

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

hongwei hou

houhw@ihb.ac.cn

Institute of Hydrobiology Chinese Academy of Sciences <https://orcid.org/0000-0003-4823-0270>

Xuyao Zhao

Institute of Hydrobiology Chinese Academy of Sciences

Xiaozhe Li

Institute of Hydrobiology Chinese Academy of Sciences

Zuoliang Sun

Institute of Hydrobiology Chinese Academy of Sciences

Gaojie Li

Institute of Hydrobiology Chinese Academy of Sciences

Wenjun Guo

Institute of Hydrobiology Chinese Academy of Sciences

Yan Chen

Institute of Hydrobiology Chinese Academy of Sciences

Manli Xia

Institute of Hydrobiology Chinese Academy of Sciences

Yimeng Chen

Institute of Hydrobiology Chinese Academy of Sciences

Xiaoyu Wang

Institute of Hydrobiology Chinese Academy of Sciences

Yixian Li

Institute of Hydrobiology Chinese Academy of Sciences

Kangsheng Luo

Institute of Hydrobiology Chinese Academy of Sciences

Mingfei Ji

Nanyang Normal University

Pengfei Duan

Nanyang Normal University

Jingjing Yang

Research Article

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

Posted Date: April 22nd, 2024

DOI: <https://doi.org/10.21203/rs.3.rs-4251537/v1>

License:   This work is licensed under a Creative Commons Attribution 4.0 International License.
[Read Full License](#)

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. Aquatic plants adapt themselves to the more complicated 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 various nutrient conditions. We found that the similar response mechanisms are shared between terrestrial plants and *S. polyrhiza*. Encouragingly, novel findings have been found. The dramatic accumulation of starch or protein without significantly growth inhibition under nutrient deficiencies, improve the crop output of *S. polyrhiza*. miR399 is the dual-function regulator in Pi homeostasis of *S. polyrhiza* through targets to *SpPHO2*, *SpPHT1;1* and lncRNAs. The N assimilation process explained the prioritizing usage of ammonium (NH_4^+)-N in duckweeds, enhancing its application to phytoremediation of NH_4^+ waste water.

Key Message

This study analyzed the adaptation mechanisms of different nutrient stresses in macrophyte Spirodela polyrhiza.

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, et al. 2010), freshwater (Elser, et al. 2007; Elser, et al. 2000) and marine (Allgeier, et al. 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 and Leyser 2020). However, overuse of fertilizers allows environmental nutrient release for the low level of N use efficiency (NUE, 18–49%) (Cassman, et al. 2002) and use efficiency (PUE, 15–30%) (Veneklaas, et al. 2012) in modern agriculture, and leads to pollution in the soil and water (Oldroyd and 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.

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 (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 (Acosta, et al. 2021; Cheng and 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 (Cheng and Stomp 2009). *S. polyrhiza* possesses the smallest genome (size of approximately 158 Mb) in duckweed plants (An, et al. 2019; Harkess, et al. 2021; Xu, et al. 2019), the stable genetic transformation and manipulation systems have been established (Liu, et al. 2019; Yang, 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).

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 and 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 and 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 and Leyser 2020; Rietra, et al. 2017; Schachtman and Shin 2007; Xu, et al. 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. 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 and Jackson 1987), P or/and potassium (K) fertilizer increased N uptake and yield of rice (*Oryza sativa*) and wheat (*Triticum aestivum*) (Duan, et al. 2014), P starvation decreased N uptake and assimilation in maize (de Magalhaes, et al. 1998), bean (*Phaseolus vulgaris*) (Gniazdowska and 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, et al. 2021; Maeda, et al. 2018; Medici, et al. 2015; Ueda, et al. 2020; Wang, et al. 2020).

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 H₂O), 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

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 (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 (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[™] 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.

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, et al. 2015) and assembled into transcripts using StringTie software (Pertea, et al. 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.

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/>) (Li, et al. 2014), and LGC (<https://ngdc.cncb.ac.cn/lgc/>) (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.

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 and 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).

Quantitative real-time PCR (qRT-PCR) analysis

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 PrimeScript™ 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 $2^{-\Delta\Delta CT}$ method was used to analyze the data (Pfaffl 2001).

Results

The growth performance of duckweed under different nutrient stresses

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 H₂O (totally nutrient starvation). Compared to control and +N/-P groups, the fronds were blanch in -N/+P, -N/-P, and H₂O treatment groups (Fig. 1). The fresh weight (FW) of duckweed was significantly

decreased in + N/-P and H₂O 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 H₂O 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 (Li, et al. 2021; Sun, et al. 2022; Yu, et al. 2017; Zhao, et al. 2015), high salt (de Moraes, et al. 2019; Fu, et al. 2019), and metal ion stress (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, H₂O 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, H₂O 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, H₂O vs + N/+P: 3.32), but increased in - N/+P treatment group (Fig. 2j). Protein contain was increased in + N/-P and H₂O treatment groups (Fig. 2l).

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

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 data 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), "H₂O 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 H₂O 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), "H₂O vs - N/+P" (3387 DE-mRNAs and 249 DE-lncRNAs), "-N/-P vs + N/-P" (2491 DE-mRNAs and 153 DE-lncRNAs), "H₂O vs + N/-P" (4113 DE-mRNAs and 316 DE-lncRNAs) and "H₂O 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).

Table 1

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

Gene ID	+P/-N versus + P/+N	-P/+N versus + P/+N	-P/-N versus + P/+N	H ₂ O versus + P/+N	Description
Transport-related genes					
<i>Spo002678</i>	NC	NC	-1.11	-1.35	<i>Aluminum-activated malate transporter 1</i>
<i>Spo000056</i>	4.65	NC	3.90	3.91	<i>Ammonium transporter</i>
<i>Spo003051</i>	3.63	1.36	2.83	3.76	<i>Ammonium transporter</i>
<i>Spo003052</i>	1.19	NC	2.59	2.76	<i>Ammonium transporter</i>
<i>Spo008823</i>	2.74	NC	1.55	NC	<i>Ammonium transporter</i>
<i>Spo011001</i>	NC	-4.84	NC	NC	<i>Ammonium transporter</i>
<i>Spo014926</i>	4.83	-1.11	4.76	3.68	<i>High affinity nitrate transporter 2.5</i>
<i>Spo007410</i>	-1.09	NC	-1.05	-1.53	<i>High affinity nitrate transporter 2.5-like</i>
<i>Spo009682</i>	1.36	NC	NC	NC	<i>Nitrate excretion transporter 1</i>
<i>Spo005973</i>	NC	NC	1.29	NC	<i>Nitrate transporter 1.2</i>
<i>Spo012599</i>	3.11	NC	1.74	NC	<i>Nitrate transporter 1.5</i>
<i>Spo015407</i>	-1.83	NC	-1.58	-1.93	<i>Nitrite transporter</i>
<i>Spo002641</i>	NC	NC	-1.06	-1.72	<i>Peptide/nitrate transporter</i>
<i>Spo008033</i>	1.93	NC	2.25	1.66	<i>Peptide/nitrate transporter</i>
<i>Spo016073</i>	-3.57	NC	-3.66	-2.85	<i>Peptide/nitrate transporter</i>
<i>Spo001318</i>	2.51	NC	2.05	1.22	<i>Peptide/nitrate transporter</i>
<i>Spo014118</i>	NC	3.56	2.79	1.03	<i>Inorganic phosphate transporter 1-4</i>
<i>Spo014119</i>	NC	7.30	4.48	NC	<i>Inorganic phosphate transporter 1-4</i>
<i>Spo014121</i>	NC	4.74	3.62	NC	<i>Inorganic phosphate transporter 1-4</i>
<i>Spo014122</i>	1.21	1.31	1.28	NC	<i>Inorganic phosphate transporter 1-4</i>
<i>Spo002956</i>	NC	NC	NC	-1.08	<i>Phosphate transporter PHO1-2</i>
<i>Spo003100</i>	NC	NC	NC	-1.02	<i>Phosphate transporter PHO1-2</i>
<i>Spo010611</i>	2.39	1.06	3.35	4.36	<i>Potassium transporter</i>
<i>Spo012861</i>	NC	NC	1.14	NC	<i>Potassium transporter</i>

