output to feed the expanding human population, they also have been used to mitigate eutrophication and upgrade the water quality. Aquatic plants adapt themselves to the more complicated, changeable and unstable conditions compared to terrestrial plants, especially the fluctuated nutrient environments. Nitrogen (N) and phosphorus (P) are the key nutrient elements for plants, and their cycles have been massively altered by anthropogenic activities in diverse ecosystems. However, there is still a lack of comprehensive understanding about the adapt mechanisms of N and P stress in aquatic plants. Therefore, we investigated the response mechanisms at the molecular, physiological, and morphological levels in the macrophyte *Spirodela polyrhiza* under N deficiency, P deficiency, combined N and P deficiency, and total nutrient deficiency using RNA-seq, physiological, and biochemical measurements in this study. We found that the similar response mechanisms are shared between terrestrial plants and this tiny aquatic plant, such as nutrient deficiency-induced root system architecture (RSA) changes and photosynthetic inhibition, interacting of N/P signaling networks and uptake, and the consistent changes of gene expression profiles at transcriptional level. Encouragingly, novel findings have been found in*S. polyrhiza* . The dramatic accumulation of starch or protein without significantly growth inhibition under nutrient deficiencies, improve the crop output of *S. polyrhiza* . It has a more complex P-signaling network, which is made up of miR399, PHO2, PHT1 and lncRNAs, and miR399 should be a dual-function regulator in Pi homeostasis of*S. polyrhiza.* The N assimilation process explained the prioritizing usage of ammonium (NH_4^+)-N in duckweed, enhancing its application to phytoremediation of NH_4^+ waste water.

Keywords : aquatic plant, *Spirodela polyrhiza* , nitrogen starvation, phosphorus starvation, ssRNA-seq

INTRODUCTION

N and P are the key macronutrients required for plant growth and development (Fageria, 2001). The growth and reproduction of photosynthetic biota are frequently limited by supplies of N or P in both of terrestrial (Elser et al., 2007; Vitousek, Porder, Houlton, & Chadwick, 2010), freshwater (Elser et al., 2007; Elser et al., 2000) and marine (Allgeier, Yeager, & Layman, 2013; Elser et al., 2007) environments. On the one hand, plants have evolved the elaborate mechanisms to adapt the deficiencies of N or P, which are called nitrogen starvation response (NSR) and phosphorus starvation response (PSR). Although lots of research focusing on NSR and PSR in model plants has been reported, we still know little about that in crops during field production, as well as in higher aquatic plants. On the other hand, excessive inorganic fertilizers are used in the process of crop production to satisfy the increasing demand of food and feedstuff, such as nitrate (NO_3^-), NH_4^+ and inorganic phosphate (Pi) (Oldroyd & Leyser, 2020). However, overuse of fertilizers allows environmental nutrient release for the low level of N use efficiency (NUE, 18–49%) (Cassman, Dobermann, & Walters, 2002) and P use efficiency (PUE, 15–30%) (Veneklaas et al., 2012) in modern agriculture, and leads to pollution in the soil and water (Oldroyd & Leyser, 2020). Therefore, it is necessary to explore the mechanisms of NSR and PSR of plants in different habits, improve NUE and PUE of crops, lower the dependency on inorganic fertilizers of crop production, and reduce the financial and environmental stress.

Liebig's law of the minimum states that plants' growth is dictated by the scarcest nutrient, and has been applied as a basic principle in various ecological, and agronomic studies on N and P (Krouk & Kiba, 2020; Paris, 1992). Previous studies have been focused mainly on investigating plant responses to single mineral availability, and led to an in-depth understanding of how plants perceive and adapt to N or P fluctuations (Krouk & Kiba, 2020). Plant changes their morphology, physiology properties, and gene expression profiles in NSR and PSR, such as modifications to root architecture, carbon metabolism, ion uptake, and hormone signaling (Oldroyd & Leyser, 2020; Rietra, Heinen, Dimkpa, & Bindraban, 2017; Schachtman & Shin, 2007; G. Xu, Fan, & Miller, 2012). However, besides single deprivation of N or P, there are complex nutrient environments for plants, such as combined N and P starvation, and even other nutrient elements. It is important to explore plant performance and response to the various nutrient environments. There is a set of molecular-physiological-morphological response in plants to accommodate nutrient deficiencies. To date, many studies suggest that N and P are interacting at ecological, physiological, and molecular levels.

Posted on 23 Nov 2022 — The copyright holder is the author/funder. All rights reserved. No reuse without permission. — https://doi.org/10.22541/au.166920214.45888392/v1 — This a preprint and has not been peer reviewed. Data may be preliminary.

Synergistic effects of combined N and P enrichment are common to aquatic and terrestrial ecosystems (Elser et al., 2007), while synergistic co-limitation of N and P is common across aquatic and terrestrial ecosystems (Harpole et al., 2011). N stimulated the uptake and translocation of P in maize (*Zea mays*) (Smith & Jackson, 1987), P or/and potassium (K) fertilizer increased N uptake and yield of rice (*Oryza sativa*) and wheat (*Triticum aestivum*) (Duan, Shi, Li, Sun, & He, 2014), P starvation decreased N uptake and assimilation in maize (de Magalhaes et al., 1998), bean (*Phaseolus vulgaris*) (Gniazdowska & Rychter, 2000), and chickpea (*Cicer arietinum*) (Esfahani et al., 2021). A series of studies have revealed that NSR and PSR are integrated by nitrate–NTR1–SPX cascade and transcription factor NIGT1 in model plants, including root development, the signaling pathway and acquisition of N and P (Hu et al., 2019; Ludewig, Vatov, Hedderich, & Neuhauser, 2021; Maeda et al., 2018; Medici et al., 2015; Ueda, Kiba, & Yanagisawa, 2020; X. Wang et al., 2020).

NSR is triggered by nitrate sensor AtNRT1.1/CHL1 (in *Arabidopsis thaliana*) (Ho, Lin, Hu, & Tsay, 2009) and OsNRT1.1B (in rice) (Hu et al., 2015), and then the expression of NSR genes is activated by the central transcription factor AtNLP7 (in *Arabidopsis thaliana*) (Marchive et al., 2013) and OsNLP3 (in rice) (Hu et al., 2019). Nitrate transporter 1 (NRT1) mediates the ubiquitination degradation of SYG1-Pho81-XPR1 (SPX) protein, thereby releases Nodule Inception (NIN)-like protein (NLP) into the nucleus, promotes the expression of NSR genes (Y. N. Cui et al., 2019; Medici et al., 2019). Inositol phosphates (InsP(8)) acts as intracellular Pi signal in plants, its content level positively correlates with cellular Pi concentration (Dong et al., 2019). SPX proteins are receptors for InsP(8), and release Phosphate Starvation Response (PHR) proteins (AtPHR1 in Arabidopsis and OsPHR2 in rice) into nucleus to promoting the expression of PSR genes (Y. N. Cui et al., 2019; M. X. Huang et al., 2019; Medici et al., 2019; Osorio et al., 2019). Nitrate-Inducible Garp-Type Transcriptional Repressor 1 (NIGT1) proteins are induced by environmental nitrate and low Pi stress, are involved in both N and P sensing, uptake, and assimilation to balance N and P content in plants (Maeda et al., 2018; X. Zhao et al., 2022).

S. polyrhiza (also named giant duckweed) is a rapidly reproducing (doubling time < 30 h under the optimal growth conditions) free-floating aquatic plant, which is distributed in various fresh water environments throughout the world (Y. L. Xu et al., 2015). *S. polyrhiza* has been considered as the ideal plant for the phytoremediation of eutrophic water to recover nutrients (N and P) from wastewaters (Cheng & Stomp, 2009). It also has been used as the aquatic crop to product food, feedstuff, feedstocks for biofuel and biogas productions, and could be developed as a major crop (Acosta et al., 2021; Cheng & Stomp, 2009). *S. polyrhiza* possesses the smallest genome (size of approximately 158 Mb) in duckweed plants (An et al., 2019; Harkess et al., 2021; S. Q. Xu et al., 2019), the stable genetic transformation and manipulation systems have been established (Y. Liu et al., 2019; J. J. Yang, Lia, et al., 2018). Therefore,*S. polyrhiza* is regarded as a model plant in the research of phytophysiology, molecular biology, genetics, and evolutionary biology (Acosta et al., 2021).

To investigating the mechanisms of NSR and PSR of plants in aquatic habits. *S. polyrhiza* were cultured in various nutrient environments (+N/-P, -N/+P, -N/-P, and H_2O), and their response at the molecular, physiological, and morphological levels were analyzed using RNA-seq, physiological, and biochemical measurements. We identified the NSR and PSR genes involved to nutrient deficiencies, and combined their expression changes with physiological and morphological changes. We found the similar but more flexible and complex response mechanisms in *S. polyrhiza* compared to terrestrial plants. The study will offer vital insight into the mechanism of N and P response in aquatic plants, and provide useful information to improve the utilization of N and P and alleviate water eutrophication.

MATERIALS AND METHODS

Plant growth conditions and nutrients limitation treatment

Giant duckweed (*S.polyrhiza* L. strain No. 7498) was obtained from National Aquatic Biological Resource Center (*http://www.nabrc.ihb.ac.cn/*) and cultivated with 1/2 strength MS (Murashige and Skoog) liquid medium at pH 5.8 in an artificial climate Chamber for 2 weeks, under the condition of 16/8 h photoperiod

Posted on 23 Nov 2022 — The copyright holder is the author/funder. All rights reserved. No reuse without permission. — https://doi.org/10.22541/au.166920214.45888392/v1 — This a preprint and has not been peer reviewed. Data may be preliminary.

(day/night) and temperature of 25/15°C. To reveal the influence of nutrient starvation on duckweed growth, 0.5 g duckweed fronds were inoculated with six replicates in a 250 mL flask containing 150 mL basal nutrient solution prepared following the recipe described in Table S1. A suitable concentration of KCl was added to the P-deficient or/and N-deficient treatment solutions to avoid K deficiency. The nutrient starvation treatments were continued one week.

Then, samples were collected with three replicates and washed in deionized water for three times and used for survey the physiological traits. The fresh weight (FW), frond size and root size were measured immediately. Dry weight, Chlorophyll (Chl) content, total P content, starch content, and protein content were measured as the previous study (J. M. Li et al., 2021; Sun et al., 2022). To investigate the transcriptome changes of duckweed in response to different nutrient starvation, the samples with the same treatment were collected with three replicates were immediately frozen in liquid nitrogen and stored at -80°C for RNA-seq.

RNA Extraction, library preparation and sequencing

Total RNA was extracted using a Ominiplant RNA Kit (CoWin Biosciences, Beijing, China), transcriptome libraries preparation, and RNA sequencing were conducted by the Origin Gene Biomedical Technology Corporation (Shanghai, China). The integrity and quality of total RNA were checked by NanoDrop2000 spectrophotometer (Thermo Scientific Inc., USA) and Agilent 2100 bioanalyzer (Agilent, USA). The strand-specific libraries were produced using Illumina Truseq^{TM} RNA sample prep Kit (Illumina, San Diego, CA, USA) and sequenced using Illumina Hiseq2000 (Illumina, San Diego, CA, USA). The RNA-seq data were deposited in NCBI Sequence Read Archive (SRA,*http://www.ncbi.nlm.nih.gov/Traces/sra/*) with accession numbers of PRJNA724886.

2.3 RNA-seq data analysis

Low-quality and contaminated reads were discarded from the raw reads, the clean reads were aligned with the *S. polyrhiza* reference genome (version 3 of strain 7498) using Hisat2 (Kim, Landmead, & Salzberg, 2015) and assembled into transcripts using StringTie software (Pertea, Kim, Pertea, Leek, & Salzberg, 2016). Gene expression levels were normalized as Fragments Per Kilobase of exon per Million fragments mapped using (FPKM) using Stringtie software (Pertea et al., 2016). Significance of expression level differences of certain genes was evaluated by ANOVA at a significance level of 0.05.

2.4 LncRNA prediction

As previously described, a vigorous pipeline was used for lncRNA identification: 1) the transcripts with ORF length $>$ 300, transcript length $<$ 200 bp, minimal reads coverage $<$ 3, or that overlapped with protein encoding genes on the same nucleotide strand were discarded; 2) the transcripts with protein-coding potential were also excluded according to the evaluation of Coding Potential Calculator (CPC,*http://cpc2.gao-lab.org/*) (Kang et al., 2017), PLEK1.2 (*https://sourceforge.net/projects/plek/files/*) (A. Li, Zhang, & Zhou, 2014), and LGC (*https://ngdc.cncb.ac.cn/lgc/*) (G. Wang et al., 2019), and the overlap transcripts of three tools were chosen for the next step; 3) the transcripts with well known protein domains were also removed based on the Pfam-hidden Markov models. The remaining transcripts were regarded as reliably expressed lncRNAs. Differentially expressed (DE) lncRNAs were determined by setting |fold-change (FC)| [?] 2 and false discovery rate (FDR) $<$ 0.05.

2.5 LncRNA target prediction

LncRNA can directly regulates the expression of the neighboring targets though *cis* -acting, on the other hand, it also could function as the competitive endogenous RNA (CeRNA) of miRNAs to regulate the expression of the miRNA targeted mRNA. The weighted correlation network analysis (WGCNA) were performed based on the expression data of mRNAs and lncRNAs in 15 RNA-seqs (Langfelder & Horvath, 2008). The*cis* -regulatory target mRNAs of Spo-lncRNA were predicted using the methods as the previous research in giant duckweed (Fu et al., 2020).

2.6 Quantitative real-time PCR (qRT-PCR) analysis

Posted on 23 Nov 2022 — The copyright holder is the author/funder. All rights reserved. No reuse without permission. — https://doi.org/10.22541/au.166920214.45888392/v1 — This a preprint and has not been peer reviewed. Data may be preliminary.

To validated the RNA-seq results, the expression of several mRNAs and lncRNAs were analyzed by qRT-PCR. Primers used for qRT-PCR were listed in Table S2. The *actin1* (ACT1) gene was used as an internal control. The first-strand cDNA was synthesized using a PrimeScriptTM RT reagent Kit (TaKaRa, Dalian, China). Then, qRT-PCR was performed using the same method in previous research (Zhao et al., 2021). Each reaction was analyzed in triplicate and the 2^{-T} method was used to analyze the data (Pfaffl, 2001).

RESULTS

The growth performance of duckweed under different nutrient stresses

Plants have evolved a diverse array of strategies in the level of morphology and physiology to obtain adequate N and P under limiting conditions (Cassman et al., 2002; Vance, Uhde-Stone, & Allan, 2003). The luxury absorption of mineral elements (N, P, K, S, etc) is a rather common phenomenon in plants (Koide, 1991; Schachtman & Shin, 2007). Therefore, the growth of plants does not be significantly inhibited when they are exposed to N or P deficiency for short-term from the optimal environment (Schachtman & Shin, 2007). In duckweeds, the appropriate treatments of N and P deficiency were used to improve the content of starch without the growth inhibition (Guo et al., 2020; Sun et al., 2022; Tao et al., 2013; C. Yu et al., 2017), indicating that the luxury absorption of N and P is also occurring in duckweeds. In the study, *S. polyrhiza*was cultured in five groups for seven days: +N/+P (N- and P-sufficient, control), -N/+P (N-deficient and P-sufficient), +N/-P (N-sufficient and P-deficient), -N/-P (N- and P-deficient), and H_2O (totally nutrient starvation). Compared to control and +N/-P groups, the fronds were blanch in -N/+P, -N/-P, and H_2O treatment groups. The fresh weight (FW) of duckweed was significantly decreased in +N/-P and H_2O treatment groups while that in -N/+P and -N/-P was not influenced (Fig. 2a). The biomass (dry weight, DW) of duckweed were similar to the results of FW (Fig. 2b). When calculating the biomass of fronds and root separately, it was found that there were more obvious differences between treatments, especially the bioaccumulation of roots (Fig. 2c). The biomass of roots in the -N/+P treatment group was the highest, followed by -N/-P and H_2O treatment groups, which was also consistent with the changes in the configuration of *S. polyrhiza* roots under different treatments (Fig. 2d-e). The number and length of roots in the -N/+P and -N/-P treatment groups were significantly higher than those of the control group, although the number of roots under the +N/-P treatment did not have significantly change. However, different types of nutrient deficiencies have led to an increase in the length of *S. polyrhiza* root and an increase in the root/frond (RF) ratio. The leaf surface area of the fronds under +N/-P treatment was also significantly lower than that of the other four groups.