Gene ID	+P/-N versus + P/+N	-P/+N versus + P/+N	-P/-N versus + P/+N	H ₂ O versus + P/+N	Description
<i>Spo016521</i>	NC	NC	1.00	1.05	<i>Potassium transporter</i>
<i>Spo008974</i>	-1.32	NC	-2.87	-3.06	<i>Magnesium transporter</i>
<i>Spo009129</i>	NC	NC	1.04	2.62	<i>Magnesium transporter</i>
<i>Spo006667</i>	NC	NC	1.57	-2.99	<i>Sulfate transporter</i>
<i>Spo010875</i>	1.64	NC	NC	NC	<i>Sulfate transporter 2.1</i>
<i>Spo006793</i>	1.57	NC	NC	1.74	<i>Zinc transporter 4</i>
<i>Spo018242</i>	1.50	NC	1.93	3.20	<i>Zinc transporter 4</i>
<i>Spo013803</i>	1.01	NC	NC	NC	<i>Yellow stripe 1A transporter</i>
<i>Spo017391</i>	NC	NC	-1.51	NC	<i>Yellow stripe 1A transporter</i>
<i>Spo012321</i>	1.14	NC	1.24	2.22	<i>Sodium-coupled neutral amino acid transporter 6</i>
<i>Spo017654</i>	-1.27	NC	-1.87	-2.25	<i>Anion transporter 4</i>
<i>Spo018152</i>	-1.38	NC	NC	-2.42	<i>Boron transporter 4</i>
<i>Spo009324</i>	NC	NC	-1.29	-1.98	<i>Putative boron transporter 2</i>
<i>Spo006616</i>	1.10	NC	NC	NC	<i>molybdate transporter 2</i>
<i>Spo000255</i>	NC	NC	-1.60	NC	<i>Copper transporter 6-like protein</i>
<i>Spo018676</i>	3.69	NC	2.73	NC	<i>Vacuolar amino acid transporter 1</i>
<i>Spo004013</i>	6.99	NC	6.62	1.76	<i>Vacuolar iron transporter</i>
<i>Spo015465</i>	-1.21	NC	-1.88	-3.98	<i>Vacuolar iron transporter 1.1</i>
<i>Spo002693</i>	NC	NC	-1.10	-3.10	<i>Vacuolar iron transporter 4</i>
<i>Spo016817</i>	1.06	NC	NC	NC	<i>Vacuolar iron transporter 4</i>
Pi-signaling and N-signaling networks-related genes					
<i>Spo006549</i>	NC	3.04	1.81	2.89	<i>SPX domain-containing protein 1</i>
<i>Spo000337</i>	-1.15	NC	-1.56	NC	<i>Vacuolar cation/proton exchanger 1a</i>
<i>Spo001571</i>	NC	NC	NC	1.16	<i>Vacuolar cation/proton exchanger 2</i>
<i>Spo004802</i>	NC	NC	NC	-1.38	<i>Vacuolar cation/proton exchanger 5</i>
<i>Spo015649</i>	1.55	NC	1.11	2.07	<i>Vacuolar cation/proton exchanger 2</i>

Gene ID	+P/-N versus + P/+N	-P/+N versus + P/+N	-P/-N versus + P/+N	H ₂ O versus + P/+N	Description
<i>Spo014991</i>	-1.86	NC	-1.53	NC	<i>Two-component response regulator ARR1</i>
Pi or N scavenging/remobilization/recycling-related genes					
<i>Spo001264</i>	-1.09	NC	-1.76	-4.28	<i>Purple acid phosphatase</i>
<i>Spo001265</i>	NC	2.21	NC	NC	<i>Purple acid phosphatase</i>
<i>Spo001654</i>	1.41	3.75	1.99	3.44	<i>Purple acid phosphatase</i>
<i>Spo002829</i>	NC	NC	NC	-2.90	<i>Purple acid phosphatase</i>
<i>Spo009071</i>	NC	NC	NC	1.11	<i>Purple acid phosphatase</i>
<i>Spo010600</i>	NC	2.64	NC	3.62	<i>Purple acid phosphatase</i>
<i>Spo011727</i>	1.18	NC	1.02	1.23	<i>Purple acid phosphatase</i>
<i>Spo016760</i>	2.11	NC	NC	NC	<i>Purple acid phosphatase</i>
<i>Spo018669</i>	NC	2.97	NC	3.48	<i>purple acid phosphatase 15-like</i>
<i>Spo000596</i>	NC	NC	NC	-1.54	<i>Ribonuclease 3</i>
<i>Spo001015</i>	1.16	NC	1.21	NC	<i>ribonucleases P/MRP protein subunit POP1 isoform X1</i>
<i>Spo001250</i>	-1.22	NC	NC	-1.64	<i>Putative deoxyribonuclease TATDN1</i>
<i>Spo001850</i>	NC	NC	NC	-2.64	<i>Extracellular ribonuclease LE</i>
<i>Spo002478</i>	NC	NC	NC	1.19	<i>Ribonuclease 2</i>
<i>Spo005957</i>	NC	NC	NC	-1.28	<i>Mini-ribonuclease 3</i>
<i>Spo005963</i>	NC	NC	NC	1.60	<i>Endoribonuclease Dicer 3a</i>
<i>Spo009025</i>	NC	NC	NC	-1.22	<i>Ribonuclease J</i>
<i>Spo006300</i>	NC	NC	NC	-1.57	<i>ATP-dependent helicase/deoxyribonuclease subunit B</i>
<i>Spo012139</i>	NC	NC	NC	-1.46	<i>Ribonuclease Z</i>
<i>Spo012607</i>	NC	NC	NC	1.11	<i>5'-3' exoribonuclease 2</i>
<i>Spo014291</i>	NC	NC	NC	-1.52	<i>Ribonuclease HII</i>
<i>Spo014680</i>	NC	NC	1.26	NC	<i>Nuclear ribonuclease Z</i>
<i>Spo016431</i>	NC	NC	NC	-1.40	<i>Ribonuclease UK114</i>

Gene ID	+P/-N versus + P/+N	-P/+N versus + P/+N	-P/-N versus + P/+N	H ₂ O versus + P/+N	Description
<i>Spo016774</i>	NC	NC	-1.32	-1.28	<i>Ribonuclease</i>
<i>Spo017302</i>	NC	NC	-1.35	-1.50	<i>Poly (U)-specific endoribonuclease-B</i>
<i>Spo017311</i>	NC	NC	NC	-1.17	<i>Ribonuclease Y</i>
<i>Spo005234</i>	-1.01	NC	-1.63	-1.73	<i>Soluble inorganic pyrophosphatase 1</i>
<i>Spo006838</i>	NC	2.16	NC	4.19	<i>Inorganic pyrophosphatase 2</i>
<i>Spo008192</i>	NC	NC	1.64	4.85	<i>Soluble inorganic pyrophosphatase</i>
<i>Spo008751</i>	1.76	2.02	2.07	2.38	<i>Soluble inorganic pyrophosphatase</i>
<i>Spo008753</i>	NC	NC	NC	-1.13	<i>Soluble inorganic pyrophosphatase</i>
<i>Spo003262</i>	NC	NC	1.40	3.39	<i>Putative isoaspartyl peptidase/L-asparaginase 4</i>
<i>Spo012203</i>	3.11	-1.06	2.67	NC	<i>probable isoaspartyl peptidase/L-asparaginase 2 isoform X1</i>
<i>Spo011909</i>	NC	NC	NC	1.06	<i>Glutamate dehydrogenase</i>
Reprogramming of lipid metabolism-related genes					
<i>Spo000329</i>	1.66	NC	1.60	1.86	<i>Non-specific phospholipase C1</i>
<i>Spo000682</i>	NC	NC	NC	1.75	<i>Phospholipase D p1</i>
<i>Spo000953</i>	1.23	NC	NC	2.93	<i>Phospholipase A1-Igama1</i>
<i>Spo000955</i>	1.80	NC	1.20	3.98	<i>Phospholipase A1-Igama1</i>
<i>Spo000977</i>	-1.15	NC	-1.46	NC	<i>non-specific phospholipase C2-like</i>
<i>Spo002339</i>	NC	NC	1.92	2.86	<i>Phospholipase D</i>
<i>Spo003613</i>	NC	NC	NC	-1.22	<i>Phospholipase D</i>
<i>Spo007119</i>	2.74	NC	NC	NC	<i>Phospholipase A1-II 1</i>
<i>Spo014611</i>	2.96	NC	3.91	6.85	<i>phospholipase A1-Igama1, chloroplastic-like</i>
<i>Spo015347</i>	NC	NC	NC	-1.08	<i>Patatin-like phospholipase domain-containing protein 2</i>
<i>Spo016725</i>	2.17	NC	1.74	1.42	<i>phospholipase A1-Igama1, chloroplastic-like</i>
<i>Spo016726</i>	1.60	NC	1.30	NC	<i>phospholipase A1-Igama1, chloroplastic-like</i>