Chlorophyll is the main pigment involved in photosynthesis of thylakoids, playing a key role in the process of light absorption and energy transfer. Chl a and Chl b are the main photosynthetic pigments in high plants. A variety of abiotic stresses can lead to a decrease in chlorophyll content in duckweeds, such as nutrient deprivation (J. M. Li et al., 2021; Sun et al., 2022; C. Yu et al., 2017; Z. Zhao et al., 2015), high salt (de Morais, Barbosa-Neto, Willadino, Ulisses, & Calsa, 2019; Fu, Ding, Sun, & Zhang, 2019), and metal ion stress (D. Q. Chen et al., 2020). Abiotic stress in duckweeds also leads to the accumulation of starch in chloroplasts, further destroying the structural integrity of chloroplasts, and ultimately inhibiting photosynthesis. We found that the chlorophyll content (Fig. 2i) was decreased in different degrees when *S. polyrhiza* subjected to nutritional stresses (-N/+P vs +N/+P: 0.65, +N/-P vs +N/+P: 0.93, -N/-P vs +N/+P: 0.68, H_2O vs +N/+P: 0.81), and the content of starch (Fig. 2k) was increased significantly (-N/+P vs +N/+P: 2.27, +N/-P vs +N/+P: 2.19, -N/-P vs +N/+P: 3.11, H_2O vs +N/+P: 3.32). The results showed that nutritional stresses affect the photosynthesis and carbon cycle of *S. polyrhiza* , single N deficiency seriously brought down the content of Chl and repressed the photosynthesis, even surpassed the combined N/P deficiency and totally nutrient starvation. TP content was significantly decreased in P deficient groups (+N/-P vs +N/+P: 2.19, -N/-P vs +N/+P: 3.11, H_2O vs +N/+P: 3.32), but increased in -N/+P treatment group (Fig. 2j). Protein contain was increased in +N/-P and H_2O treatment groups (Fig. 2l).

Comparative transcriptome analyses of *S. polyrhiza* under nutrient deficiencies

Posted on 23 Nov 2022 — The copyright holder is the author/funder. All rights reserved. No reuse without permission. — https://doi.org/10.22541/au.166920214.45888392/v1 — This a preprint and has not been peer reviewed. Data may be preliminary.

To gain comprehensive insights into molecular modulations in duckweed subjected to nutrient deficiency stresses, the strand-specific RNA sequencing (ssRNA-seq) of *S. polyrhiza* under different nutrient stresses were performed, as showed in our experimental design. The*S. polyrhiza* strain 7498 v3 genome date were used as reference genome, 14,036 out of 18,708 coding genes were detected in the 15 RNA libraries. Then, 1770 Spo-lncRNAs origin from 1246 lncRNA genes were discovered, including 477 reported Spo-lncRNA genes and 769 novel Spo-lncRNA genes (Table S3).

The DE-mRNAs and DE-lncRNAs between the control group (+N/+P) and nutrient stress groups were identified, including "-N/+P vs +N/+P" (3197 DE-mNRAs and 193 DE-lncRNAs), "+N/-P vs +N/+P" (343 DE-mRNAs and 27 DE-lncRNAs), "-N/-P vs +N/+P" (3706 DE-mNRAs and 225 DE-lncRNAs), "H_2O vs +N/+P" (5992 DE-mRNAs and 369 DE-lncRNAs) (Fig. 3a). A total of 7410 DEGs (6956 DE-mRNAs and 454 DE-lncRNAs) were identified in all treatments, and 205 DEGs (194 DE-mRNAs and 11 DE-lncRNAs) shared in these four nutrient treatment groups. There are most DEGs between H_2O and +N/+P treatments, while fewest DEGs between +N/-P and +N/+P treatments. DEGs between the nutrient stress groups also be identified, including "+N/-P vs -N/+P" (2043 DE-mRNAs and 136 DE-lncRNAs), "-N/-P vs -N/+P" (619 DE-mRNAs and 42 DE-lncRNAs), "H_2O vs -N/+P" (3387 DE-mRNAs and 249 DE-lncRNAs), "-N/-P vs +N/-P" (2491 DE-mRNAs and 153 DE-lncRNAs), "H_2O vs +N/-P" (4113 DE-mRNAs and 316 DE-lncRNAs) and "H_2O vs -N/-P" (2289 DE-mRNAs and 177 DE-lncRNAs) (Fig. 3a-e). The GO enrichment (Fig. S1a-d), KEGG enrichment (Fig. S2a-d), and interactive Pathways Explorer (iPath, Fig. S3a-d) of DEGs suggested that most of the DEGs are involved in the ion uptake, transcription regulation, carbon metabolism, and amino acid metabolism.

To fully interpret the molecular mechanism of responding to N or/and P deficiencies, the DEGs which participated in PSR, NSP were screened and classified. A total of 212 candidate DEGs were classified into eight subgroups, including N- and P-signaling networks, nutrient elements uptake and transport, scavenging/remobilization/recycling of N and P, hormone synthesis and signaling, transcription regulation, antioxidant system, protein metabolism, and carbon metabolism/photosynthesis (Table 1).

DEGs involved in N- and P-signaling networks

NRT1 acts as the major sensor of NO_3^-in the environment, and mediates the degradation of SPX proteins by the 26S protease complex pathway. Subsequently, NLP and PHR proteins are released into the nucleus to activate NSR and PSR genes (Hu & Chu, 2020; Osorio et al., 2019; Ueda et al., 2020). At the same time, NLP/PHR can activate the expression of*NIGT1* genes at the transcriptional level, which are the activator of PSR genes but the repressor of NSR genes (Hu & Chu, 2020; Hu et al., 2019; J. J. Yang et al., 2022). *NRT1.1* (*Spo012599*) was upregulated under -N/+P and -N/-P treatments, *SPX1*(*Spo006549*) was upregulated under different treatments, especially under +N/-P and H_2O treatments, which is consistent with previous studies in Arabidopsis and chickpea (Esfahani et al., 2021). Three *NLP* genes (*Spo005803* ,*Spo011159* , and *Spo016898*) were upregulated under -N/+P, -N/-P, and H_2O treatments (Fig. 4 and Table 1). Two*NIGT1* genes (*Spo014991* and *Spo018029*) were upregulated under +N/-P treatment, and downregulated under -N/+P, -N/-P, and H_2O treatments. Vacuolar cation/proton exchanger (VCPA) transmitted the P/N signal by altering the pH, ion concentration, and osmotic pressure in the cytoplasm. Several VCPA encoding genes (*Spo000337* , *Spo0001571* , *Spo004802* , and*Spo015649*) were differential expressing under nutrient stresses, indicated their critical roles in N-signaling and P-signaling networks.

DEGs involved in ion uptake

Plants absorb various elements from the environment through active transport/diffusion through different transporter/ion channels and transport them between tissues/cells (Y. F. Chen, Wang, & Wu, 2008; Reid & Hayes, 2003). Nitrate (NO_3^-) and ammonium (NH_4^+) are the main forms of N source in environment. Ammonium transporter 1 (AMT1) is the major high-affinity NH_4^+ transporters in plants, mediate the transmembrane uptake of ammonium, while nitrate transporter 2 (NRT2) is responsible for the absorption of NO_3^-. There were six DE-AMT1 genes under the four nutrient stress treatments, AMT1 encoded gene*Spo003051* was upregulated under both +N/-P, -N/+P, -N/-P, and H_2O treatments. *Spo000056* ,

Posted on 23 Nov 2022 — The copyright holder is the author/funder. All rights reserved. No reuse without permission. — https://doi.org/10.22541/au.166920214.45888392/v1 — This a preprint and has not been peer reviewed. Data may be preliminary.

Spo003052 , and*Spo008823* were upregulated under -N/+P, -N/-P, and H_2O treatments, and the transcriptional expression level were not influenced by the individual P starvation. *Spo002678* was downregulated under -N/-P and H_2O treatments. NRT2 encoding gene *Spo014926* was downregulated under +N/-P treatment, and upregulated under other three nutrient stress treatments. Two NRT1 encoding genes were upregulated under different nutrient stresses,*Spo005973* was upregulated under combined N and P deprivation,*Spo012599* was upregulated under -N/+P and -N/-P treatments (Fig. 5 and Table 1).

Pi is the main form of P source in environment for plant growth. Phosphate transporter (PHT) is a class of Pi/H^+symporters responsible for the absorption and translocation of Pi (Roch, Maharajan, Ceasar, & Ignacimuthu, 2019; Srivastava et al., 2018). Nineteen *SpPHT* genes have been identified in *S. polyrhiza* , including five *SpPHT1s* , one *SpPHT2* , four *SpPHT3s* , six *SpPHT4s* , one *SpPHT5* , and two *SpPHO1s* (Zhao et al., 2021). PHT1 is a high-affinity PHT system which is responsible for Pi absorption, and Phosphate1 (PHO1) protein is responsible for the xylem loading of Pi in the root (Hamburger, Rezzonico, Petetot, Somerville, & Poirier, 2002; Secco, Baumann, & Poirier, 2010). As shown in Fig. 5, three *PHT1* genes were upregulated under +N/-P and -N/-P treatments, including*SpPHT1;2* (*Spo014118*), *SpPHT1;4* (*Spo014121*), and *SpPHT1;5* (*Spo014122*). The expression of *SpPHT1;2 and SpPHT1;5* was upregulated under H_2O treatment, while SpPHT1;5 was upregulated under -N/+P treatment. The expression of*PHO1* genes (*Spo003100* and *Spo007435*) was slightly downregulated under H_2O treatment, however, there were no significant changes under the other three nutritional stresses, possibly because the fronds are the main organs for nutrient absorption in duckweed, and the root's ability to obtain nutrients from the environment is weak, resulting in a weakened role of PHO1 in mediating the transport of Pi from root to frond (Wege et al., 2016).

Besides PHT1s, PHO1s, AMT1s, and NRT1/2s, other nutrient element transporter encoding genes were influenced by the N or/and P deprivations in *S. polyrhiza* , such as K, sulfur (S), magnesium (Mg), and zinc (Zn). There K^+ transporter (KUP/HAK/KT) encoding genes were differential expressing under nutrient stresses (Table 1), *Spo010611* was upregulated under four treatments,*Spo012861* and *Spo016521* were upregulated under -N/-P and H_2O treatments, respectively (Table 3). Two differential expressed magnesium transporter (MGT) encoding genes were presented under the nutrient treatments, *Spo008974* was downregulated when N was deprived, *Spo009129* was upregulated under -N/-P and H_2O treatments. Sulfate transporter (Sultr) encoding gene *Spo006667* was upregulated under -N/-P treatment and downregulated under H_2O treatment, while*Spo010875* was upregulated under -N/+P. Zn transporter (ZnT) proteins are engaged in zinc influx, efflux, and intracellular compartmentalization, two ZnT encoding DEGs were detected in the nutrient treatments: *Spo018242* was upregulated under -N/+P, -N/-P, and H_2O treatments; *Spo006793* was upregulated under -N/+P and H_2O treatments. We also found vacuolar transporters which involved in elements storage and reuse from the candidate DEGs, such as vacuolar amino acid transporter 1 (AVT1) encoding gene (*Spo018676*) was significantly upregulated under -N/+P and -N/-P treatments, the expression of vacuolar iron transporter (VIT) encoding genes (*Spo004013* , *Spo015465* ,*Spo002693* , and *Spo016817*) were affected by the individual Pi starvation.

DEGs involved in N assimilation and P assimilation

In plants, NH_4^+ is a form of nitrogen that plants can directly utilize, and NO_3^- needs to be converted to NH_4^+ under the catalysis of nitrate reductase (NR) and nitrite reductase (NiR) before being further utilized by plants (Tegeder & Masclaux-Daubresse, 2018). Pi can be used directly after entering the cell, it can also be processed into organophosphates such as phospholipids as a constituent component of cells, or in the form of polyphosphate inositol to participate in the transmission of molecular signals and efficient storage of P (Cridland & Gillaspy, 2020; Hernando et al., 2017).

As shown in Fig. 5 and Table 1, the genes encoding enzymes involved in N and P assimilation were significantly affected by the environmental N and P contents. In the process of N assimilation, the expression of NR/NiR encoding genes was down-regulated under -N/+P, -N/-P and H_2O treatments, the expression of NR gene (*Spo015334*) and NiR gene (*Spo015293*) was down-regulated by 10-80 times in N deficient treatment groups. In previous study,*NR* gene (*XM_004500543.1*) was upregulated in the shoots

Posted on 23 Nov 2022 — The copyright holder is the author/funder. All rights reserved. No reuse without permission. — https://doi.org/10.22541/au.166920214.45888392/v1 — This a preprint and has not been peer reviewed. Data may be preliminary.

of*Cicer arietinum* under -N/+P, +N/-P and -P/.N treatments, and the expression of that in roots did not change; *NiR* gene (*XM_004505163*) was down-regulated in the roots under -N/+P, +N/-P and -N/-P treatments, and the expression in the shoots was unchanged or down-regulated (Esfahani et al., 2021). It suggests that duckweed has different priorities for the absorption and assimilation of different forms of nitrogen compared to terrestrial plants, also explained the prioritizing usage of NH_4^+ in duckweed (Petersen et al., 2021). In the subsequent assimilation process of NH_4^+, the expression of glutamine synthetase (GD) encoding genes was down-regulated in N deficient environments, while that of other enzymes was mostly upregulated under different treatments, such as isocitrate dehydrogenase (ICDH), NADH-dependent glutamine-2-oxoglutarate aminotransferase (NADH-GOGAT), glutamate dehydrogenase (GDH), aspartate aminotransferase (AAT), asparagine synthetase, and asparaginase. These findings implied that duckweed synthesize aspartic acid/asparagine through other routes to make up for the lack of NH_4^+ in the cell. In the process of P assimilation, myo-inositol phosphate synthase (MIPS), Inositol-Pentakisphosphate 2-kinase (IPK1), Inositol hexakisphosphate and diphosphoinositol-Pentakisphosphate kinase (VIP), that catalyze the biosynthesis of Inositol Pyrophosphate InsP8 from Pi, the transcriptional expressions of their encoding genes were significantly upregulated under -N/+P and -N/-P treatments, slightly upregulated under +N/-P and H_2O treatments. Inositol-tetrakisphosphate 1-kinase (ITPK) encoding gene (*Spo005248*) was upregulated under +N/-P treatment, and downregulated under other three nutrient treatments.