Gene ID	+P/-N versus + P/+N	-P/+N versus + P/+N	-P/-N versus + P/+N	H ₂ O versus + P/+N	Description
<i>Spo016951</i>	NC	NC	NC	1.49	<i>Phospholipase A(1) LCAT3</i>
<i>Spo015069</i>	-1.17	NC	-1.52	-3.13	<i>Phosphoethanolamine N-methyltransferase 1</i>
<i>Spo002214</i>	NC	1.51	NC	1.83	<i>Glycerophosphodiester phosphodiesterase GDE1</i>
<i>Spo001513</i>	NC	NC	-1.18	-1.57	<i>Putative 1-acyl-sn-glycerol-3 - Phosphate acyltransferase 5</i>
<i>Spo009021</i>	-1.18	NC	NC	-1.35	<i>1-acyl-sn-glycerol-3 - Phosphate acyltransferase 2</i>
<i>Spo012718</i>	NC	NC	NC	1.17	<i>Glycerol-3 - Phosphate acyltransferase</i>
<i>Spo014615</i>	NC	NC	NC	-1.90	<i>Glycerol-3 - Phosphate acyltransferase 6</i>
<i>Spo016417</i>	NC	NC	NC	-1.22	<i>1-acyl-sn-glycerol-3 - Phosphate acyltransferase</i>
<i>Spo012305</i>	4.33	4.45	4.33	4.72	<i>Putative monogalactosyldiacylglycerol synthase 3</i>
<i>Spo013217</i>	NC	NC	-1.17	-1.68	<i>Monogalactosyldiacylglycerol synthase</i>
<i>Spo000565</i>	NC	1.48	NC	NC	<i>UDP-sulfoquinovose synthase, chloroplastic</i>
Carbon metabolism-related genes					
<i>Spo002002</i>	2.45	NC	2.34	3.05	<i>probable galactinol-sucrose galactosyltransferase 2</i>
<i>Spo004513</i>	NC	NC	NC	-1.58	<i>(Fructose-bisphosphate aldolase)-lysine N-methyltransferase</i>
<i>Spo010480</i>	NC	NC	NC	1.58	<i>2,3-bisphosphoglycerate-dependent phosphoglycerate mutase</i>
<i>Spo015191</i>	NC	NC	NC	1.96	<i>2,3-bisphosphoglycerate-dependent phosphoglycerate mutase</i>
<i>Spo013844</i>	NC	NC	1.05	2.03	<i>2 - Phosphoglycerate kinase</i>
<i>Spo006266</i>	NC	NC	NC	1.32	<i>6 - Phosphofructokinase 2</i>
<i>Spo014743</i>	2.46	NC	2.84	2.86	<i>6 - Phosphofructokinase 3</i>

Gene ID	+P/-N versus + P/+N	-P/+N versus + P/+N	-P/-N versus + P/+N	H ₂ O versus + P/+N	Description
<i>Spo005912</i>	1.38	NC	1.48	NC	<i>6 - Phosphofructokinase 4</i>
<i>Spo016102</i>	NC	NC	NC	2.93	<i>D-3 - Phosphoglycerate dehydrogenase</i>
<i>Spo015300</i>	NC	NC	-1.14	-1.24	<i>Enolase</i>
<i>Spo010225</i>	NC	NC	NC	-1.06	<i>Fructose-bisphosphate aldolase</i>
<i>Spo011373</i>	-1.01	NC	-1.13	-1.86	<i>Fructose-bisphosphate aldolase</i>
<i>Spo017380</i>	NC	NC	-1.25	-1.96	<i>Fructose-bisphosphate aldolase</i>
<i>Spo007418</i>	-1.11	NC	NC	NC	<i>Galactinol-sucrose galactosyltransferase</i>
<i>Spo018468</i>	1.69	NC	1.86	1.84	<i>Glucose-1 - Phosphate adenylyltransferase</i>
<i>Spo018109</i>	NC	NC	1.18	NC	<i>Glucose-1 - Phosphate adenylyltransferase</i>
<i>Spo000868</i>	-1.53	NC	-2.60	-3.47	<i>Glucose-1 - Phosphate adenylyltransferase</i>
<i>Spo001108</i>	NC	NC	NC	-1.06	<i>Glucose-6 - Phosphate 1- dehydrogenase</i>
<i>Spo002191</i>	-1.76	NC	-1.59	-1.57	<i>Glucose-6 - Phosphate 1- dehydrogenase</i>
<i>Spo010623</i>	NC	NC	1.40	1.74	<i>Glucose-6 - Phosphate 1- dehydrogenase</i>
<i>Spo014375</i>	1.14	NC	1.33	NC	<i>Glucose-6 - Phosphate/phosphate translocator 1</i>
<i>Spo001888</i>	-1.38	NC	-2.14	-3.10	<i>Glyceraldehyde-3 - Phosphate dehydrogenase</i>
<i>Spo003439</i>	-1.18	NC	-1.45	-1.47	<i>Glyceraldehyde-3 - Phosphate dehydrogenase</i>
<i>Spo017145</i>	NC	NC	NC	-1.06	<i>Glyceraldehyde-3 - Phosphate dehydrogenase</i>
<i>Spo017211</i>	1.64	NC	2.62	1.06	<i>Glyceraldehyde-3 - Phosphate dehydrogenase</i>
<i>Spo008380</i>	NC	NC	NC	-1.58	<i>KHG/KDPG aldolase</i>
<i>Spo002942</i>	NC	NC	1.47	1.17	<i>L-allo-threonine aldolase</i>

Gene ID	+P/-N versus + P/+N	-P/+N versus + P/+N	-P/-N versus + P/+N	H ₂ O versus + P/+N	Description
<i>Spo011290</i>	-1.10	NC	-1.23	-1.44	<i>Malate dehydrogenase</i>
<i>Spo001854</i>	NC	NC	-1.28	-2.19	<i>Malate dehydrogenase</i>
<i>Spo014756</i>	NC	NC	1.49	1.86	<i>Malic enzyme</i>
<i>Spo010817</i>	NC	NC	NC	2.60	<i>Phospho-2-dehydro-3-deoxyheptonate aldolase</i>
<i>Spo011471</i>	NC	NC	NC	-1.71	<i>Phospho-2-dehydro-3-deoxyheptonate aldolase</i>
<i>Spo017908</i>	2.46	NC	2.18	NC	<i>Phosphoenolpyruvate carboxylase 4</i>
<i>Spo002560</i>	-1.86	NC	-2.35	-2.74	<i>Phosphoenolpyruvate carboxylase family protein</i>
<i>Spo002932</i>	NC	NC	1.08	NC	<i>Phosphoenolpyruvate carboxylase kinase 1</i>
<i>Spo018065</i>	NC	NC	NC	-1.51	<i>Phosphoglycerate kinase</i>
<i>Spo018323</i>	NC	NC	-1.37	-2.37	<i>Phosphoglycerate kinase</i>
<i>Spo010425</i>	NC	1.63	NC	NC	<i>Sucrose - Phosphate synthase 4</i>
<i>Spo004983</i>	NC	NC	NC	-2.22	<i>6 - Phosphogluconolactonase 2</i>
<i>Spo014865</i>	NC	NC	-1.10	-1.22	<i>Bifunctional methylthioribulose-1 - Phosphate dehydratase/enolase - Phosphatase E1 1</i>
<i>Spo012162</i>	1.06	NC	NC	NC	<i>Galactinol - sucrose galactosyltransferase 1</i>
<i>Spo004293</i>	-1.24	NC	-1.63	-2.89	<i>Glucose-6 - Phosphate 1-epimerase</i>
<i>Spo007027</i>	NC	NC	NC	-1.55	<i>Glucose-6 - Phosphate 1-epimerase</i>
<i>Spo012436</i>	-1.09	NC	-1.11	-1.70	<i>Phosphoglycerate mutase DET1</i>
<i>Spo002861</i>	-1.10	NC	-1.41	-1.82	<i>Phosphoglycerate mutase GpmB</i>
<i>Spo010425</i>	NC	1.63	NC	NC	<i>Sucrose - Phosphate synthase 4</i>
<i>Spo001399</i>	NC	NC	1.45	1.55	<i>Succinate dehydrogenase [ubiquinone] flavoprotein subunit</i>
<i>Spo008894</i>	NC	NC	NC	1.20	<i>Succinate dehydrogenase [ubiquinone] iron-sulfur subunit</i>
<i>Spo017648</i>	NC	NC	NC	-1.28	<i>Sucrose nonfermenting 4-like protein</i>

Gene ID	+P/-N versus + P/+N	-P/+N versus + P/+N	-P/-N versus + P/+N	H ₂ O versus + P/+N	Description
<i>Spo004483</i>	NC	NC	NC	1.58	<i>Sucrose synthase</i>
<i>Spo010656</i>	NC	NC	NC	1.40	<i>Sucrose synthase</i>
<i>Spo017757</i>	1.61	NC	NC	2.00	<i>Sucrose synthase</i>
<i>Spo000698</i>	-1.84	NC	-1.01	-2.10	<i>Sucrose synthase</i>
<i>Spo011281</i>	NC	NC	NC	-1.25	<i>Transaldolase</i>
<i>Spo001453</i>	1.46	NC	1.43	3.03	<i>UDP-glucose 6-dehydrogenase</i>
<i>Spo005030</i>	1.72	NC	1.44	NC	<i>UDP-glucose 6-dehydrogenase</i>
Chlorophyll biosynthetic-related genes					
<i>Spo005442</i>	NC	1.01	1.00	2.93	<i>Glutamyl-tRNA reductase</i>
<i>Spo018490</i>	-1.48	NC	-1.88	-2.31	<i>Glutamyl-tRNA reductase</i>
<i>Spo012373</i>	-1.28	NC	-1.46	-1.94	<i>Porphobilinogen deaminase</i>
<i>Spo016422</i>	1.39	NC	NC	NC	<i>Porphobilinogen deaminase</i>
<i>Spo016423</i>	3.65	NC	2.43	6.03	<i>Porphobilinogen deaminase</i>
<i>Spo008668</i>	-1.21	NC	-1.63	-2.58	<i>Uroporphyrinogen decarboxylase</i>
<i>Spo012420</i>	NC	NC	-1.27	-1.61	<i>Coproporphyrinogen-III oxidase</i>
<i>Spo008359</i>	NC	NC	NC	-1.10	<i>Protoporphyrinogen oxidase</i>
<i>Spo016683</i>	NC	NC	NC	-1.55	<i>Protoporphyrinogen oxidase</i>
<i>Spo010281</i>	2.12	1.03	2.48	4.18	<i>Ferrochelatase</i>
<i>Spo001033</i>	-1.65	NC	-1.71	-2.46	<i>Mg-protoporphyrin IX chelatase</i>
<i>Spo007142</i>	NC	NC	-1.50	-2.13	<i>Mg-protoporphyrin IX chelatase</i>
<i>Spo011174</i>	-1.19	NC	-1.80	-2.55	<i>Uncharacterized protein</i>
<i>Spo000589</i>	-1.54	NC	-1.84	-2.80	<i>Uncharacterized protein</i>
<i>Spo003304</i>	-1.98	NC	-3.61	-5.71	<i>Uncharacterized protein</i>
<i>Spo014065</i>	NC	NC	-1.35	-2.10	<i>WD repeat-containing protein 44</i>
<i>Spo000074</i>	NC	NC	-1.13	-1.87	<i>Chlorophyll synthase</i>
<i>Spo010326</i>	-1.26	NC	-1.53	-1.6	<i>Chlorophyllide a oxygenase</i>
<i>Spo000074</i>	NC	NC	-1.13	-1.87	<i>Chlorophyll synthase</i>

Gene ID	+P/-N versus + P/+N	-P/+N versus + P/+N	-P/-N versus + P/+N	H ₂ O versus + P/+N	Description
Protein-metabolism-related genes					
<i>Spo017964</i>	NC	NC	1.68	NC	<i>26S protease regulatory subunit 10B homolog A</i>
<i>Spo000653</i>	NC	NC	1.01	1.13	<i>AMSH-like ubiquitin thioesterase 3</i>
<i>Spo000095</i>	1.03	NC	1.62	NC	<i>ATP-dependent Clp protease ATP-binding subunit ClpL</i>
<i>Spo000097</i>	1.28	NC	1.79	NC	<i>ATP-dependent Clp protease ATP-binding subunit ClpL</i>
<i>Spo008143</i>	1.29	NC	1.48	NC	<i>ATP-dependent Clp protease ATP-binding subunit ClpL</i>
<i>Spo011319</i>	-1.4	NC	-1.52	-1.92	<i>ATP-dependent Clp protease ATP-binding subunit CLPT1</i>
<i>Spo013060</i>	2.63	NC	1.82	1.77	<i>Carboxyl-terminal - Processing protease</i>
<i>Spo007224</i>	2.93	NC	1.61	1.46	<i>Eukaryotic aspartyl protease family protein</i>
<i>Spo004667</i>	10.36	NC	NC	NC	<i>Lon protease 2</i>
<i>Spo001904</i>	NC	NC	1.04	1.21	<i>BOI-related E3 ubiquitin - Protein ligase 3 isoform X2</i>
<i>Spo004697</i>	NC	NC	3.57	5.71	<i>Protease inhibitor 2</i>
<i>Spo005564</i>	1.05	NC	NC	1.21	<i>Ubiquitin fusion degradation protein 1</i>
<i>Spo014386</i>	1.12	NC	1.09	1.73	<i>Ubiquitin-conjugating enzyme variant</i>
Hormone metabolism-related genes					
<i>Spo010019</i>	3.02	1.43	3.47	5.21	<i>Auxin-induced in root cultures protein 12</i>
<i>Spo011759</i>	NC	NC	NC	2.04	<i>Auxin-induced in root cultures protein 12</i>
<i>Spo010580</i>	NC	NC	NC	-2.39	<i>Auxin-induced in root cultures protein 12</i>
<i>Spo006906</i>	1.43	NC	1.46	-2.89	<i>Carotenoid cleavage dioxygenase 8</i>
<i>Spo006911</i>	3.61	1.20	NC	NC	<i>Carotenoid cleavage dioxygenase 8</i>
<i>Spo006913</i>	5.19	NC	NC	NC	<i>Carotenoid cleavage dioxygenase 8</i>

Gene ID	+P/-N versus + P/+N	-P/+N versus + P/+N	-P/-N versus + P/+N	H ₂ O versus + P/+N	Description
<i>Spo006908</i>	1.02	NC	NC	-2.36	<i>Carotenoid cleavage dioxygenase 8</i>
<i>Spo011055</i>	2.38	1.99	2.93	5.08	<i>Ethylene response factor</i>
<i>Spo017677</i>	2.04	1.48	3.06	2.33	<i>Ethylene response factor 10</i>
<i>Spo017291</i>	-1.61	NC	-1.61	-2.49	<i>Gibberellin 20 oxidase</i>
<i>Spo009147</i>	NC	NC	-2.93	-4.01	<i>Gibberellin 20 oxidase 2</i>
<i>Spo018050</i>	1.78	NC	2.23	4.26	<i>Gibberellin 2-beta-dioxygenase</i>
<i>Spo013914</i>	NC	NC	1.16	2.09	<i>Histidine kinase 1</i>
<i>Spo002714</i>	2.58	1.81	3.79	4.20	<i>Carotenoid cleavage dioxygenase 4</i>
<i>Spo014331</i>	4.39	NC	NC	2.65	<i>Carotenoid cleavage dioxygenase 4</i>
Hormone response-related genes					
<i>Spo000620</i>	NC	NC	1.58	2.64	<i>Auxin efflux carrier component</i>
<i>Spo001052</i>	2.8	NC	2.2	NC	<i>Auxin-induced protein 5NG4</i>
<i>Spo001587</i>	NC	NC	-2.11	-2.91	<i>Auxin-induced protein X10A</i>
<i>Spo002350</i>	NC	NC	-1.36	NC	<i>Auxin-responsive protein</i>
<i>Spo003503</i>	NC	NC	-1.04	NC	<i>Auxin response factor</i>
<i>Spo005902</i>	2.58	NC	3.03	3.68	<i>Auxin-responsive protein</i>
<i>Spo007442</i>	1.42	NC	1.83	1.38	<i>Auxin efflux carrier component</i>
<i>Spo007797</i>	4.07	NC	3.32	NC	<i>Auxin-induced protein 5NG4</i>
<i>Spo009550</i>	NC	NC	1.31	1.54	<i>Auxin transporter protein 1</i>
<i>Spo009957</i>	3.46	NC	3.74	2.03	<i>Auxin-responsive protein</i>
<i>Spo010314</i>	-1.36	NC	NC	NC	<i>Auxin transporter-like protein 3</i>
<i>Spo011064</i>	1.46	NC	NC	NC	<i>Auxin-responsive protein SAUR21-like</i>
<i>Spo011369</i>	2.83	1.5	3.25	4.55	<i>Auxin-induced protein 10A5</i>
<i>Spo013826</i>	1.11	1.03	1.84	1.93	<i>Auxin-induced protein X10A</i>
<i>Spo016482</i>	1.94	NC	1.08	1.23	<i>Auxin-repressed 12.5 kDa protein</i>
<i>Spo016484</i>	2.04	NC	1.03	1.23	<i>Auxin-repressed 12.5 kDa protein</i>
<i>Spo016486</i>	2.09	NC	1.39	NC	<i>Auxin-repressed 12.5 kDa protein</i>