DEGs involved in scavenging/remobilization/recycling of N and P

In response to nutrient deprivations, plants mobilize the endogenous resources sufficiently to achieve ionic homeostasis through the reuse/redistribution of nutrients, speed the nutrients cycle up, as well as enhance the absorption of nutrients from environment adaptively. The catabolism of P-containing compounds (such as phospholipid membranes, nucleic acid molecules, organophosphorus such as inositol phosphate) was accelerated in plants under Pi deprivation to achieve the reuse/redistribution of Pi. Purple acid phosphatase (PAP) releases Pi by catalyzing the enzymatic lysis of a variety of phosphate compounds (P. D. Liu, Xue, Chen, Liu, & Tian, 2016; Zhang, Wang, Tian, Li, & Shou, 2011). As shown in Table 1, there were nine differential expressing*PAP* genes in the treatment groups, most of which changed under H_2O treatment, *Spo001654* was upregulated in all four treatment groups; *Spo001264* was downregulated under -N/+P, -N/+P, and H_2O treatments; *Spo011727* was upregulated under -N/+P, -N/+P, and H_2O treatments;*Spo010600* and *Sp018669* were significantly upregulated in +N/-P and H_2O treatment groups; Spo001265 was raised only in +N/-P treatment group; and *Spo016760* was raised in -N/+P treatment group. The expression of Ribonucleases encoding genes (*Spo000596* , *Spo001015* , and *Spo002478*) were upregulated in -N/-P and H_2O treatment groups. Inorganic pyrophosphatase accelerates the cycling of Pi by hydrolysis of intracellular pyrophosphate (PPi), *Spo008751* was upregulated in all four treatment groups, and *Spo006838* was upregulated in the +N/-P and H_2O treatment groups.

DEGs involved in carbon cycling

Plants produce hexose (C6) by photosynthesis to fix CO_2, which is then stored in different forms such as sucrose and starch, and consumed and provided with energy in respiration. Photosynthetic products are mainly used for vegetative growth in duckweed under the suitable environment, and when subjected to environmental pressures such as nutritional stress (J. M. Li et al., 2021; Sun et al., 2022; C. Yu et al., 2017), heavy metal stress (H. Xu et al., 2018; J. Yang et al., 2022; J. J. Yang, Li, et al., 2018), cold stress (Bovet, Kammer, Suter, & Brunold, 2000), and high salt stress (Fu et al., 2019; Sree, Adelmann, Garcia, Lam, & Appenroth, 2015), carbon metabolism in duckweed is disturbed, and a large amount of starch accumulates in chloroplast. Nutrient stresses affect the photosynthesis and carbon cycle of *S. polyrhiza* , single N deficiency seriously brought down the content of Chl and repressed the photosynthesis, even surpassed the combined N/P deficiency and totally nutrient starvation.

A total of 15 enzymes involved in the synthesis of Chl a from Glu-tRNA have been identified in *A. thaliana* (Beale, 2005). Accordingly, we analyzed the expression changes of the coding genes of these enzymes in *S. polyrhiza* under different nutritional stresses, to discovered the relationship between nutritional stress and light and pigment content was analyzed. As shown in Fig. 6, the expression of 14 enzymes were dramatically

Posted on 23 Nov 2022 — The copyright holder is the author/funder. All rights reserved. No reuse without permission. — https://doi.org/10.22541/au.166920214.45888392/v1 — This a preprint and has not been peer reviewed. Data may be preliminary.

downregulated under individual/combined N deficiency (-N/+P, -N/-P, and H_2O treatments), except that of Ferrochelatase (FeCH) who catalyze the synthesis of Heme from Protoporphyrinogen IX (Proto IX). And few DEGs were upregulated in treatment groups, such as Glutamyl-tRNA reductase (HEMA) encoding gene*Spo005442,* Porphobilinogen deaminase (HEMC) encoding gene*Spo016423* , and FeCH encoding gene *Spo010281.*

C6 (mainly glucose and fructose) also can be converted from the stored starch and sucrose to supply the plants with energy besides photosynthesis. Starch is the main form of stored carbohydrate in duckweed, and is mainly stored in the chloroplast of fronds (J. M. Li et al., 2021). Alpha-amylase and beta-amylase catalyzes the hydrolysis of starch into glucose, then glucokinase catalyzes glucose to Glucose-6-Phosphate (G6P) which could be transported transmembrane. Starch phosphorylase catalyzes the hydrolysis of starch into Glucose-1-Phosphate (G1P), and then phosphoglucomutase (PGM) catalyzes G1P to G6P. The expression of the genes encoding enzymes that involved in starch degradation in different treatment groups changed with the accumulation of starch (Fig. 7). The expression of genes encoding α-amylase was upregulated in the -N/-P treatment group and down-regulated in the H_2O treatment group. There were two DE-α-amylase genes: *Spo011231* was upregulated in different treatment groups, with the highest expression in the -N/-P treatment group and the lowest expression in the +N/-P treatment group; *Spo002790* was down-regulated in the treatment groups except +N/-P and decreased significantly in the -N/-P and H_2O treatment groups. Three β-Amylase genes (*Spo000804* ,*Spo009506* , and *Spo014185)* were upregulated under every nutrient stresses, while Spo016333 only be upregulated under +N/-P stress. Starch phosphorylase (*Spo005034*) was upregulated under -N/+P and -N/-P treatments, phosphoglucomutase (PGM) encoding gene *Spo017307* was downregulated in -N/+P, -N/-P, and H_2O treatment groups.

Sucrose is the main format of photosynthetic products in some plants, can be transmitted in plant, it also be involved as a signaling factor in regulating the expression of sugar synthesis and decomposition related genes (Yoon, Cho, Tun, Jeon, & An, 2021). Sucrose can be dissociated into one molecule of glucose and one molecule of fructose under the action of alkaline invertase (AI) or sucrose synthase (SS). In this study, we did not detect the expression of AI encoding genes in the RNA-seq data, and the total expression of SS encoding genes were upregulated under all the treatments, especially the H_2O treatment.*Spo004483* , *Spo010656* , and *Spo017757* were upregulated in all treatment groups, Spo000698 was downregulated under -N/+P, -N/-P, and H_2O treatment. The fructokinase coding genes (*Spo002432, Spo004982* , *Spo008257* , *Spo015361*) and PGM gene (*Spo017307*) were downregulated in the treatment groups.

G6P is the common substrates of glycolysis and pentose phosphorylation metabolic pathways. Glycolysis released the sugar molecule and transferred it to adenosine triphosphate (ATP) and the reduced coenzyme nicotinamide adenine dinucleotide (NADH). The early steps of glycolysis from G6P to 3-P-glycerate were inhibited under individual/combined N deficiency (-N/+P, -N/-P, and H_2O treatments), and the expressions of related enzymes were downregulated. Such as ATP-dependent phosphofructokinase (ATP-PFK) encoding gene*Spo013128* , *aldolase* genes (*Spo010225* ,*Spo011373* , *Spo017380*), enolase encoding gene*Spo015300* , and pyruvate dehydrogenase complex (PDC) encoding gene*Spo007769* . Though the total expression level of*glyceraldehyde-3-Phosphate dehydrogenas* e (*GADPH*) and*phosphoglycerate kinase* (*PGK*) genes were downregulated, they both had the DEGs with different expression profiles. Such as*Spo008868* and Spo017211 were upregulated in all the treatment groups; *Spo018308* was downregulated under +N/-P treatment and upregulated in -N/-P treatment group; *Spo001888* ,*Spo003439* , and *Spo017145* were downregulate under individual/combined N deficiency (-N/+P, -N/-P, and H_2O treatments). What is really interesting is that the expression of*non-phosphorylating glyceraldehyde-3-phosphate dehydrogenase*(*GAPN*) genes was upregulated which catalysis glyceraldehude-3-P to 3-P-glycerate and product one molecule of NADPH in all the treatment groups. It suggested that the NADPH that provides reducing force is more required than ATP and NADH that provide energy in *S. polyrhiza* under nutrient stresses, especially the individual/combined N deficiency. In the latter steps from 3-P-glycerate to pyruvate were enhanced, and the genes encoding 2,3-bisphosphoglycerate-independent phosphoglycerate mutase (iPGAM), Pyruvate kinase, and pyruvate phosphate dikinase (PPDK) were upregulated under all the treatments.

Posted on 23 Nov 2022 — The copyright holder is the author/funder. All rights reserved. No reuse without permission. — https://doi.org/10.22541/au.166920214.45888392/v1 — This a preprint and has not been peer reviewed. Data may be preliminary.

Pentose phosphate pathway provides critical productions such as ribose-5-phosphate (R5P) that is vital for synthesis of nucleotides and coenzymes, and NADPH that is kept ready to donate electrons in biosynthetic reactions. In individual/combined N deficiency (-N/+P, -N/-P, and H_2O) treatment groups, the main enzymes coding genes were upregulated, such as glucose-6-Phosphate dehydrogenase (G6PDH) encoding gene *Spo010623* , 6-Phosphogluconate dehydrogenase (6PGDH) *Spo012893* . And the latter steps for production R5P and other C4/5/7 were inhibited individual/combined N deficiency (-N/+P, -N/-P, and H_2O treatments), the genes encoding ribose 5-phosphate isomerase (RPI), ribulose-5-phosphate-3-epimerase (RPE), transketolase, and transaldolase were significantly downregulated.

DEGs involved in hormone synthesis and signaling

Phytohormones play critical roles in helping the plants optimal response to environmental stresses (Fujita et al., 2006). Phytohormones synthesis and signaling are influenced by nutrient stresses in *S. polyrhiza* at transcriptional level, including auxin, Gibberellin acid (GA), salicylic acid (SA), abscisic acid (ABA), strigolactones (SLs), and ethylene (ET) (Table 1). GA is one of the major growth promoting hormones and involved in response to environmental stresses (Yamaguchi, 2008). Several GA synthesis related genes were downregulated in *S. polyrhiza* under nutrient stresses, including two *Gibberellin 20 oxidase* (*GA 20-ox*) genes (*Spo009147 and Spo017291*) which were downregulated in +N/-P, -N/-P, and H_2O treatment groups. SLs act as sensors during early plant responses to both N and phosphate starvation and mediating the N–P signaling interplay (Gamir et al., 2020; Marro et al., 2022). Carotenoid cleavage dioxygenase 7/8 (CCD7/8) is the key enzymes in the synthesis of SLs (Al-Babili & Bouwmeester, 2015). The expression of four *CCD8*genes were changed in the treatment groups, *Spo006906* was upregulated under -N/+P and -N/-P treatment while downregulated under H_2O treatment, *Spo006911* was upregulated in -N/+P and +N/-P treatment groups,*Spo006908* was upregulated under -N/+P treatment while downregulated in H_2O treatment group, *Spo006913*was upregulated under -N/+P treatment. SA is one of the major plant defense response hormones, *Spo009600* that encode salicylic acid carboxyl methyltransferase (SAMT) involved in salicylic acid synthesis was upregulated in -N/-P group.

There were lots of DEGs involved in hormone signaling in *S. polyrhiza* under nutrient stresses, especially in -N/-P and H_2O treatment groups. Auxin efflux carrier coding gene*Spo000620* was upregulated under -N/-P and H_2O treatments, and *Spo007442* was upregulated in -N/+P, -N/-P and H_2O treatment groups. Auxin-induced in root cultures protein 12 (AIR12) coding gene Spo010019 was upregulated under all the treatments, it also explains the morphological changes of *S. polyrhiza* under different nutritional stresses, especially the number and length of the roots. ABA-insensitive (ABI) are a class of transcription factors that play a negative regulatory role in the abscisic acid signal transduction pathway (Fujita et al., 2006), ABI5 (*Spo016764*) was upregulated in -N/+P, -N/-P and H_2O treatment groups. Four Ethylene response factor (ERF) coding genes (*Spo011055* , *Spo017677* ,*Spo011055* , and *Spo017677*) were upregulated under all the treatments, and had the relative high expression level in -N/-P and H_2O treatment groups.

DEGs involved in antioxidant system

Environmental stresses cause the accumulation of reactive oxygen species (ROS) in plants, and these excess ROS can cause oxidative damage if not cleaned up in time (Gill & Tuteja, 2010; Mittler, Vanderauwera, Gollery, & Van Breusegem, 2004). The expression levels of several Superoxide dismutase (SOD) encoding genes were changed under nutritional stresses, *Spo002187* was downregulated in -N/+P and H_2O treatment groups, *Spo009504 was upregulated in* -N/+P and -N/-P treatment groups. Glutathione (GST) is the main form of antioxidant in plants, provides reducing power and be involved in plant response to various stresses (Mittler, 2002). Expression of three glutathione transferase (GST) encoding genes were changed in treatment groups, *Spo000611* was upregulated in +N/-P, -N/-P, and H_2O treatment groups, *Spo000612* and*Spo004760* were upregulated in -N/+P, -N/-P, and H_2O treatment groups. Glutathione peroxidase (GPX) encoding genes的*Spo010444* was upregulated in -N/+P, -N/-P, and H_2O treatment groups, *Spo012043* was downregulated in -N/+P, -N/-P, and H_2O treatment groups. The above results show that due to the weak inhibition of photosynthesis in the +N/-P treatment group, the peroxide accumulation in *S. polyrhiza*was less than that in -N/+P, -N/-P, and H_2O treatment groups (Mittler et al., 2004).

Posted on 23 Nov 2022 — The copyright holder is the author/funder. All rights reserved. No reuse without permission. — https://doi.org/10.22541/au.166920214.45888392/v1 — This a preprint and has not been peer reviewed. Data may be preliminary.

3.10 Transcription factors

Transcription factors (TFs) play key regulatory roles in the growth and development of plant, and adapt to the stimulation of the external environment through altering the gene expression profile of their target genes and subsequent physiological and morphological responses. Some TFs involved in nutritional adaptations have been well studied, such as PHRs and NIGT1s belonging to GARP superfamily (Bari, Pant, Stitt, & Scheible, 2006; Safi et al., 2017; Ueda et al., 2020), NLPs belonging to RWP-RK TF family (K.-h. Liu et al., 2017; Marchive et al., 2013), WRKY6/42/75 (Y.-F. Chen et al., 2009; Devaiah, Karthikeyan, & Raghothama, 2007; Su et al., 2015), bZIP member ELONGATED HYPOCOTYL5 (HY5) (X. Chen et al., 2016; L. Huang, Zhang, Zhang, Deng, & Wei, 2015). To reveal the TFs involved in the response to N/P starvation, a total of 358 differential expressed TFs belonged to 41 TF families were identified in *S. polyrhiza* under nutrient stresses (Fig. 8 and Table S4). In which, bHLH (56), bZIP (24), ERF (28), MYB (39), NAC (24), WRKY (27) TF families had the most DEG genes (Fig. 9). Server TFs are the critical nodes in N- and P-signaling networks, such as NIGT1s, PHRs, and NLPs (Fig. 4). The expressions of two NIGT1 and three NLP genes were influenced by N and P deficiencies in *S. polyrhiza* . However, the expressions of*SpPHR1 -Spo003067* and *SpPHR2 -Spo010995* did not changed under nutrient deficiencies, which also similar to the previous studies in Arabidopsis (Bari et al., 2006) and rice (J. Zhou et al., 2008). *HY5* homologous *Spo013126* , *WRKY42*homologous *Spo015050* , *WRKY75*homologous genes *Spo002165*and *Spo010511* were upregulate in all the treatments. OsPTF1 (rice Pi starvation-induced transcription factor 1) is a bHLH TF induced by P deficiency to enhance the tolerance of Pi starvation in rice (Yi et al., 2005), TabHLH1 (in *Triticum aestivum*) is induced by N and P deprivation, regulates the expression of *NRT2* and *PHT1* to enhance the acquisition of N and P (T. R. Yang et al., 2016). We also found that some *SpbHLH* genes were significantly upregulated under nutrient stresses, including *Spo001307* , *Spo007425* ,*Spo013334* , and *Spo013581* .