Gene ID	+P/-N versus + P/+N	-P/+N versus + P/+N	-P/-N versus + P/+N	H ₂ O versus + P/+N	Description
<i>Spo016488</i>	5.72	NC	1.33	5.09	<i>Auxin-repressed 12.5 kDa protein</i>
<i>Spo017385</i>	1.91	NC	1.17	-2.16	<i>Auxin-induced protein 10A5</i>
<i>Spo016764</i>	1.65	NC	2.19	2.41	<i>Protein ABSCISIC ACID-INSENSITIVE 5</i>
<i>Spo009600</i>	NC	NC	-1.43	NC	<i>S-adenosyl-L-methionine:salicylic acid carboxyl methyltransferase</i>
Antioxidant-related genes					
<i>Spo002187</i>	-1.03	NA	NA	-1.20	<i>Superoxide dismutase 1 copper chaperone</i>
<i>Spo002187</i>	-1.03	NA	NA	-1.20	<i>Superoxide dismutase 1 copper chaperone</i>
<i>Spo002195</i>	NA	NA	NA	-1.24	<i>Superoxide dismutase [Fe]</i>
<i>Spo009464</i>	NA	NA	-1.39	-2.04	<i>Superoxide dismutase 1 copper chaperone</i>
<i>Spo009504</i>	2.18	NA	1.50	NA	<i>Superoxide dismutase [Cu-Zn]</i>
<i>Spo012688</i>	NA	1.39	-1.34	NA	<i>Hydrogen peroxide induced protein 1</i>
<i>Spo017298</i>	-1.13	NA	-1.14	-2.14	<i>Iron superoxide dismutase</i>
<i>Spo017300</i>	-1.54	NA	-1.18	-1.15	<i>Superoxide dismutase [Fe]</i>
<i>Spo000611</i>	NA	1.53	1.81	1.12	<i>Glutathione transferase GST 23</i>
<i>Spo000612</i>	1.67	NA	2.23	2.53	<i>Glutathione transferase GST 23</i>
<i>Spo001688</i>	NA	NA	NA	-1.88	<i>Glutathione S-transferase DHAR3</i>
<i>Spo003174</i>	NA	NA	NA	-1.27	<i>Lactoylglutathione lyase</i>
<i>Spo003619</i>	-1.58	NA	-1.88	-2.61	<i>Glutathione S-transferase F11</i>
<i>Spo003620</i>	NA	NA	-1.77	-2.67	<i>Glutathione S-transferase</i>
<i>Spo003621</i>	NA	NA	NA	-1.04	<i>Glutathione S-transferase F8</i>
<i>Spo004760</i>	1.70	NA	2.52	1.06	<i>Glutathione S-transferase U17</i>
<i>Spo005888</i>	NA	NA	-1.13	-1.73	<i>Lactoylglutathione lyase</i>
<i>Spo007734</i>	NA	NA	NA	-1.38	<i>Hydroxyacylglutathione hydrolase 3</i>
<i>Spo008502</i>	-2.07	NA	-1.58	-4.73	<i>Glutathione S-transferase</i>

Gene ID	+P/-N versus + P/+N	-P/+N versus + P/+N	-P/-N versus + P/+N	H ₂ O versus + P/+N	Description
<i>Spo008503</i>	-1.42	NA	-1.67	-3.97	<i>Glutathione S-transferase</i>
<i>Spo009201</i>	NA	NA	NA	-1.39	<i>Glutathione S-transferase 2</i>
<i>Spo009873</i>	NA	NA	NA	-1.23	<i>Glutathione reductase</i>
<i>Spo010329</i>	NA	NA	-1.13	NA	<i>Lactoylglutathione lyase / glyoxalase I family protein</i>
<i>Spo010444</i>	1.45	NA	2.13	3.20	<i>Glutathione peroxidase</i>
<i>Spo010922</i>	NA	NA	1.77	1.48	<i>Glutathione S-transferase U10</i>
<i>Spo012043</i>	-1.01	NA	-1.09	-1.64	<i>Glutathione peroxidase</i>
<i>Spo017937</i>	NA	NA	-1.36	-2.41	<i>Glutathione transport system permease protein gsiD</i>

DEGs involved in N- and P-signaling networks

As shown in Fig. 4 and Table 1, *NRT1.1* (*Spo012599*) was upregulated under - N/+P and - N/-P treatments. *SPX1* (*Spo006549*) was upregulated under different treatments, especially under + N/-P and H₂O 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 H₂O treatments. Two *NIGT1* genes (*Spo014991* and *Spo018029*) were upregulated under + N/-P treatment, and downregulated under - N/+P, -N/-P, and H₂O treatments. Several vacuolar cation/proton exchanger (VCPA) encoding genes (*Spo000337*, *Spo0001571*, *Spo004802*, and *Spo015649*) were differential expressing under nutrient stresses, which transmitted the P/N signal by altering the pH, ion concentration, and osmotic pressure in the cytoplasm.

DEGs involved in ion uptake

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 H₂O treatments. *Spo000056*, *Spo003052*, and *Spo008823* were upregulated under - N/+P, -N/-P, and H₂O treatments, and the transcriptional expression level were not influenced by the individual P starvation. *Spo002678* was downregulated under - N/-P and H₂O 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, et al. 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, et al. 2002; Secco, et al. 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

As shown in Fig. 5 and Table 1, the genes 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 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 (Cridland and Gillaspay 2020; 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 synthesizes 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 H₂O 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 (Liu, et al. 2016; Zhang, et al. 2011). As shown in Table 1, there were nine differential expressing *PAP* genes in the treatment groups, most of which changed under H₂O treatment, *Spo001654* was upregulated in all four treatment groups; *Spo001264* was downregulated under – N/+P, –N/+P, and H₂O treatments; *Spo011727* was upregulated under – N/+P, –N/+P, and H₂O treatments; *Spo010600* and *Spo018669* were significantly upregulated in + N/–P and H₂O 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 H₂O 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 H₂O treatment groups.

DEGs involved in carbon cycling

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 discover the relationship between nutritional stress and light and pigment content was analyzed. As shown in Fig. 6, the expression of 14 enzymes were dramatically downregulated under individual/combined N deficiency (– N/+P, –N/–P, and H₂O 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 (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 H₂O 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 H₂O 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 H₂O 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, et al. 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 H₂O treatment. *Spo004483*, *Spo010656*, and *Spo017757* were upregulated in all treatment groups, *Spo000698* was downregulated under – N/+P, –N/–P, and H₂O 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 H₂O 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 dehydrogenase* (*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 H₂O treatments). The expression of *non-phosphorylating glyceraldehyde-3-phosphate dehydrogenase* (*GAPN*) genes was upregulated which catalysis glyceraldehyde-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.

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 H₂O) 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 H₂O 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 H₂O 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 and 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 H₂O treatment, *Spo006911* was upregulated in – N/+P and + N/–P treatment groups, *Spo006908* was upregulated under – N/+P treatment while downregulated in H₂O 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 H₂O treatment groups. Auxin efflux carrier coding gene *Spo000620* was upregulated under – N/–P and H₂O treatments, and *Spo007442* was upregulated in – N/+P, –N/–P and H₂O 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 H₂O 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 H₂O treatment groups.

DEGs involved in antioxidant system

The expression levels of several Superoxide dismutase (SOD) encoding genes were changed under nutritional stresses, *Spo002187* was downregulated in – N/+P and H₂O treatment groups, *Spo009504* was upregulated in – N/+P and – N/–P treatment groups. Expression of three glutathione transferase (GST) encoding genes were changed in treatment groups, *Spo000611* was upregulated in + N/–P, –N/–P, and H₂O treatment groups, *Spo000612* and *Spo004760* were upregulated in – N/+P, –N/–P, and H₂O treatment groups. Glutathione peroxidase (GPX) encoding gene *Spo010444* was upregulated in – N/+P, –N/–P, and H₂O treatment groups, *Spo012043* was downregulated in – N/+P, –N/–P, and H₂O 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 H₂O treatment groups (Mittler, et al. 2004).

DEGs involved in transcriptional regulation

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 (Zhou, et al. 2008). *HY5* homologous *Spo013126*, *WRKY42* homologous *Spo015050*, *WRKY75* homologous genes *Spo002165* and *Spo010511* were upregulated 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 (Yang, et al. 2016). We also found that some *SpbHLH* genes were significantly upregulated under nutrient stresses, including *Spo001307*, *Spo007425*, *Spo013334*, and *Spo013581*.

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.

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, et al. 2009; Hell and Hillebrand 2001; 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 and Lin 2011; Cordell, et al. 2009; Yuan and 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, et al. 2010; Yuan and 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, et al. 2011), and low N inhibits the lateral root development (Kellermeier, et al. 2014). In maize, Pi deficiency can promote the development of lateral roots, increase the total root length and root-to-shoot (R/S) ratio, and promotes the formation of mycorrhizae (Yu, et al. 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 (Li, et al. 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 RSA 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, et al. 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 (Huang, et al. 2004; Mghase, et al. 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 H₂O treatment groups, and less than that in + N/-P and - N/-P treatment groups. The biomass of fronds in treatment groups was less than that in control group, biomass of fronds in - N/-P treatment group was greater than - N/+P, +N/-P, and H₂O 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 (Medici, et al. 2019).

Nutrient deficiency causes the decreasing of chlorophyll content, especially N deficiency (Cui, et al. 2019; Huang, et al. 2004). In this study, we found individual/combined N deficiency (- N/+P, -N/-P, and H₂O treatments) both caused the bleaching and decreasing of chlorophyll content in frond, as well as the previous studies in duckweeds under N starvation (Liu, et al. 2018; Sun, et al. 2022; 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 (Li, et al. 2021; Zhao, et al. 2015) and H₂O 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 H₂O 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* (Yu, et al. 2017) and *punctata* (Zhao, et al. 2015). In this study, protein content in control was higher than that in - N/+P and - N/-P treatment groups, and lower than that in + N/-P and H₂O 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 (Li, et al. 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 (Chen, et al. 2008). On the other hand, the absorption and assimilation process of different elements is influenced each other in the plants (Cochavi, et al. 2020; Oldroyd and 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 (Li, et al. 2021; 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, et al. 2018). There was an increased affinity to 30 fold for NH_4^+ compared with NO_3^- in *Zostera noltii* (Alexandre, et al. 2011). Previous studies reported that duckweed plants *S. polyrhiza* (Caicedo, et al. 2000; Zhou, et al. 2022), *L. punctata* (Fang, et al. 2007; Zhou, et al. 2022), *L. aequinoctialis* (Zhou, et al. 2022), *L. minor* (Cedergreen and Madsen 2002; Petersen, et al. 2021; Zhou, et al. 2022), *L. turionifera* (Zhou, et al. 2022), *Wolffiella hyaline* (Petersen, et al. 2021), and *Woffila globosa* (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 (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 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 (Huang, et al. 2004; Muller, et al. 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 and Lin 2011; Khan, et al. 2016; Perez-Torres, et al. 2008; 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.

Declarations

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]; National Key R & D Program [Grant numbers, 2020YFD0900305]; Zhongke Technology Achievement Transfer and Transformation Center of Henan Province [2024128]; Future Partnership Network Special Program of Chinese Academy of Sciences for 2024 [075GJHZ2023015FN]; National Aquatic Biological Resource Center.

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, Mingfei Ji** and **Pengfei Duan**: 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

¹The 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. ²Henan 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. ³University of Chinese Academy of Sciences, Beijing 100049, China. ⁴College of Fisheries and Life Science, Dalian Ocean University, Dalian 116023, Liaoning, China.

References

1. Acosta K, Appenroth KJ, Borisjuk L, Edelman M, Heinig U, Jansen MAK, Oyama T, Pasaribu B, Schubert I, Sorrels S, Sree KS, Xu S, Michael TP, Lam E (2021) Return of the Lemnaceae: duckweed

- as a model plant system in the genomics and postgenomics era. *Plant Cell* 33:3207-3234
2. Al-Babili S, Bouwmeester HJ (2015) Strigolactones, a novel carotenoid-derived plant hormone. *Annu Rev Plant Biol* 66:161-186
 3. Alexandre A, Silva J, Bouma TJ, Santos R (2011) Inorganic nitrogen uptake kinetics and whole-plant nitrogen budget in the seagrass *Zostera noltii*. *J Exp Mar Biol Ecol* 401:7-12
 4. Allgeier JE, Yeager LA, Layman CA (2013) Consumers regulate nutrient limitation regimes and primary production in seagrass ecosystems. *Ecology* 94:521-529
 5. An D, Zhou Y, Li CS, Xiao Q, Wang T, Zhang YT, Wu YR, Li YB, Chao DY, Messing J, Wang WQ (2019) Plant evolution and environmental adaptation unveiled by long-read whole-genome sequencing of *Spirodela*. *Proc Natl Acad Sci U S A* 116:18893-18899
 6. Balzergue C, Darteville T, Godon C, Laugier E, Meisrimler C, Teulon J-M, Creff A, Bissler M, Bouchoud C, Hagege A, Muller J, Chiarenza S, Javot H, Becuwe-Linka N, David P, Peret B, Delannoy E, Thibaud M-C, Armengaud J, Abel S, Pellequer J-L, Nussaume L, Desnos T (2017) Low phosphate activates STOP1-ALMT1 to rapidly inhibit root cell elongation. *Nat Commun* 8:15300
 7. Bari R, Pant BD, Stitt M, Scheible WR (2006) PHO2, microRNA399, and PHR1 define a phosphate-signaling pathway in plants. *Plant Physiol* 141:988-999
 8. Beale SI (2005) Green genes gleaned. *Trends Plant Sci* 10:309-312
 9. Caicedo JR, Van der Steen NP, Arce O, Gijzen HJ (2000) Effect of total ammonia nitrogen concentration and pH on growth rates of duckweed (*Spirodela polyrrhiza*). *Water Res* 34:3829-3835
 10. Calabrese S, Kohler A, Niehl A, Veneault-Fourrey C, Boller T, Courty PE (2017) Transcriptome analysis of the *Populus trichocarpa*-*Rhizophagus irregularis* Mycorrhizal Symbiosis: Regulation of Plant and Fungal Transportomes under Nitrogen Starvation. *Plant Cell Physiol* 58:1003-1017
 11. Cassman KG, Dobermann A, Walters DT (2002) Agroecosystems, nitrogen-use efficiency, and nitrogen management. *Ambio* 31:132-140
 12. Cedergreen N, Madsen TV (2002) Nitrogen uptake by the floating macrophyte *Lemna minor*. *New Phytol* 155:285-292
 13. Chen DQ, Zhang H, Wang QL, Shao M, Li XY, Chen DM, Zeng RS, Song YY (2020) Intraspecific variations in cadmium tolerance and phytoaccumulation in giant duckweed (*Spirodela polyrrhiza*). *J Hazard Mater* 395:122672
 14. Chen YF, Wang Y, Wu WH (2008) Membrane transporters for nitrogen, phosphate and potassium uptake in plants. *J Integr Plant Biol* 50:835-848
 15. Cheng JJ, Stomp AM (2009) Growing Duckweed to Recover Nutrients from Wastewaters and for Production of Fuel Ethanol and Animal Feed. *Clean-Soil Air Water* 37:17-26
 16. Chiou TJ, Lin SI (2011) Signaling network in sensing phosphate availability in plants. *Annu Rev Plant Biol* 62:185-206
 17. Cochavi A, Cohen IH, Rachmilevitch S (2020) The role of different root orders in nutrient uptake. *Environ Exp Bot* 179:104212