3.11 Function prediction of lncRNAs

LncRNA can regulate the expression of mRNAs through *cis* - and*trans* -actions. To identify the regulatory networks between lncRNAs and mRNAs. In our study, the genome localization and WGCNA analysis of mRNA and lncRNA genes were conducted. A total of 323 coding genes were *cis* -acting regulated by 254 lncRNAs (Fig. 10). In the trans-acting networks, 23 lncRNA and 154 mRNAs were interacting with 24 miRNAs as ceRNA (Fig. 11). Some lncRNAs were involved in the NSR or PSR, such as *XLOC_-015142* which was the cis-acting regulator of*SpPHT1;2* (*Spo014118*) involved in Pi uptake (Zhao et al., 2021). *XLOC_005099* was the *cis* -acting regulator of four neighbor coding genes: *reversibly glycosylated polypeptide 1*(*PGP1* , *Spo002163*) which participates in the synthesis of cell walls*, Spo002164* , the key TF involved in low phosphorus response *SpWRKY22* (*Spo002165*) (Zhao et al., 2021), and plastocyanin-like domain containing protein (*Spo002166*).*XLOC_001243 -Spo000953* (phospholipase A, PLA) and*XLOC_-016922 -Spo016725*(phospholipase A, PLA)*cis* -acting gene pairs were the recovery and reuse of intracellular P (Nakamura et al., 2009; Nguyen et al., 2016). In the miRNA-mRNA-lncRNA ceRNA networks, *XLOC_005639* ,*XLOC_006842* , *XLOC_013682* , and *XLOC_007237* were found regulated by miR399, functioned as the ceRNAs of*dicarboxylate transporter 1*(*DiT1* , *Spo003189*),*SpPHT1;1* (*Spo003133*)*, and SpPHO2*(*Spo007243*). DiT1 is an integral membrane protein involved in exchange of Pi, sulfate, and thiosulfate (Taniguchi et al., 2002). PHO2 and PHT1 are critical components of P signaling and uptake in plants (Bari et al., 2006). miR399 always target to *AtPHO2* homologs in plants (Bari et al., 2006), but *ZePHT1;7* and *ZePHT3* in maize (Pei et al., 2013). We found Spo-miR399 target to *SpPHO2*and *SpPHT1.1* , indicated miR399 could be a dual-function regulator in Pi homeostasis in *S. polyrhiza* (Zhao et al., 2021). In summary, lncRNA responds to different nutritional stresses by regulating gene expression in *S. polyrhiza* by regulating *cis* - and*trans* -acting, and further research is needed to elucidate the molecular mechanisms in it.

3.12 Quantitative RT-PCR validation of transcriptional expression

To verify the RNA-seq results, seven mRNAs (*SpNIGT1.1/2* ,*SpSPX1* and *SpPHO2* involved in N and P signaling,*SpPHT1;5* involved in Pi uptake, SpAMT2;1 involved in NH_4^+ uptake, *SpSS1* involved in starch synthesis) and five lncRNAs (*SPOL_LNC003113* ,*SPOL_LNC002711* , *SPOL_LNC003124* , *SPOL_LNC002128* ,*SPOL_LNC002845*) were chosen to perform qRT-PCR. As shown in Fig. 12, the relative expression levels

of 12 genes were basically consistent under different nutritional conditions between qRT-PCR and RNA-seq results.

DISCUSSION

Nutrient deficiency is a common abiotic stress in the process of plant growth and development. N and P are essential macro-elements for plant growth, and N/P fertilizer should be applied in agricultural production to ensure food security (Cordell, Drangert, & White, 2009; Hell & Hillebrand, 2001; G. Xu et al., 2012). An in-depth understanding of the molecular mechanism of plants' adaptation to low N and low P environments can provide theoretical basis and data support for improving the N and P utilization rate of plants and reducing the amount of fertilizer. This study analyzed the physiological and RNA-seq data of*S. polyrhiza* under different nutritional conditions to analyze the adaptation mechanism of *S. polyrhiza* to different nutrient stresses.

When plants perceive the lack of nutrients in the environment, they respond through a series of adaptive behaviors, including from the molecular level, physiological level to plant morphology changes (Chiou & Lin, 2011; Yuan & Liu, 2008). The root system is the main organ of terrestrial plants that sense and take nutrients, it is also the organ first to perceive the environmental stimuli and respond to the absence of nutrients (Balzergue et al., 2017; Rouached, Arpat, & Poirier, 2010; Yuan & Liu, 2008). The physiological and morphological plasticity of root types is particularly important in the response to nutrient deficiency. In Arabidopsis, the growth of the primary root is inhibited, while the formation and growth of lateral roots is strengthened under low Pi stress (Kellermeier et al., 2014; Peret, Clement, Nussaume, & Desnos, 2011), and low N inhibits the lateral root development (Kellermeier et al., 2014). In maize, Pi deficiency can promotes the development of lateral roots, increase the total root length and root-to-shoot (R/S) ratio, and promotes the formation of mycorrhizae (P. Yu, Gutjahr, Li, & Hochholdinger, 2016). In *Lupinus albus* , Pi deficiency promotes the growth of lateral root and forms a large number of cluster roots (Neumann et al., 2000). The root system architecture (RSA) of *S. polyrhiza* is relatively simple, but it still presents the typical RSA changes of monocotyledon under different N/P deficiencies, mainly showed in the increase of the number and length of adventitious root (X. X. Li, Zeng, & Liao, 2016). The root number, root length, biomass of root, and R/F ratio were the lowest in *S. polyrhiza* when N and P were adequately supplied in the control group, and RAS changed dramatically under nutrient deficiencies, especially in the -N/+P and -N/-P treatment groups. It suggested that the root development is enhanced and the allocation of resources is inclined to the roots when suffering from N/P deficiency in duckweeds, as that in terrestrial plants (Esfahani et al., 2021; Gruber, Giehl, Friedel, & von Wiren, 2013). Though fronds are the organ for nutrient absorption in *S. polyrhiza* (An et al., 2019), roots play the vital complementary role for the uptake of nutrients under the impoverished environment (Zhao et al., 2021). The phenotype plasticity also presents in the aerial parts of plants when suffering nutrient stresses, including the configuration of the plant, the size, color, and anthocyanin content of the leaves. N deficiency leads to plant miniaturization, decreases tillering, photosynthetic pigment content, and ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBPCO) in rice, resulting in a decrease in photosynthetic efficiency (Z. A. Huang, Jiang, Yang, Sun, & Jin, 2004; Mghase, Shiwachi, Takahashi, & Irie, 2011). P deficiency leads to a decrease of leaf width the accumulation of anthocyanins in the leaves, the formation of upright leaves at the angle of the leaves to reduce photosynthetic efficiency, reduce the tillering in rice and cause yield reduction (Hu et al., 2011; Mghase et al., 2011). Fronds undertake various functions such as photosynthesis, physical support, absorption, storage, and proliferation in duckweeds (An et al., 2019), each frond can be considered as a complete individual. The size of fronds in control group was greater than that in -N/+P and H_2O treatment groups, and less than that in +N/-P and -N/-P treatment groups. The biomass of fronds in treatment groups was less that in control group, biomass of fronds in -N/-P treatment group was greater than -N/+P, +N/-P, and H_2O treatment groups. The growth inhibition of fronds caused by individual P deficiency does not appear in -N/-P treatment group. It indicated that PSR may be dependent on environmental N resource in *S. polyrhiza* , as well as in terrestrial plants (Y.-N. Cui et al., 2019; Medici et al., 2019).

Nutrient deficiency causes the decreasing of chlorophyll content, especially N deficiency (Z. A. Huang et

Posted on 23 Nov 2022 — The copyright holder is the author/funder. All rights reserved. No reuse without permission. — https://doi.org/10.22541/au.166920214.45888392/v1 — This a preprint and has not been peer reviewed. Data may be preliminary.

Posted on 23 Nov 2022 — The copyright holder is the author/funder. All rights reserved. No reuse without permission. — https://doi.org/10.22541/au.166920214.45888392/v1 — This a preprint and has not been peer reviewed. Data may be preliminary.

al., 2004). In this study, we found individual/combined N deficiency (-N/+P, -N/-P, and H_2O treatments) both caused the bleaching and decreasing of chlorophyll content in frond, as well as the previous studies in duckweeds under N starvation (Y. Liu et al., 2018; Sun et al., 2022; C. Yu et al., 2017). However, individual P deficiency did not bring obvious changes to the color and chlorophyll content, perhaps the storage of P in duckweed and the accelerated P cycling offset the shortage of P in a short period. N/P deficiency also disturbs the photosynthesis and carbon cycling in plants, inhibits the transport of photosynthate from chloroplasts to cytoplasm, causes the biosynthesis and accumulation of starch in chloroplasts. The previous study found that individual N/P starvation (J. M. Li et al., 2021; Z. Zhao et al., 2015) and H_2O treatment (Tao et al., 2013) improve the content of starch in *Landoltia punctata* . We found the similar phenomenon in *S. polyrhiza* under nutrient deficiency stresses, especially in -N/-P and H_2O treatment groups, which can reach above 40%. It showed that the combined N/P deficiency and an overall deficiency of nutrients can act on different metabolic pathways and eventually aggravated the disturbances in carbon metabolism and the accumulation of starch. N is an essential element for amino acid synthesis in plants, N deficiency interferes with the synthesis of proteins. N starvation leads to the decreasing of protein content in duckweed plant *Lemna aequinoctialis* (C. Yu et al., 2017) and *L. punctata* (Z. Zhao et al., 2015). In this study, protein contain in control was higher than that in -N/+P and -N/-P treatment groups, and lower than that in +N/-P and H_2O treatment groups. It indicated that individual P deficiency and nutrient starvation are efficient methods for improve the protein content in duckweeds.

N and P deficiencies both can alter the expression profiles of ion transporting related protein coding genes. P deficiency leads to the upregulation of *PHT1s* in rice, and the downregulation of one*AMT* and three *NRT* genes (L. H. Li, Liu, & Lian, 2010). In*Populus trichocarpa* , N starvation affects the expression of*AMT* , *NRT* , *amino acid transporter* (*AAT*),*urea transporter* (*UT*), *PHT1* , *ZnT* ,*divalent cation transporter* (*DCT*), and *ATP binding cassette* (*ABC*) *transporters* genes (Calabrese et al., 2017). In this study, a variety of transporter genes exhibited alterations in *S. polyrhiza* under nutrient deficiency stresses, such as *PHT1* , *PHO1* , *NTR1* , *NRT2* , *AAT* ,*Sultr* , *ZnT* , *KT* , *magnesium transporter*(*MagT*), and *copper transporter* (*Ctr*). On the one hand, it may be due to the increase of the R/F ratio, and ion transporters always are relatively higher expressing in roots than shoots (Y. F. Chen et al., 2008; Cochavi, Cohen, & Rachmilevitch, 2020). On the other hand, the absorption and assimilation process of different elements is influenced each other in the plants (Oldroyd & Leyser, 2020). Meanwhile, lots of vacuole transporters involving amino acid and ion transport were upregulated in the N deficient treatment groups, suggesting that vacuole regulate intracellular ion concentrations by controlling the storage and transfer out of ions under the stress of N and other element deficiencies, thereby responding to different types of nutritional stress (J. M. Li et al., 2021; L. Xu et al., 2019).

Terrestrial plants absorb NO_3^- mainly from environment to meet their requirement of N, while some aquatic botany exhibits a preference to NH_4^+. The uptake rates of NH_4^+ was about 6 times of NO_3^- in seagrass *Zostera nigricaulis* when it was cultured with ^{15}N labelled NO_3^- and NH_4^+ (Nayar, Loo, Tanner, Longmore, & Jenkins, 2018). There was an increased affinity to 30 fold for NH_4^+ compared with NO_3^- in *Zostera noltii*(Alexandre, Silva, Bouma, & Santos, 2011). Previous studies reported that duckweed *S. polyrhiza* (Caicedo, Van der Steen, Arce, & Gijzen, 2000; Y. Z. Zhou et al., 2022), *L. punctata* (Fang, Babourina, Rengel, Yang, & Pu, 2007; Y. Z. Zhou et al., 2022), *L. aequinoctialis* (Y. Z. Zhou et al., 2022), *L. minor* (Cedergreen & Madsen, 2002; Petersen et al., 2021; Y. Z. Zhou et al., 2022),*L. turionifera* (Y. Z. Zhou et al., 2022), *Wolffiella hyaline* (Petersen et al., 2021), and *Woffila globosa* (Y. Z. Zhou et al., 2022) both performed the preferential uptake of NH_4^+ over NO_3^-. *NR* was upregulated significantly in the root of *C. arietinum* under the -N/+P (56.8 folds to control) and -N/-P (38.6 folds to control) treatment groups (Esfahani et al., 2021). However, *NR* genes were downregulated significantly in duckweed *L. aequinoctialis and L. punctata* under N starvation (C. Yu et al., 2017). In this study, *NR* and*NiR* genes that involved in nitrification were significantly downregulated in *S. polyrhiza* under -N/+P, -N/-P, and H_2O. The implication was that *S. polyrhiza* have a priority for absorption and assimilation of NH_4^+-N to NO_3^--N, just as the previous studies in duckweed plants.

In the process of carbon metabolism, the genes encoding the enzyme involved in catabolism of starch to G6P

Posted on 23 Nov 2022 — The copyright holder is the author/funder. All rights reserved. No reuse without permission. — https://doi.org/10.22541/au.166920214.45888392/v1 — This a preprint and has not been peer reviewed. Data may be preliminary.

were upregulated under different nutrient deficient stresses, while that involved in catabolism of sucrose to G6P were inhibited. And the expression of that the subsequent TCA cycle related genes was upregulated. It possibly due to the accumulation of photosynthate in chloroplast which leads to the accumulation of glyceraldehyde 3-phosphate (G3P) and starch and the inhibition of sucrose synthesis, and eventually affect the genes which involved in the sucrose catabolic process. In pentose phosphorylation pathway, the upper reactions from G6P to Ribulose-5-phosphate (R5P) were enhanced to product NADPH, and the later reaction that product C4/5/7 were inhibited. The results indicated that duckweed supplemented the loss of reductive force caused by photosynthetic inhibition through the pentose phosphorylation pathway to resist intracellular excess Reactive Oxygen Species (ROS) under nutrient deficiencies (Z. A. Huang et al., 2004; Muller, Morant, Jarmer, Nilsson, & Nielsen, 2007). And, withal, the synthesis of superoxide dismutase (SOD) and glutathione (GSH) were enhanced to cope with the ROS stress. The synthesis of chlorophyll was repressed, which explained the phenomenon of chlorosis and decreased chlorophyll content under nutritional stress in duckweed. The synthesis of phytohormones is regulated in response to N/P starvation (Chiou & Lin, 2011; Khan, Vogiatzaki, Glauser, & Poirier, 2016; Perez-Torres et al., 2008; G. Xu et al., 2012). The genes related to the synthesis and signal transduction of auxin, SA and SL were differential expressed in *S. polyrhiza* under N/P deprivation, indicated that these phytohormones were involved in the response to nutrient stresses, and the supplementation of exogenous hormones may alleviate the stress response or improve the utilization rate of nutrients in the plant.

ACKNOWLEDGMENTS

We thank Xin Wang at The Analysis and Testing Center of Institute of Hydrobiology, Chinese Academy of Sciences for microwave digestion experimental support. This work was supported by the National Natural Science Foundation of China [Grant number, 32201285, 32001107, 31870384]; National Key R & D Program [Grant numbers, 2020YFD0900305, 2018YFD0900801, 2017YFE0128800]; International Partnership Program of Chinese Academy of Sciences [152342KYSB20200021], Open project of State Key Laboratory of Freshwater Ecology and Biotechnology [2021FB04].

CONFLICT OF INTEREST

The authors declare that they have no competing interests.

AUTHOR CONTRIBUTION STATEMENT

Xuyao Zhao : Conceptualization, Investigation, Writing-original draft. **Xiaozhe Li** : Investigation, Data curation and Methodology. **Zuoliang Sun** and **Gaojie Li** : Investigation, Data curation. **Wenjun Guo** and **Chen Yan** : Methodology, Formal analysis. **Manli Xia** and **Yimeng Chen** : Methodology, Conceptualization. **Xiaoyu Wang** and**Yixian Li** : Methodology, Data curation. **Kangsheng Luo** : Writing-review & editing. **Jingjing Yang** and **Hongwei Hou** : Writing-review & editing, Funding acquisition, Supervision, Project administration. All authors read and approved the manuscript.

AUTHORS' INFORMATION

[1]The State Key Laboratory of Freshwater Ecology and Biotechnology, The Key Laboratory of Aquatic Biodiversity and Conservation of Chinese Academy of Sciences, Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan 430072, China. [2]University of Chinese Academy of Sciences, Beijing 100049, China.[3]Henan Key Laboratory of Ecological Security for Water Source Region of Mid-line of South-to-North Diversion Project of Henan Province, Collaborative Innovation Center of Water Security for Water Source Region of Mid-line of South-to-North Diversion Project of Henan Province, Nanyang Normal University, Nanyang 473061, China.

References:

Acosta, K., Appenroth, K. J., Borisjuk, L., Edelman, M., Heinig, U., Jansen, M. A. K., . . . Lam, E. (2021). Return of the Lemnaceae: Duckweed as a model plant system in the genomics and post-genomics era. *The Plant cell . 33* (10), 3207-3234.

Posted on 23 Nov 2022 — The copyright holder is the author/funder. All rights reserved. No reuse without permission. — https://doi.org/10.22541/au.166920214.45888392/v1 — This a preprint and has not been peer reviewed. Data may be preliminary.

Al-Babili, S., & Bouwmeester, H. J. (2015). Strigolactones, a Novel Carotenoid-Derived Plant Hormone. *Annual Review of Plant Biology, Vol 66, 66* , 161-186.

Alexandre, A., Silva, J., Bouma, T. J., & Santos, R. (2011). Inorganic nitrogen uptake kinetics and whole-plant nitrogen budget in the seagrass Zostera noltii. *Journal of Experimental Marine Biology and Ecology, 401* (1-2), 7-12.

Allgeier, J. E., Yeager, L. A., & Layman, C. A. (2013). Consumers regulate nutrient limitation regimes and primary production in seagrass ecosystems. *Ecology, 94* (2), 521-529.

An, D., Zhou, Y., Li, C. S., Xiao, Q., Wang, T., Zhang, Y. T., . . . Wang, W. Q. (2019). Plant evolution and environmental adaptation unveiled by long-read whole-genome sequencing of Spirodela.*Proceedings of the National Academy of Sciences of the United States of America, 116* (38), 18893-18899.

Balzergue, C., Dartevelle, T., Godon, C., Laugier, E., Meisrimler, C., Teulon, J.-M., . . . Desnos, T. (2017). Low phosphate activates STOP1-ALMT1 to rapidly inhibit root cell elongation. *Nature Communications, 8* , 15300.

Bari, R., Pant, B. D., Stitt, M., & Scheible, W. R. (2006). PHO2, microRNA399, and PHR1 define a phosphate-signaling pathway in plants.*Plant Physiology, 141* (3), 988-999.

Beale, S. I. (2005). Green genes gleaned. *Trends in Plant Science, 10* (7), 309-312.

Bovet, L., Kammer, P. M., Suter, M., & Brunold, C. (2000). Effect of mannitol and cold treatments on phosphate uptake and protein phosphorylation in Lemna minor (L.) plants. *Journal of Plant Physiology, 157* (4), 375-382.

Caicedo, J. R., Van der Steen, N. P., Arce, O., & Gijzen, H. J. (2000). Effect of total ammonia nitrogen concentration and pH on growth rates of duckweed (Spirodela polyrrhiza). *Water Research, 34* (15), 3829-3835.

Calabrese, S., Kohler, A., Niehl, A., Veneault-Fourrey, C., Boller, T., & Courty, P. E. (2017). Transcriptome analysis of the Populus trichocarpa-Rhizophagus irregularis Mycorrhizal Symbiosis: Regulation of Plant and Fungal Transportomes under Nitrogen Starvation. *Plant and Cell Physiology, 58* (6), 1003-1017.

Cassman, K. G., Dobermann, A., & Walters, D. T. (2002). Agroecosystems, nitrogen-use efficiency, and nitrogen management. *Ambio, 31* (2), 132-140.

Cedergreen, N., & Madsen, T. V. (2002). Nitrogen uptake by the floating macrophyte Lemna minor. *New Phytologist, 155* (2), 285-292.

Chen, D. Q., Zhang, H., Wang, Q. L., Shao, M., Li, X. Y., Chen, D. M., . . . Song, Y. Y. (2020). Intraspecific variations in cadmium tolerance and phytoaccumulation in giant duckweed (*Spirodela polyrhiza*).*Journal of Hazardous Materials, 395* , 122672.

Chen, X., Yao, Q., Gao, X., Jiang, C., Harberd, N. P., & Fu, X. (2016). Shoot-to-root mobile transcription factor HY5 coordinates plant carbon and nitrogen acquisition. *Current Biology, 26* (5), 640-646.

Chen, Y.-F., Li, L.-Q., Xu, Q., Kong, Y.-H., Wang, H., & Wu, W.-H. (2009). The WRKY6 transcription factor modulates PHOSPHATE1 expression in response to low pi stress in Arabidopsis. *Plant Cell, 21* (11), 3554-3566.

Chen, Y. F., Wang, Y., & Wu, W. H. (2008). Membrane transporters for nitrogen, phosphate and potassium uptake in plants. *Journal of Integrative Plant Biology, 50* (7), 835-848.

Cheng, J. J., & Stomp, A. M. (2009). Growing Duckweed to recover nutrients from wastewaters and for production of fuel ethanol and animal feed. *Clean-Soil Air Water, 37* (1), 17-26.

Chiou, T. J., & Lin, S. I. (2011). Signaling network in sensing phosphate availability in plants. *Annual Review of Plant Biology, Vol 62, 62* , 185-206.

Posted on 23 Nov 2022 — The copyright holder is the author/funder. All rights reserved. No reuse without permission. — https://doi.org/10.22541/au.166920214.45888392/v1 — This a preprint and has not been peer reviewed. Data may be preliminary.

Cochavi, A., Cohen, I. H., & Rachmilevitch, S. (2020). The role of different root orders in nutrient uptake. *Environmental and Experimental Botany, 179* , 104212.

Cordell, D., Drangert, J.-O., & White, S. (2009). The story of phosphorus: Global food security and food for thought. *Global Environmental Change-Human and Policy Dimensions, 19* (2), 292-305.

Cridland, C., & Gillaspy, G. (2020). Inositol pyrophosphate pathways and mechanisms: What can we learn from plants? *Molecules, 25* (12), 2789.

Cui, Y.-N., Li, X.-T., Yuan, J.-Z., Wang, F.-Z., Wang, S.-M., & Ma, Q. (2019). Nitrate transporter NPF7.3/NRT1.5 plays an essential role in regulating phosphate deficiency responses in Arabidopsis.*Biochemical and Biophysical Research Communications, 508* (1), 314-319.

de Magalhaes, J. V., Alves, V. M. C., de Novais, R. F., Mosquim, P. R., Magalhaes, J. R., Bahia, A. F. C., & Huber, D. M. (1998). Nitrate uptake by corn under increasing periods of phosphorus starvation.*Journal of Plant Nutrition, 21* (12), 2707-2707.

de Morais, M. B., Barbosa-Neto, A. G., Willadino, L., Ulisses, C., & Calsa, T. (2019). Salt stress induces increase in starch accumulation in duckweed (*Lemna aequinoctialis* , Lemnaceae): Biochemical and physiological aspects. *Journal of Plant Growth Regulation, 38* (2), 683-700.

Devaiah, B. N., Karthikeyan, A. S., & Raghothama, K. G. (2007). WRKY75 transcription factor is a modulator of phosphate acquisition and root development in arabidopsis. *Plant Physiology, 143* (4), 1789-1801.

Dong, J., Ma, G., Sui, L., Wei, M., Satheesh, V., Zhang, R., . . . Lei, M. (2019). Inositol pyrophosphate InsP(8) acts as an intracellular phosphate signal in Arabidopsis. *Molecular Plant, 12* (11), 1463-1473.

Duan, Y. H., Shi, X. J., Li, S. L., Sun, X. F., & He, X. H. (2014). Nitrogen use efficiency as affected by phosphorus and potassium in long-term rice and wheat experiments. *Journal of Integrative Agriculture, 13* (3), 588-596.

Elser, J. J., Bracken, M. E. S., Cleland, E. E., Gruner, D. S., Harpole, W. S., Hillebrand, H., . . . Smith, J. E. (2007). Global analysis of nitrogen and phosphorus limitation of primary producers in freshwater, marine and terrestrial ecosystems. *Ecology Letters, 10* (12), 1135-1142.

Elser, J. J., Fagan, W. F., Denno, R. F., Dobberfuhl, D. R., Folarin, A., Huberty, A., . . . Sterner, R. W. (2000). Nutritional constraints in terrestrial and freshwater food webs. *Nature, 408* (6812), 578-580.

Esfahani, M. N., Inoue, K., Nguyen, K. H., Chu, H. D., Watanabe, Y., Kanatani, A., . . . Tran, L. S. P. (2021). Phosphate or nitrate imbalance induces stronger molecular responses than combined nutrient deprivation in roots and leaves of chickpea plants. *Plant Cell and Environment, 44* (2), 574-597.

Fageria, V. D. (2001). Nutrient interactions in crop plants.*Journal of Plant Nutrition, 24* (8), 1269-1290.

Fang, Y. Y., Babourina, O., Rengel, Z., Yang, X. E., & Pu, P. M. (2007). Ammonium and nitrate uptake by the floating plant Landoltia punctata. *Annals of Botany, 99* (2), 365-370.

Fu, L. L., Ding, Z. H., Sun, X. P., & Zhang, J. M. (2019). Physiological and transcriptomic analysis reveals distorted ion homeostasis and responses in the freshwater plant *Spirodela polyrhiza* L. under salt stress.*Genes, 10* (10), 743.

Fu, L. L., Ding, Z. H., Tan, D. G., Han, B. Y., Sun, X. P., & Zhang, J. M. (2020). Genome-wide discovery and functional prediction of salt-responsive lncRNAs in duckweed. *BMC Genomics, 21* (1), 212.

Fujita, M., Fujita, Y., Noutoshi, Y., Takahashi, F., Narusaka, Y., Yamaguchi-Shinozaki, K., & Shinozaki, K. (2006). Crosstalk between abiotic and biotic stress responses: a current view from the points of convergence in the stress signaling networks. *Current Opinion in Plant Biology, 9* (4), 436-442.

Posted on 23 Nov 2022 — The copyright holder is the author/funder. All rights reserved. No reuse without permission. — https://doi.org/10.22541/au.166920214.45888392/v1 — This a preprint and has not been peer reviewed. Data may be preliminary.

Gamir, J., Torres-Vera, R., Rial, C., Berrio, E., Campos, P. M. D., Varela, R. M., . . . Lopez-Raez, J. A. (2020). Exogenous strigolactones impact metabolic profiles and phosphate starvation signalling in roots.*Plant Cell and Environment, 43* (7), 1655-1668.

Gill, S. S., & Tuteja, N. (2010). Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants.*Plant Physiology and Biochemistry, 48* (12), 909-930.

Gniazdowska, A., & Rychter, A. M. (2000). Nitrate uptake by bean (*Phaseolus vulgaris* L.) roots under phosphate deficiency.*Plant and Soil, 226* (1), 79-85.

Gruber, B. D., Giehl, R. F., Friedel, S., & von Wiren, N. (2013). Plasticity of the Arabidopsis root system under nutrient deficiencies.*Plant Physiology, 163* (1), 161-179.

Guo, L., Jin, Y. L., Xiao, Y., Tan, L., Tian, X. P., Ding, Y. Q., . . . Zhao, H. (2020). Energy-efficient and environmentally friendly production of starch-rich duckweed biomass using nitrogen-limited cultivation. *Journal of Cleaner Production, 251* , 119726.

Hamburger, D., Rezzonico, E., Petetot, J. M. C., Somerville, C., & Poirier, Y. (2002). Identification and characterization of the Arabidopsis *PHO1* gene involved in phosphate loading to the xylem.*Plant Cell, 14* (4), 889-902.

Harkess, A., McLoughlin, F., Bilkey, N., Elliott, K., Emenecker, R., Mattoon, E., . . . Michael, T. P. (2021). Improved *Spirodela polyrhiz* a genome and proteomic analyses reveal a conserved chromosomal structure with high abundance of chloroplastic proteins favoring energy production. *Journal of Experimental Botany, 72* (7), 2491-2500.

Harpole, W. S., Ngai, J. T., Cleland, E. E., Seabloom, E. W., Borer, E. T., Bracken, M. E. S., . . . Smith, J. E. (2011). Nutrient co-limitation of primary producer communities. *Ecology Letters, 14* (9), 852-862.

Hell, R., & Hillebrand, H. (2001). Plant concepts for mineral acquisition and allocation. *Current Opinion in Biotechnology, 12* (2), 161-168.

Hernando, N., Ruminska, J., Myakala, K., Knopfel, T., Biber, J., Murer, H., & Wagner, C. A. (2017). Phosphate homeostasis: role of renal and intestinal transporters. *Acta Physiologica, 219* , 9-9.

Ho, C.-H., Lin, S.-H., Hu, H.-C., & Tsay, Y.-F. (2009). CHL1 functions as a nitrate sensor in plants. *Cell, 138* (6), 1184-1194.

Hu, B., & Chu, C. C. (2020). Nitrogen-phosphorus interplay: old story with molecular tale. *New Phytologist, 225* (4), 1455-1460.

Hu, B., Jiang, Z., Wang, W., Qiu, Y., Zhang, Z., Liu, Y., . . . Chu, C. (2019). Nitrate-NRT1.1B-SPX4 cascade integrates nitrogen and phosphorus signalling networks in plants. *Nature Plants, 5* (4), 401-413.

Hu, B., Wang, W., Ou, S. J., Tang, J. Y., Li, H., Che, R. H., . . . Chu, C. C. (2015). Variation in NRT1.1B contributes to nitrate-use divergence between rice subspecies. *Nature Genetics, 47* (7), 834-838.

Hu, B., Zhu, C., Li, F., Tang, J., Wang, Y., Lin, A., . . . Chu, C. (2011). LEAF TIP NECROSIS1 plays a pivotal role in the regulation of multiple phosphate starvation responses in rice. *Plant Physiology, 156* (3), 1101-1115.

Huang, L., Zhang, H., Zhang, H., Deng, X. W., & Wei, N. (2015). HY5 regulates nitrite reductase 1 (NIR1) and ammonium transporter1;2 (AMT1;2) in Arabidopsis seedlings. *Plant Science, 238* , 330-339.