18. Cordell D, Drangert JO, White S (2009) The story of phosphorus: Global food security and food for thought. *Global Environ Chang* 19:292-305
19. Cridland C, Gillaspay G (2020) Inositol Pyrophosphate Pathways and Mechanisms: What Can We Learn from Plants? *Molecules* 25:11
20. 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. *Biochem Biophys Res Commun* 508:314-319
21. de Magalhaes JV, Alves VMC, de Novais RF, Mosquim PR, Magalhaes JR, Bahia AFC, Huber DM (1998) Nitrate uptake by corn under increasing periods of phosphorus starvation. *J Plant Nutr* 21:1753-1763
22. de Moraes MB, Barbosa-Neto AG, Willadino L, Ulisses C, Calsa T (2019) Salt Stress Induces Increase in Starch Accumulation in Duckweed (*Lemna aequinoctialis*, Lemnaceae): Biochemical and Physiological Aspects. *J Plant Growth Regul* 38:683-700
23. Duan YH, Shi XJ, Li SL, Sun XF, He XH (2014) Nitrogen Use Efficiency as Affected by Phosphorus and Potassium in Long-Term Rice and Wheat Experiments. *J Integr Agr* 13:588-596
24. Elser JJ, Bracken MES, Cleland EE, Gruner DS, Harpole WS, Hillebrand H, Ngai JT, Seabloom EW, Shurin JB, Smith JE (2007) Global analysis of nitrogen and phosphorus limitation of primary producers in freshwater, marine and terrestrial ecosystems. *Ecol Lett* 10:1135-1142
25. Elser JJ, Fagan WF, Denno RF, Dobberfuhl DR, Folarin A, Huberty A, Interlandi S, Kilham SS, McCauley E, Schulz KL, Siemann EH, Sterner RW (2000) Nutritional constraints in terrestrial and freshwater food webs. *Nature* 408:578-580
26. Esfahani MN, Inoue K, Nguyen KH, Chu HD, Watanabe Y, Kanatani A, Burritt DJ, Mochida K, Tran LSP (2021) Phosphate or nitrate imbalance induces stronger molecular responses than combined nutrient deprivation in roots and leaves of chickpea plants. *Plant Cell Environ* 44:574-597
27. Fageria VD (2001) Nutrient interactions in crop plants. *J Plant Nutr* 24:1269-1290
28. Fang YY, Babourina O, Rengel Z, Yang XE, Pu PM (2007) Ammonium and nitrate uptake by the floating plant *Landoltia punctata*. *Ann Bot* 99:365-370
29. Fu LL, Ding ZH, Sun XP, Zhang JM (2019) Physiological and Transcriptomic Analysis Reveals Distorted Ion Homeostasis and Responses in the Freshwater Plant *Spirodela polyrrhiza* L. under Salt Stress. *Genes* 10:24
30. Fu LL, Ding ZH, Tan DG, Han BY, Sun XP, Zhang JM (2020) Genome-wide discovery and functional prediction of salt-responsive lncRNAs in duckweed. *BMC Genomics* 21:14
31. 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. *Curr Opin Plant Biol* 9:436-442
32. Gamir J, Torres-Vera R, Rial C, Berrio E, Campos PMD, Varela RM, Macias FA, Pozo MJ, Flors V, Lopez-Raez JA (2020) Exogenous strigolactones impact metabolic profiles and phosphate starvation signalling in roots. *Plant Cell Environ* 43:1655-1668

33. Gniazdowska A, Rychter AM (2000) Nitrate uptake by bean (*Phaseolus vulgaris* L.) roots under phosphate deficiency. *Plant Soil* 226:79-85
34. Gruber BD, Giehl RF, Friedel S, von Wiren N (2013) Plasticity of the Arabidopsis root system under nutrient deficiencies. *Plant Physiol* 163:161-179
35. Hamburger D, Rezzonico E, Petetot JMC, Somerville C, Poirier Y (2002) Identification and characterization of the Arabidopsis *PHO1* gene involved in phosphate loading to the xylem. *Plant Cell* 14:889-902
36. Harkess A, McLoughlin F, Bilkey N, Elliott K, Emenecker R, Mattoon E, Miller K, Czymmek K, Vierstra RD, Meyers BC, Michael TP (2021) Improved *Spirodela polyrhiza* genome and proteomic analyses reveal a conserved chromosomal structure with high abundance of chloroplastic proteins favoring energy production. *J Exp Bot* 72:2491-2500
37. Harpole WS, Ngai JT, Cleland EE, Seabloom EW, Borer ET, Bracken MES, Elser JJ, Gruner DS, Hillebrand H, Shurin JB, Smith JE (2011) Nutrient co-limitation of primary producer communities. *Ecol Lett* 14:852-862
38. Hell R, Hillebrand H (2001) Plant concepts for mineral acquisition and allocation. *Curr Opin Biotechnol* 12:161-168
39. Hu B, Jiang Z, Wang W, Qiu Y, Zhang Z, Liu Y, Li A, Gao X, Liu L, Qian Y, Huang X, Yu F, Kang S, Wang Y, Xie J, Cao S, Zhang L, Wang Y, Xie Q, Kopriva S, Chu C (2019) Nitrate-NRT1.1B-SPX4 cascade integrates nitrogen and phosphorus signalling networks in plants. *Nat Plants* 5:401-413
40. Hu B, Zhu C, Li F, Tang J, Wang Y, Lin A, Liu L, Che R, Chu C (2011) LEAF TIP NECROSIS1 Plays a Pivotal Role in the Regulation of Multiple Phosphate Starvation Responses in Rice. *Plant Physiol* 156:1101-1115
41. Huang ZA, Jiang DA, Yang Y, Sun JW, Jin SH (2004) Effects of nitrogen deficiency on gas exchange, chlorophyll fluorescence, and antioxidant enzymes in leaves of rice plants. *Photosynthetica* 42:357-364
42. 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 Res* 45:W12-W16
43. Kellermeier F, Armengaud P, Seditas TJ, Danku J, Salt DE, Amtmann A (2014) Analysis of the Root System Architecture of Arabidopsis Provides a Quantitative Readout of Crosstalk between Nutritional Signals. *Plant Cell* 26:1480-1496
44. Khan GA, Vogiatzaki E, Glauser G, Poirier Y (2016) Phosphate Deficiency Induces the Jasmonate Pathway and Enhances Resistance to Insect Herbivory. *Plant Physiol* 171:632-644
45. Kim D, Landmead B, Salzberg SL (2015) HISAT: a fast spliced aligner with low memory requirements. *Nat Methods* 12:357-U121
46. Krouk G, Kiba T (2020) Nitrogen and Phosphorus interactions in plants: from agronomic to physiological and molecular insights. *Curr Opin Plant Biol* 57:104-109
47. Langfelder P, Horvath S (2008) WGCNA: an R package for weighted correlation network analysis. *BMC Bioinformatics* 9:559

48. 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
49. Li JM, Du AP, Liu PH, Tian XP, Jin YL, Yi ZL, He KZ, Fang Y, Zhao H (2021) High starch accumulation mechanism and phosphorus utilization efficiency of duckweed (*Landoltia punctata*) under phosphate starvation. *Ind Crop Prod* 167:113529
50. Li LH, Liu C, Lian XM (2010) Gene expression profiles in rice roots under low phosphorus stress. *Plant Mol Biol* 72:423-432
51. Li XX, Zeng RS, Liao H (2016) Improving crop nutrient efficiency through root architecture modifications. *J Integr Plant Biol* 58:193-202
52. Liu PD, Xue YB, Chen ZJ, Liu GD, Tian J (2016) Characterization of purple acid phosphatases involved in extracellular dNTP utilization in *Stylosanthes*. *J Exp Bot* 67:4141-4154
53. Liu Y, Wang XH, Fang Y, Huang MJ, Chen XY, Zhang Y, Zhao H (2018) The effects of photoperiod and nutrition on duckweed (*Landoltia punctata*) growth and starch accumulation. *Ind Crop Prod* 115:243-249
54. Liu Y, Wang Y, Xu SQ, Tang XF, Zhao JS, Yu CJ, He G, Xu H, Wang SM, Tang YL, Fu CX, Ma YB, Zhou GK (2019) Efficient genetic transformation and CRISPR/Cas9-mediated genome editing in *Lemna aequinoctialis*. *Plant Biotechnol J* 17:2143-2152
55. 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. *J Exp Bot* 72:3500-3503
56. Maeda Y, Konishi M, Kiba T, Sakuraba Y, Sawaki N, Kurai T, Ueda Y, Sakakibara H, Yanagisawa S (2018) A NIGT1-centred transcriptional cascade regulates nitrate signalling and incorporates phosphorus starvation signals in *Arabidopsis*. *Nat Commun* 9:1376
57. Marro N, Lidoy J, Chico MA, Rial C, Garcia J, Varela RM, Macias FA, Pozo MJ, Janouskova M, Lopez-Raez JA (2022) Strigolactones: New players in the nitrogen-phosphorus signalling interplay. *Plant Cell Environ* 45:512-527
58. Medici A, Marshall-Colon A, Ronzier E, Szponarski W, Wang R, Gojon A, Crawford NM, Ruffel S, Coruzzi GM, Krouk G (2015) AtNIGT1/HRS1 integrates nitrate and phosphate signals at the *Arabidopsis* root tip. *Nat Commun* 6:6274
59. Medici A, Szponarski W, Dangeville P, Safi A, Dissanayake IM, Saenchai C, Emanuel A, Rubio V, Lacombe B, Ruffel S, Tanurdzic M, Rouached H, Krouk G (2019) Identification of Molecular Integrators Shows that Nitrogen Actively Controls the Phosphate Starvation Response in Plants. *Plant Cell* 31:1171-1184
60. Mghase JJ, 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)*
61. Mittler R, Vanderauwera S, Gollery M, Van Breusegem F (2004) Reactive oxygen gene network of plants. *Trends Plant Sci* 9:490-498