Huang, M. X., Zhong, Y. S., Ma, X. Y., Hu, Q. X., Fu, M. H., & Han, Y. L. (2019). Analysis of codon usage in the mitochondrion genome of*Spirodela polyrhiza* . *Aquatic Botany, 156* , 65-72.

Huang, Z. A., Jiang, D. A., Yang, Y., Sun, J. W., & Jin, S. H. (2004). Effects of nitrogen deficiency on gas exchange, chlorophyll fluorescence, and antioxidant enzymes in leaves of rice plants.*Photosynthetica, 42* (3), 357-364.

Posted on 23 Nov 2022 — The copyright holder is the author/funder. All rights reserved. No reuse without permission. — https://doi.org/10.22541/au.166920214.45888392/v1 — This a preprint and has not been peer reviewed. Data may be preliminary.

Kang, Y.-J., Yang, D.-C., Kong, L., Hou, M., Meng, Y.-Q., Wei, L., & Gao, G. (2017). CPC2: a fast and accurate coding potential calculator based on sequence intrinsic features. *Nucleic Acids Research, 45* (W1), W12-W16.

Kellermeier, F., Armengaud, P., Seditas, T. J., Danku, J., Salt, D. E., & Amtmann, A. (2014). Analysis of the root system architecture of Arabidopsis provides a quantitative readout of crosstalk between nutritional signals. *Plant Cell, 26* (4), 1480-1496.

Khan, G. A., Vogiatzaki, E., Glauser, G., & Poirier, Y. (2016). Phosphate deficiency induces the jasmonate pathway and enhances resistance to insect herbivory. *Plant Physiology, 171* (1), 632-644.

Kim, D., Landmead, B., & Salzberg, S. L. (2015). HISAT: a fast spliced aligner with low memory requirements. *Nature Methods, 12* (4), 357-U121.

Koide, R. T. (1991). Nutrient Supply, Nutrient demand and plant-response to mycorrhizal infection. *New Phytologist, 117* (3), 365-386.

Krouk, G., & Kiba, T. (2020). Nitrogen and Phosphorus interactions in plants: from agronomic to physiological and molecular insights.*Current Opinion in Plant Biology, 57* , 104-109.

Langfelder, P., & Horvath, S. (2008). WGCNA: an R package for weighted correlation network analysis. *BMC Bioinformatics, 9* , 559.

Li, A., Zhang, J., & Zhou, Z. (2014). PLEK: a tool for predicting long non-coding RNAs and messenger RNAs based on an improved k-mer scheme.*BMC Bioinformatics, 15* , 311.

Li, J. M., Du, A. P., Liu, P. H., Tian, X. P., Jin, Y. L., Yi, Z. L., . . . Zhao, H. (2021). High starch accumulation mechanism and phosphorus utilization efficiency of duckweed (*Landoltia punctata*) under phosphate starvation. *Industrial Crops and Products, 167* , 113529.

Li, L. H., Liu, C., & Lian, X. M. (2010). Gene expression profiles in rice roots under low phosphorus stress. *Plant Molecular Biology, 72* (4-5), 423-432.

Li, X. X., Zeng, R. S., & Liao, H. (2016). Improving crop nutrient efficiency through root architecture modifications. *Journal of Integrative Plant Biology, 58* (3), 193-202.

Liu, K.-h., Niu, Y., Konishi, M., Wu, Y., Du, H., Chung, H. S., . . . Sheen, J. (2017). Discovery of nitrate-CPK-NLP signalling in central nutrient-growth networks. *Nature, 545* (7654), 311-316.

Liu, P. D., Xue, Y. B., Chen, Z. J., Liu, G. D., & Tian, J. (2016). Characterization of purple acid phosphatases involved in extracellular dNTP utilization in Stylosanthes. *Journal of Experimental Botany, 67* (14), 4141-4154.

Liu, Y., Wang, X. H., Fang, Y., Huang, M. J., Chen, X. Y., Zhang, Y., & Zhao, H. (2018). The effects of photoperiod and nutrition on duckweed (*Landoltia punctata*) growth and starch accumulation.*Industrial Crops and Products, 115* , 243-249.

Liu, Y., Wang, Y., Xu, S. Q., Tang, X. F., Zhao, J. S., Yu, C. J., . . . Zhou, G. K. (2019). y Efficient genetic transformation and CRISPR/Cas9-mediated genome editing in *Lemna aequinoctialis* .*Plant Biotechnology Journal, 17* (11), 2143-2152.

Ludewig, U., Vatov, E., Hedderich, D., & Neuhauser, B. (2021). Adjusting plant nutrient acquisition to fluctuating availability: transcriptional co-regulation of the nitrate and phosphate deprivation responses in roots. *Journal of Experimental Botany, 72* (10), 3500-3503.

Maeda, Y., Konishi, M., Kiba, T., Sakuraba, Y., Sawaki, N., Kurai, T., . . . Yanagisawa, S. (2018). A NIGT1-centred transcriptional cascade regulates nitrate signalling and incorporates phosphorus starvation signals in Arabidopsis. *Nature Communications, 9* , 1376.

Posted on 23 Nov 2022 — The copyright holder is the author/funder. All rights reserved. No reuse without permission. — https://doi.org/10.22541/au.166920214.45888392/v1 — This a preprint and has not been peer reviewed. Data may be preliminary.

Marchive, C., Roudier, F., Castaings, L., Brehaut, V., Blondet, E., Colot, V., . . . Krapp, A. (2013). Nuclear retention of the transcription factor NLP7 orchestrates the early response to nitrate in plants. *Nature Communications, 4* , 1713.

Marro, N., Lidoy, J., Chico, M. A., Rial, C., Garcia, J., Varela, R. M., . . . Lopez-Raez, J. A. (2022). Strigolactones: New players in the nitrogen-phosphorus signalling interplay. *Plant, Cell & Environment, 45* (2), 512-527.

Medici, A., Marshall-Colon, A., Ronzier, E., Szponarski, W., Wang, R., Gojon, A., . . . Krouk, G. (2015). AtNIGT1/HRS1 integrates nitrate and phosphate signals at the Arabidopsis root tip. *Nature Communications, 6* , 6274.

Medici, A., Szponarski, W., Dangeville, P., Safi, A., Dissanayake, I. M., Saenchai, C., . . . Krouk, G. (2019). Identification of molecular integrators shows that nitrogen actively controls the phosphate starvation response in plants. *Plant Cell, 31* (5), 1171-1184.

Mghase, J. J., Shiwachi, H., Takahashi, H., & Irie, K. (2011). Nutrient deficiencies and their symptoms in upland rice. *Journal of ISSAAS International Society for Southeast Asian Agricultural Sciences (Philippines),17* , 59-67.

Mittler, R. (2002). Oxidative stress, antioxidants and stress tolerance. *Trends in Plant Science, 7* (9), 405-410.

Mittler, R., Vanderauwera, S., Gollery, M., & Van Breusegem, F. (2004). Reactive oxygen gene network of plants. *Trends in Plant Science, 9* (10), 490-498.

Muller, R., Morant, M., Jarmer, H., Nilsson, L., & Nielsen, T. H. (2007). Genome-wide analysis of the Arabidopsis leaf transcriptome reveals interaction of phosphate and sugar metabolism. *Plant Physiology, 143* (1), 156-171.

Nakamura, Y., Koizumi, R., Shui, G. H., Shimojima, M., Wenk, M. R., Ito, T., & Ohta, H. (2009). Arabidopsis lipins mediate eukaryotic pathway of lipid metabolism and cope critically with phosphate starvation. *Proceedings of the National Academy of Sciences of the United States of America, 106* (49), 20978-20983.

Nayar, S., Loo, M. G. K., Tanner, J. E., Longmore, A. R., & Jenkins, G. P. (2018). Nitrogen acquisition and resource allocation strategies in temperate seagrass *Zostera nigricaulis* : Uptake, assimilation and translocation processes. *Scientific Reports, 8* , 17151.

Neumann, G., Massonneau, A., Langlade, N., Dinkelaker, B., Hengeler, C., Romheld, V., & Martinoia, E. (2000). Physiological aspects of cluster root function and development in phosphorus-deficient white lupin (*Lupinus albus* L.). *Annals of Botany, 85* (6), 909-919.

Nguyen, H. T. K., Kim, S. Y., Cho, K. M., Hong, J. C., Shin, J. S., & Kim, H. J. (2016). A Transcription factor gamma MYB1 binds to the P1BS*cis* -element and activates PLA(2)-gamma expression with its co-activator gamma MYB2. *Plant and Cell Physiology, 57* (4), 784-797.

Oldroyd, G. E. D., & Leyser, O. (2020). A plant's diet, surviving in a variable nutrient environment. *Science, 368* (6486), eaba0196.

Osorio, M. B., Ng, S., Berkowitz, O., De Clercq, I., Mao, C., Shou, H., . . . Jost, R. (2019). SPX4 acts on PHR1-dependent and -independent regulation of shoot phosphorus status in Arabidopsis. *Plant Physiology, 181* (1), 332-352.

Paris, Q. (1992). The return of vonliebig law of the minimum. *Agronomy Journal, 84* (6), 1040-1046.

Pei, L., Jin, Z., Li, K., Yin, H., Wang, J., & Yang, A. (2013). Identification and comparative analysis of low phosphate tolerance-associated microRNAs in two maize genotypes. *Plant Physiology and Biochemistry, 70* , 221-234.

Posted on 23 Nov 2022 — The copyright holder is the author/funder. All rights reserved. No reuse without permission. — https://doi.org/10.22541/au.166920214.45888392/v1 — This a preprint and has not been peer reviewed. Data may be preliminary.

Peret, B., Clement, M., Nussaume, L., & Desnos, T. (2011). Root developmental adaptation to phosphate starvation: better safe than sorry. *Trends in Plant Science, 16* (8), 442-450.

Perez-Torres, C.-A., Lopez-Bucio, J., Cruz-Ramirez, A., Ibarra-Laclette, E., Dharmasiri, S., Estelle, M., & Herrera-Estrella, L. (2008). Phosphate availability alters lateral root development in Arabidopsis by modulating auxin sensitivity via a mechanism involving the TIR1 auxin receptor. *Plant Cell, 20* (12), 3258-3272.

Pertea, M., Kim, D., Pertea, G. M., Leek, J. T., & Salzberg, S. L. (2016). Transcript-level expression analysis of RNA-seq experiments with HISAT, StringTie and Ballgown. *Nature Protocols, 11* (9), 1650-1667.

Petersen, F., Demann, J., Restemeyer, D., Ulbrich, A., Olfs, H. W., Westendarp, H., & Appenroth, K. J. (2021). Influence of the nitrate-N to ammonium-N ratio on relative growth rate and crude protein content in the duckweeds *Lemna minor* and *Wolffiella hyalina* .*Plants-Basel, 10* (8), 1741.

Pfaffl, M. W. (2001). A new mathematical model for relative quantification in real-time RT-PCR. *Nucleic Acids Research, 29* (9), e45.

Reid, R., & Hayes, J. (2003). Mechanisms and control of nutrient uptake in plants. In K. W. Jeon (Ed.), *International Review of Cytology - a Survey of Cell Biology, Vol 229* , 73-114.

Rietra, R. P. J. J., Heinen, M., Dimkpa, C. O., & Bindraban, P. S. (2017). Effects of nutrient antagonism and synergism on yield and fertilizer use efficiency. *Communications in Soil Science and Plant Analysis, 48* (16), 1895-1920.

Roch, G. V., Maharajan, T., Ceasar, S. A., & Ignacimuthu, S. (2019). The role of PHT1 family transporters in the acquisition and redistribution of phosphorus in plants. *Critical Reviews in Plant Sciences, 38* (3), 171-198.

Rouached, H., Arpat, A. B., & Poirier, Y. (2010). Regulation of phosphate starvation responses in plants: Signaling players and cross-talks. *Molecular Plant, 3* (2), 288-299.

Safi, A., Medici, A., Szponarski, W., Ruffel, S., Lacombe, B., & Krouk, G. (2017). The world according to GARP transcription factors.*Current Opinion in Plant Biology, 39* , 159-167.

Schachtman, D. P., & Shin, R. (2007). Nutrient sensing and signaling: NPKS. *Annual Review of Plant Biology, 58* , 47-69.

Secco, D., Baumann, A., & Poirier, Y. (2010). Characterization of the rice *PHO1* gene family reveals a key role for ospho1;2 in phosphate homeostasis and the evolution of a distinct clade in dicotyledons. *Plant Physiology, 152* (3), 1693-1704.

Smith, F. W., & Jackson, W. A. (1987). Nitrogen enhancement of phosphate-transport in roots of *Zea mays* L.: kinetic and inhibitor studies. *Plant Physiology, 84* (4), 1319-1324.

Sree, K. S., Adelmann, K., Garcia, C., Lam, E., & Appenroth, K. J. (2015). Natural variance in salt tolerance and induction of starch accumulation in duckweeds. *Planta, 241* (6), 1395-1404.

Srivastava, S., Upadhyay, M. K., Srivastava, A. K., Abdelrahman, M., Suprasanna, P., & Tran, L. S. P. (2018). Cellular and subcellular phosphate transport machinery in plants. *International Journal of Molecular Sciences, 19* (7), 1914.

Su, T., Xu, Q., Zhang, F.-C., Chen, Y., Li, L.-Q., Wu, W.-H., & Chen, Y.-F. (2015). WRKY42 modulates phosphate homeostasis through regulating phosphate translocation and acquisition in Arabidopsis. *Plant Physiology, 167* (4), 1579-U1717.

Sun, Z. L., Guo, W. J., Zhao, X. Y., Chen, Y., Yang, J. J., Xu, S. Q., & Hou, H. W. (2022). Sulfur limitation boosts more starch accumulation than nitrogen or phosphorus limitation in duckweed (*Spirodela polyrhiza*). *Industrial Crops and Products, 185* , 115098.

Posted on 23 Nov 2022 — The copyright holder is the author/funder. All rights reserved. No reuse without permission. — https://doi.org/10.22541/au.166920214.45888392/v1 — This a preprint and has not been peer reviewed. Data may be preliminary.

Taniguchi, M., Taniguchi, Y., Kawasaki, M., Takeda, S., Kato, T., Sato, S., . . . Sugiyama, T. (2002). Identifying and characterizing plastidic 2-oxoglutarate/malate and dicarboxylate transporters in *Arabidopsis thaliana* . *Plant and Cell Physiology, 43* (7), 706-717.

Tao, X., Fang, Y., Xiao, Y., Jin, Y.-l., Ma, X.-r., Zhao, Y., . . . Wang, H.-y. (2013). Comparative transcriptome analysis to investigate the high starch accumulation of duckweed (*Landoltia punctata*) under nutrient starvation. *Biotechnology for Biofuels, 6* , 72.

Tegeder, M., & Masclaux-Daubresse, C. (2018). Source and sink mechanisms of nitrogen transport and use. *New Phytologist, 217* (1), 35-53.

Ueda, Y., Kiba, T., & Yanagisawa, S. (2020). Nitrate-inducible NIGT1 proteins modulate phosphate uptake and starvation signalling via transcriptional regulation of *SPX* genes. *Plant Journal, 102* (3), 448-466.

Vance, C. P., Uhde-Stone, C., & Allan, D. L. (2003). Phosphorus acquisition and use: critical adaptations by plants for securing a nonrenewable resource. *New Phytologist, 157* (3), 423-447.

Veneklaas, E. J., Lambers, H., Bragg, J., Finnegan, P. M., Lovelock, C. E., Plaxton, W. C., . . . Raven, J. A. (2012). Opportunities for improving phosphorus-use efficiency in crop plants. *New Phytologist, 195* (2), 306-320.

Vitousek, P. M., Porder, S., Houlton, B. Z., & Chadwick, O. A. (2010). Terrestrial phosphorus limitation: mechanisms, implications, and nitrogen-phosphorus interactions. *Ecological Applications, 20* (1), 5-15.

Wang, G., Yin, H., Li, B., Yu, C., Wang, F., Xu, X., . . . Zhang, Z. (2019). Characterization and identification of long non-coding RNAs based on feature relationship. *Bioinformatics, 35* (17), 2949-2956.

Wang, X., Wang, H. F., Chen, Y., Sun, M. M., Wang, Y., & Chen, Y. F. (2020). The transcription factor NIGT1.2 modulates both phosphate uptake and nitrate influx during phosphate starvation in Arabidopsis and maize.*Plant Cell, 32* (11), 3519-3534.

Wege, S., Khan, G. A., Jung, J. Y., Vogiatzaki, E., Pradervand, S., Aller, I., . . . Poirier, Y. (2016). The EXS domain of PHO1 participates in the response of shoots to phosphate deficiency via a root-to-shoot signal. *Plant Physiology, 170* (1), 385-400.

Xu, G., Fan, X., & Miller, A. J. (2012). Plant nitrogen assimilation and use efficiency. In S. S. Merchant (Ed.), *Annual Review of Plant Biology, Vol 63* (Vol. 63, pp. 153-182).

Xu, H., Yu, C., Xia, X., Li, M., Li, H., Wang, Y., . . . Zhou, G. (2018). Comparative transcriptome analysis of duckweed (*Landoltia punctata*) in response to cadmium provides insights into molecular mechanisms underlying hyperaccumulation. *Chemosphere, 190* , 154-165.

Xu, L., Zhao, H. Y., Wan, R. J., Liu, Y., Xu, Z., Tian, W., . . . Yi, K. K. (2019). Identification of vacuolar phosphate efflux transporters in land plants. *Nature Plants, 5* (1), 84-94.

Xu, S. Q., Stapley, J., Gablenz, S., Boyer, J., Appenroth, K. J., Sree, K. S., . . . Huber, M. (2019). Low genetic variation is associated with low mutation rate in the giant duckweed. *Nature Communications, 10* , 1243.

Xu, Y. L., Ma, S., Huang, M., Peng, M., Bog, M., Sree, K. S., . . . Zhang, J. M. (2015). Species distribution, genetic diversity and barcoding in the duckweed family (Lemnaceae). *Hydrobiologia, 743* (1), 75-87.

Yamaguchi, S. (2008). Gibberellin metabolism and its regulation.*Annual Review of Plant Biology, 59* , 225-251.

Yang, J., Li, G., Xia, M., Chen, Y., Chen, Y., Kumar, S., . . . Hou, H. (2022). Combined effects of temperature and nutrients on the toxicity of cadmium in duckweed (*Lemna aequinoctialis*). *Journal of Hazardous Materials, 432* , 128646-128646.

Posted on 23 Nov 2022 — The copyright holder is the author/funder. All rights reserved. No reuse without permission. — https://doi.org/10.22541/au.166920214.45888392/v1 — This a preprint and has not been peer reviewed. Data may be preliminary.

Yang, J. J., Li, G. J., Bishopp, A., Heenatigala, P. P. M., Hu, S. Q., Chen, Y., . . . Hou, H. W. (2018). A comparison of growth on mercuric chloride for three Lemnaceae species reveals differences in growth dynamics that effect their suitability for use in either monitoring or remediating ecosystems contaminated with mercury. *Frontiers in Chemistry, 6* , 112.

Yang, J. J., Lia, G. J., Hua, S. Q., Bishopp, A., Heenatigala, P. P. M., Kumar, S., . . . Hou, H. W. (2018). A protocol for efficient callus induction and stable transformation of *Spirodela polyrhiza* (L.) Schleiden using *Agrobacterium tumefaciens* . *Aquatic Botany, 151* , 80-86.

Yang, J. J., Zhao, X. Y., Chen, Y., Li, G. J., Li, X. Z., Xia, M. L., . . . Hou, H. W. (2022). Identification, structural, and expression analyses of *SPX* genes in giant duckweed (*Spirodela polyrhiza*) reveals its role in response to low phosphorus and nitrogen stresses. *Cells, 11* (7), 1167.

Yang, T. R., Hao, L., Yao, S. F., Zhao, Y. Y., Lu, W. J., & Xiao, K. (2016). TabHLH1, a bHLH-type transcription factor gene in wheat, improves plant tolerance to Pi and N deprivation via regulation of nutrient transporter gene transcription and ROS homeostasis. *Plant Physiology and Biochemistry, 104* , 99-113.

Yi, K., Wu, Z., Zhou, J., Du, L., Guo, L., Wu, Y., & Wu, P. (2005). OsPTF1, a novel transcription factor involved in tolerance to phosphate starvation in rice. *Plant Physiology, 138* (4), 2087-2096.

Yoon, J., Cho, L. H., Tun, W., Jeon, J. S., & An, G. (2021). Sucrose signaling in higher plants. *Plant Science, 302* , 110703.

Yu, C., Zhao, X., Qi, G., Bai, Z., Wang, Y., Wang, S., . . . Zhou, G. (2017). Integrated analysis of transcriptome and metabolites reveals an essential role of metabolic flux in starch accumulation under nitrogen starvation in duckweed. *Biotechnology for Biofuels, 10* , 167.

Yu, P., Gutjahr, C., Li, C. J., & Hochholdinger, F. (2016). Genetic control of lateral root formation in cereals. *Trends in Plant Science, 21* (11), 951-961.

Yuan, H., & Liu, D. (2008). Signaling components involved in plant responses to phosphate starvation. *Journal of Integrative Plant Biology, 50* (7), 849-859.

Zhang, Q., Wang, C., Tian, J., Li, K., & Shou, H. (2011). Identification of rice purple acid phosphatases related to posphate starvation signalling. *Plant Biology, 13* (1), 7-15.

Zhao, X., Li, G., Sun, Z., Chen, Y., Guo, W., Li, Y., . . . Hou, H. (2021). Identification, structure analysis, and transcript profiling of phosphate transporters under Pi deficiency in duckweeds.*International Journal of Biological Macromolecules, 188* , 595-608.

Zhao, X., Yang, J., Li, G., Sun, Z., Hu, S., Chen, Y., . . . Hou, H. (2021). Genome-wide identification and comparative analysis of the*WRKY* gene family in aquatic plants and their response to abiotic stresses in giant duckweed (*Spirodela polyrhiza*). *Genomics, 113* (4), 1761-1777.

Zhao, X., Yang, J., Li, X., Li, G., Sun, Z., Chen, Y., . . . Hou, H. (2022). Identification and expression analysis of *GARP*superfamily genes in response to nitrogen and phosphorus stress in*Spirodela polyrhiza* . *BMC Plant Biology, 22* (1), 308.

Zhao, Z., Shi, H.-j., Wang, M.-l., Cui, L., Zhao, H., & Zhao, Y. (2015). Effect of nitrogen and phosphorus deficiency on transcriptional regulation of genes encoding key enzymes of starch metabolism in duckweed (*Landoltia punctata*). *Plant Physiology and Biochemistry, 86* , 72-81.

Zhou, J., Jiao, F. C., Wu, Z. C., Li, Y. Y., Wang, X. M., He, X. W., . . . Wu, P. (2008). OsPHR2 is involved in phosphate-starvation signaling and excessive phosphate accumulation in shoots of plants. *Plant Physiology, 146* (4), 1673-1686.

Zhou, Y. Z., Kishchenko, O., Stepanenko, A., Chen, G. M., Wang, W., Zhou, J., . . . Borisjuk, N. (2022). The dynamics of NO_3^- and NH_4^+ uptake in duckweed are coordinated with the expression of major nitrogen

Posted on 23 Nov 2022 — The copyright holder is the author/funder. All rights reserved. No reuse without permission. — https://doi.org/10.22541/au.166920214.45888392/v1 — This a preprint and has not been peer reviewed. Data may be preliminary.

assimilation genes.*Plants-Basel, 11* (1).

Table 1. List of candidate genes that might contribute to the responses to nutrient deficiency in *S. polyrhiza*.

FIGURE CAPTION

Fig. 1. The morphology of *S. polyrhiza* **under different nutrient deficiency conditions.** (a) Mass morphology; (b) the rear side (the side far away water) of fronds; (c) ventral side (the side near to water) of frond and root.

Fig. 2. The physiological index of *S. polyrhiza* under different nutrient deficiency conditions. (a) Fresh weight; (b) Dry weight; (c) Dry weight of frond; (d) Dry weight of roots; (e) Area per frond; (f) The number of roots per frond; (g) The length of roots; (h) Root-to-frond ratio; (i) Chlorophyll content; (j) Total phosphorus content; (k) Starch content;(l) Protein content. Different letters indicate a significant difference between different samples at the $P < 0.05$ level (Tukey's test).

Fig. 3. Comparative transcriptome analyses of *S. polyrhiza* under different nutritional conditions. (a) The DE-mRNA and DE-lncRNAs between different treatment groups. (b-e) UpSet plots present the overlapping DEGs in 4 comparison groups. UpSet plot of upregulated mRNAs (b). UpSet plot of downregulated mRNAs (c). UpSet plot of upregulated lncRNAs (d). UpSet plot of downregulated lncRNAs (e). Set1: -N/+P vs +N/+P, Set2: +N/-P vs +N/+P, Set3: -N/-P vs +N/+P, Set4: H_2O vs +N/+P.

Fig. 4. Changes in the expression levels of genes involved in the N- and P-signaling.

Fig. 5. Changes in the expression levels of genes involved in the processing of N and P uptake/assimilation of *S. polyrhiza* under different nutrient condition.

Fig. 6. Changes in the expression levels of genes involved in the chlorophyll biosynthetic pathways in of *S. polyrhiza* under different nutrient condition

Fig. 7. Changes in the expression levels of genes involved in the glycolytic and oxidative pentose phosphate pathways in *S. polyrhiza* under different nutrient condition

Fig. 8. Distribution of DEGs in TF gene families.

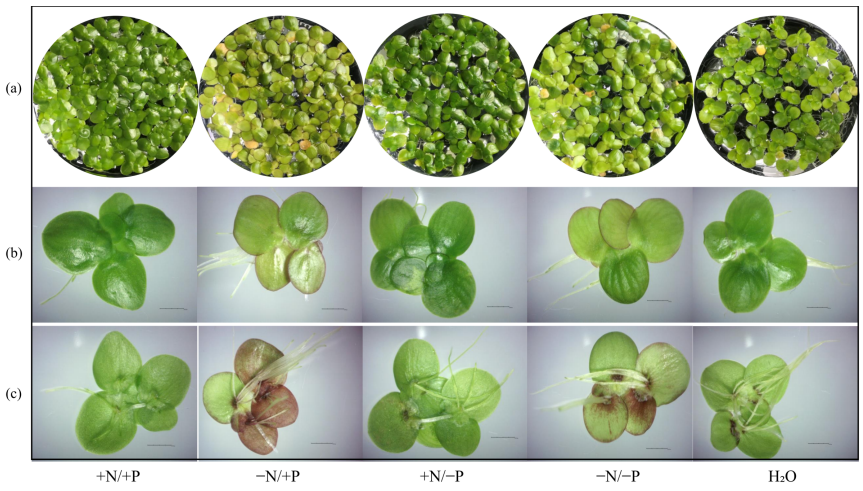
Fig. 9. TFs involved in N or/and P starvation response in *S. polyrhiza*. (a-f) changes in the expression levels of bHLH, bZIP, ERF, MYB, NAC and WRKY TF genes in *S. polyrhiza* under different nutrient condition.

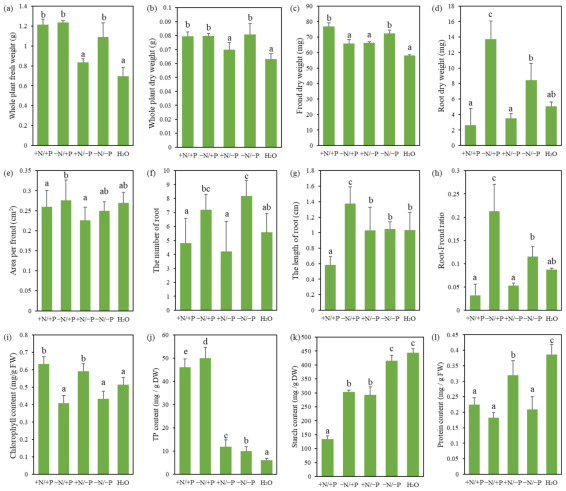
Fig. 10. Interaction networks of lncRNAs and their adjacent genes in cis-regulation , purple cycles represent coding genes, green cycles represent lncRNAs.

Fig. 11. CeRNA networks analysis of mRNA-miRNA-lncRNA in *S. polyrhiza* under N or/and P deprivation, blue cycles represent miRNAs, red cycles represent coding genes, green cycles represent lncRNAs.

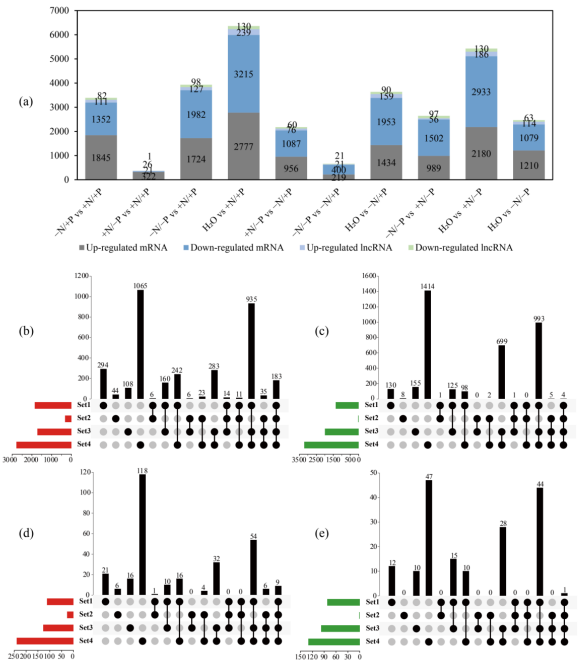
Fig. 12. Verification of the expression profiles of RNA-seq data using qRT-PCT.

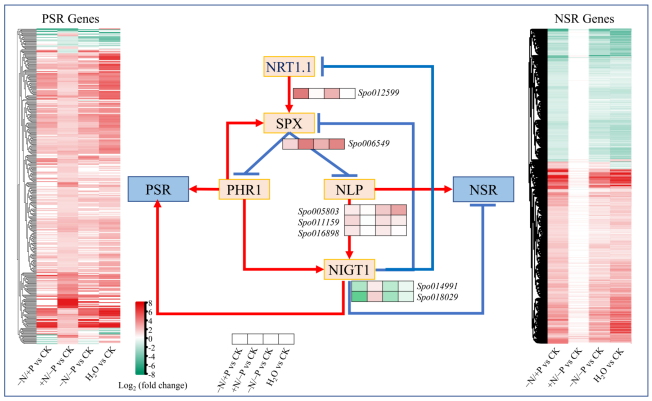
Posted on 23 Nov 2022 — The copyright holder is the author/funder. All rights reserved. No reuse without permission. — https://doi.org/10.22541/au.166920214.45888392/v1 — This a preprint and has not been peer reviewed. Data may be preliminary.



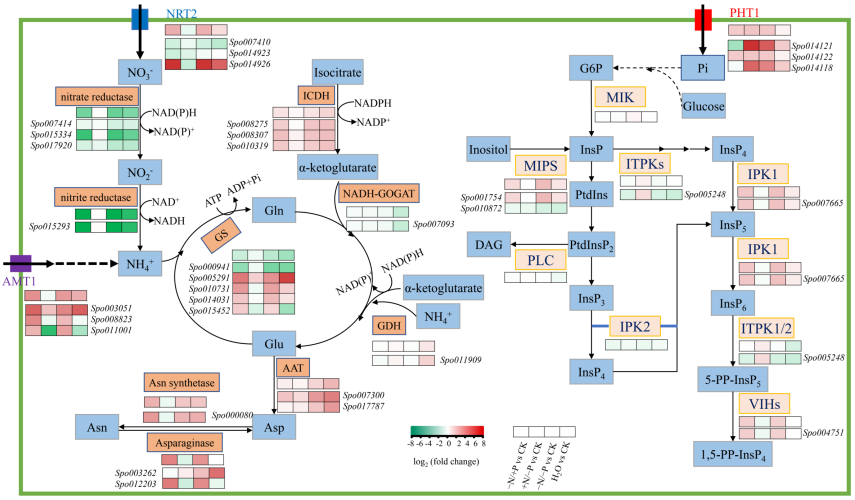


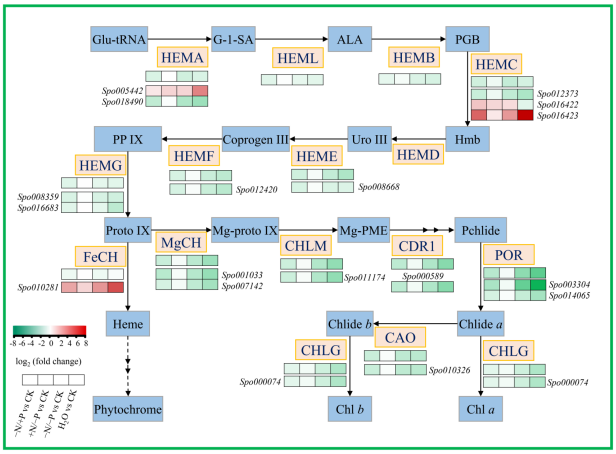
Posted on 23 Nov 2022 — The copyright holder is the author/funder. All rights reserved. No reuse without permission. — https://doi.org/10.22541/au.166920214.45888392/v1 — This a preprint and has not been peer reviewed. Data may be preliminary.



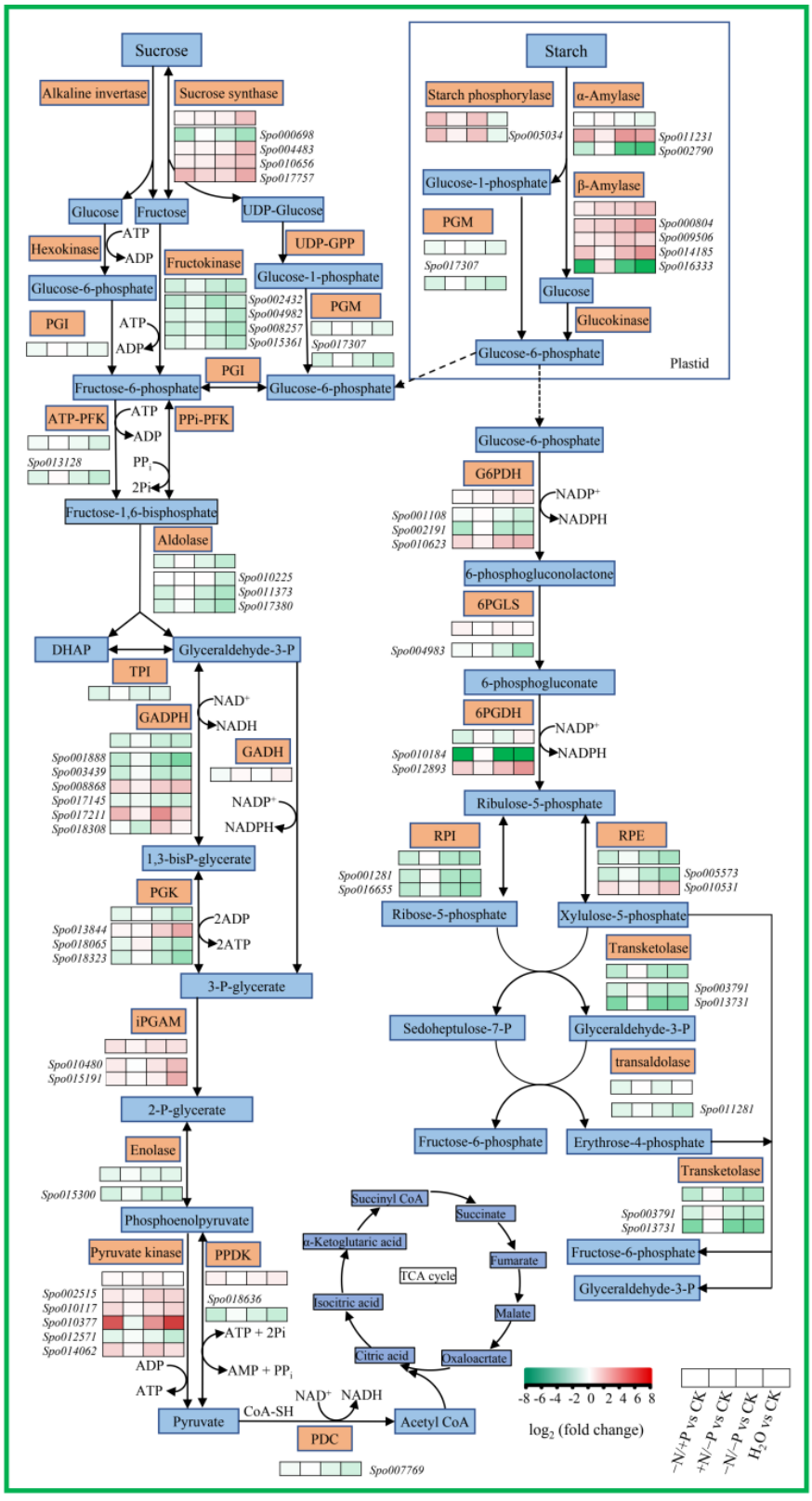


Posted on 23 Nov 2022 — The copyright holder is the author/funder. All rights reserved. No reuse without permission. — https://doi.org/10.22541/au.166920214.45888392/v1 — This a preprint and has not been peer reviewed. Data may be preliminary.

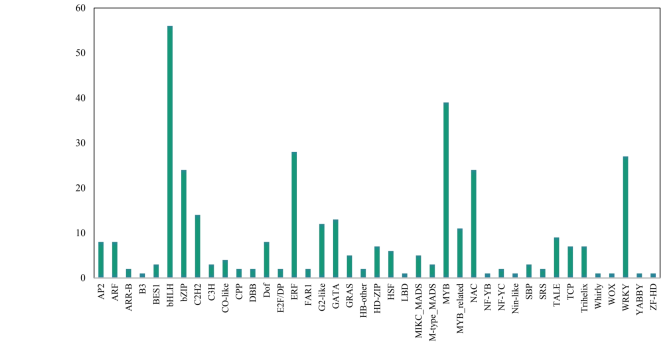


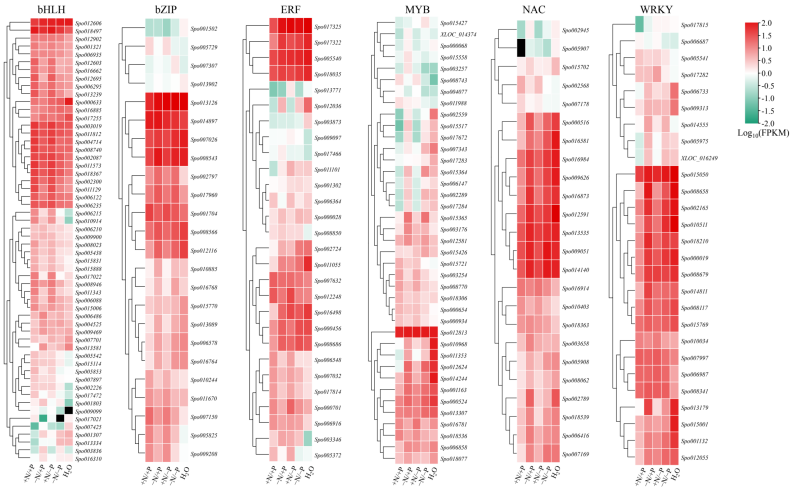


Posted on 23 Nov 2022 — The copyright holder is the author/funder. All rights reserved. No reuse without permission. — https://doi.org/10.22541/au.166920214.45888392/v1 — This a preprint and has not been peer reviewed. Data may be preliminary.

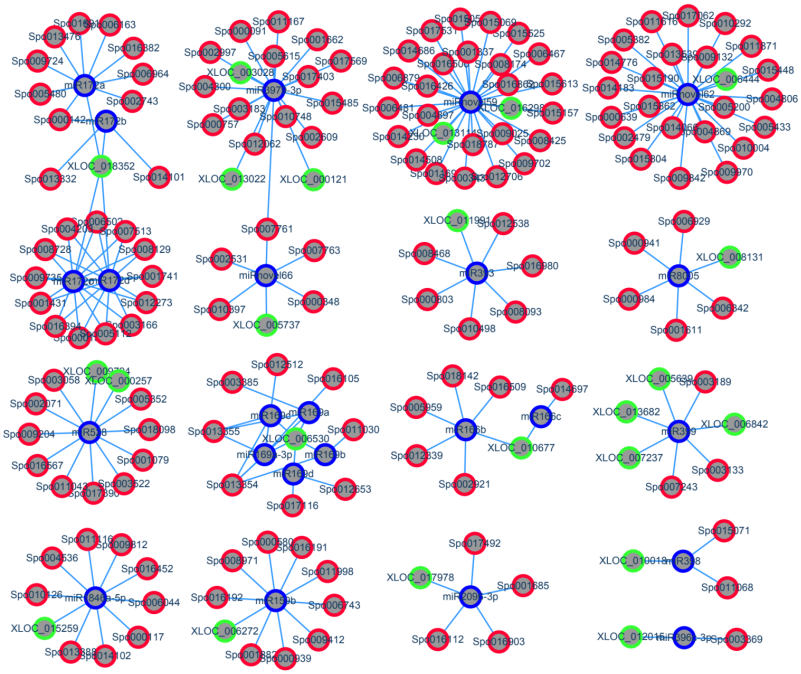


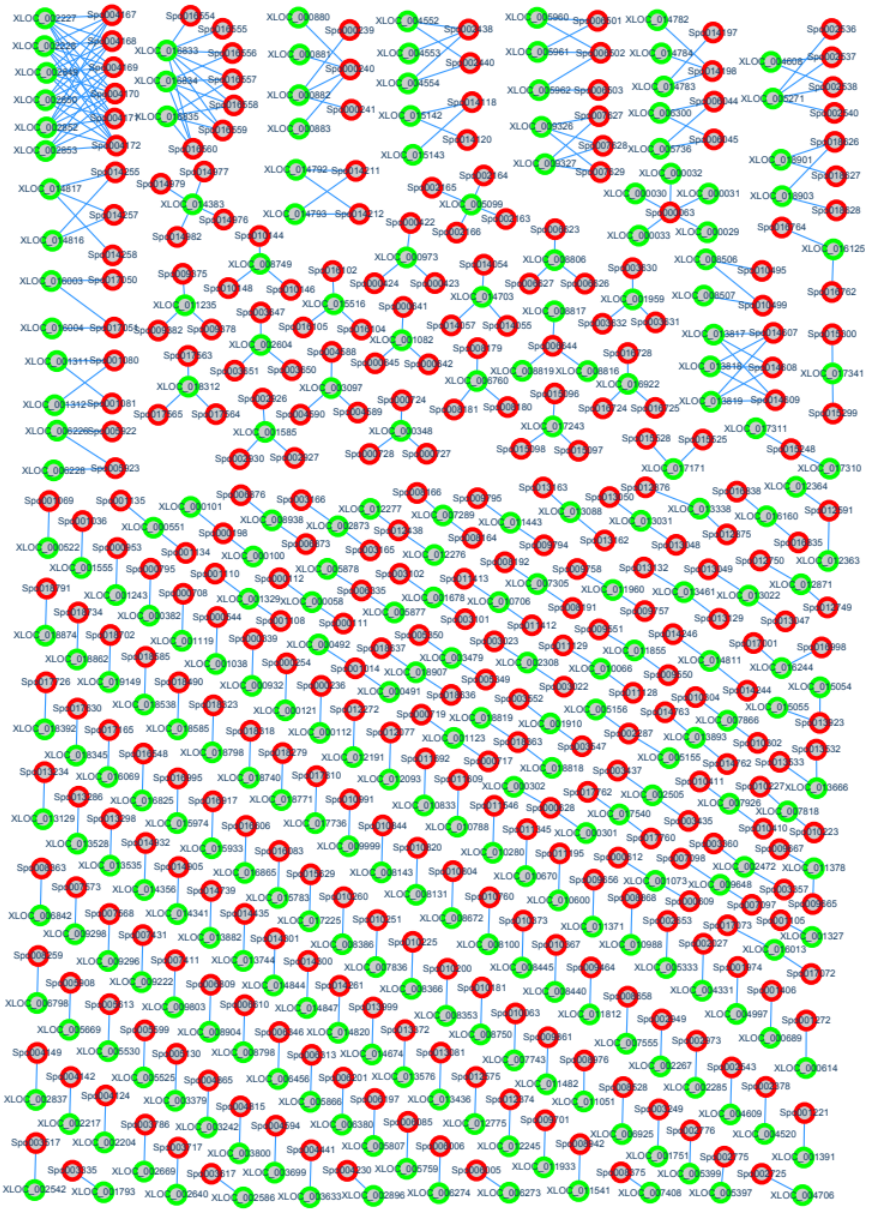
Posted on 23 Nov 2022 — The copyright holder is the author/funder. All rights reserved. No reuse without permission. — https://doi.org/10.22541/au.166920214.45888392/v1 — This a preprint and has not been peer reviewed. Data may be preliminary.



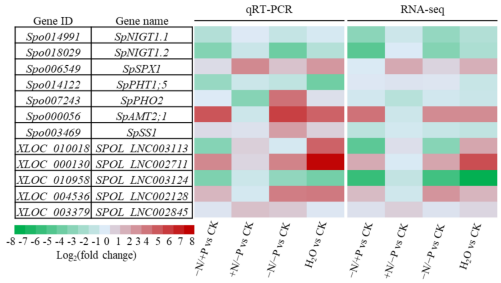


Posted on 23 Nov 2022 — The copyright holder is the author/funder. All rights reserved. No reuse without permission. — https://doi.org/10.22541/au.166920214.45888392/v1 — This a preprint and has not been peer reviewed. Data may be preliminary.





Posted on 23 Nov 2022 — The copyright holder is the author/funder. All rights reserved. No reuse without permission. — https://doi.org/10.22541/au.166920214.45888392/v1 — This a preprint and has not been peer reviewed. Data may be preliminary.



Hosted file

Table 1.docx available at https://authorea.com/users/512860/articles/601548-physiological-and-transcriptomic-analysis-reveal-the-response-mechanisms-to-nutrient-deficiencies-in-aquatic-plant-spirodela-polyrhiza

Posted on 23 Nov 2022 — The copyright holder is the author/funder. All rights reserved. No reuse without permission. — https://doi.org/10.22541/au.166920214.45888392/v1 — This a preprint and has not been peer reviewed. Data may be preliminary.