62. Muller R, Morant M, Jarmer H, Nilsson L, Nielsen TH (2007) Genome-wide analysis of the Arabidopsis leaf transcriptome reveals interaction of phosphate and sugar metabolism. *Plant Physiol* 143:156-171
63. Nakamura Y, Koizumi R, Shui GH, Shimojima M, Wenk MR, Ito T, Ohta H (2009) Arabidopsis lipins mediate eukaryotic pathway of lipid metabolism and cope critically with phosphate starvation. *Proc Natl Acad Sci U S A* 106:20978-20983
64. Nayar S, Loo MGK, Tanner JE, Longmore AR, Jenkins GP (2018) Nitrogen acquisition and resource allocation strategies in temperate seagrass *Zostera nigricaulis*: Uptake, assimilation and translocation processes. *Sci Rep* 8:17151
65. 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.). *Ann Bot* 85:909-919
66. Nguyen HTK, Kim SY, Cho KM, Hong JC, Shin JS, Kim HJ (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 Cell Physiol* 57:784-797
67. Oldroyd GED, Leyser O (2020) A plant's diet, surviving in a variable nutrient environment. *Science* 368:45-+
68. Paris Q (1992) The Return of VonLiebig Law of the Minimum. *Agron J* 84:1040-1046
69. 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 Physiol Biochem* 70:221-234
70. Peret B, Clement M, Nussaume L, Desnos T (2011) Root developmental adaptation to phosphate starvation: better safe than sorry. *Trends Plant Sci* 16:442-450
71. 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* 20:3258-3272
72. Pertea M, Kim D, Pertea GM, Leek JT, Salzberg SL (2016) Transcript-level expression analysis of RNA-seq experiments with HISAT, StringTie and Ballgown. *Nat Protoc* 11:1650-1667
73. Petersen F, Demann J, Restemeyer D, Ulbrich A, Olf HW, Westendarp H, Appenroth KJ (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* 10:1741
74. Pfaffl MW (2001) A new mathematical model for relative quantification in real-time RT-PCR. *Nucleic Acids Res* 29:e45
75. Rietra RPJJ, Heinen M, Dimkpa CO, Bindraban PS (2017) Effects of Nutrient Antagonism and Synergism on Yield and Fertilizer Use Efficiency. *Commun Soil Sci Plant Anal* 48:1895-1920
76. Roch GV, Maharajan T, Ceasar SA, Ignacimuthu S (2019) The Role of PHT1 Family Transporters in the Acquisition and Redistribution of Phosphorus in Plants. *Crit Rev Plant Sci* 38:171-198

77. Rouached H, Arpat AB, Poirier Y (2010) Regulation of Phosphate Starvation Responses in Plants: Signaling Players and Cross-Talks. *Mol Plant* 3:288-299
78. Schachtman DP, Shin R (2007) Nutrient sensing and signaling: NPKS. *Annu Rev Plant Biol* 58:47-69
79. 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 Physiol* 152:1693-1704
80. Smith FW, Jackson WA (1987) Nitrogen Enhancement of Phosphate-Transport in Roots of *Zea-Mays*-L .2. Kinetic and Inhibitor Studies. *Plant Physiol* 84:1319-1324
81. Srivastava S, Upadhyay MK, Srivastava AK, Abdelrahman M, Suprasanna P, Tran LSP (2018) Cellular and Subcellular Phosphate Transport Machinery in Plants. *Int J Mol Sci* 19:
82. Sun ZL, Guo WJ, Zhao XY, Chen Y, Yang JJ, Xu SQ, Hou HW (2022) Sulfur limitation boosts more starch accumulation than nitrogen or phosphorus limitation in duckweed (*Spirodela polyrhiza*). *Ind Crop Prod* 185:115098
83. Taniguchi M, Taniguchi Y, Kawasaki M, Takeda S, Kato T, Sato S, Tahata S, Miyake H, Sugiyama T (2002) Identifying and characterizing plastidic 2-oxoglutarate/malate and dicarboxylate transporters in *Arabidopsis thaliana*. *Plant Cell Physiol* 43:706-717
84. Tao X, Fang Y, Xiao Y, Jin YL, Ma XR, Zhao Y, He KZ, Zhao H, Wang HY (2013) Comparative transcriptome analysis to investigate the high starch accumulation of duckweed (*Landoltia punctata*) under nutrient starvation. *Biotechnol Biofuels* 6:15
85. Ueda Y, Kiba T, Yanagisawa S (2020) Nitrate-inducible NIGT1 proteins modulate phosphate uptake and starvation signalling via transcriptional regulation of *SPX* genes. *Plant J* 102:448-466
86. Veneklaas EJ, Lambers H, Bragg J, Finnegan PM, Lovelock CE, Plaxton WC, Price CA, Scheible WR, Shane MW, White PJ, Raven JA (2012) Opportunities for improving phosphorus-use efficiency in crop plants. *New Phytol* 195:306-320
87. Vitousek PM, Porder S, Houlton BZ, Chadwick OA (2010) Terrestrial phosphorus limitation: mechanisms, implications, and nitrogen-phosphorus interactions. *Ecol Appl* 20:5-15
88. Wang G, Yin H, Li B, Yu C, Wang F, Xu X, Cao J, Bao Y, Wang L, Abbasi AA, Bajic VB, Ma L, Zhang Z (2019) Characterization and identification of long non-coding RNAs based on feature relationship. *Bioinformatics* 35:2949-2956
89. Wang X, Wang HF, Chen Y, Sun MM, Wang Y, Chen YF (2020) The Transcription Factor NIGT1.2 Modulates Both Phosphate Uptake and Nitrate Influx during Phosphate Starvation in *Arabidopsis* and Maize. *Plant Cell* 32:3519-3534
90. Wege S, Khan GA, Jung JY, Vogiatzaki E, Pradervand S, Aller I, Meyer AJ, Poirier Y (2016) The EXS Domain of PHO1 Participates in the Response of Shoots to Phosphate Deficiency via a Root-to-Shoot Signal. *Plant Physiol* 170:385-400
91. Xu G, Fan X, Miller AJ (2012) Plant Nitrogen Assimilation and Use Efficiency. In: Merchant SS (ed) *Annu Rev Plant Biol*. pp 153-182

92. Xu L, Zhao HY, Wan RJ, Liu Y, Xu Z, Tian W, Ruan WY, Wang F, Deng MJ, Wang JM, Dolan L, Luan S, Xue SW, Yi KK (2019) Identification of vacuolar phosphate efflux transporters in land plants. *Nat Plants* 5:84-94
93. Xu SQ, Stapley J, Gablenz S, Boyer J, Appenroth KJ, Sree KS, Gershenzon J, Widmer A, Huber M (2019) Low genetic variation is associated with low mutation rate in the giant duckweed. *Nat Commun* 10:1243
94. Xu YL, Ma S, Huang M, Peng M, Bog M, Sree KS, Appenroth KJ, Zhang JM (2015) Species distribution, genetic diversity and barcoding in the duckweed family (Lemnaceae). *Hydrobiologia* 743:75-87
95. Yamaguchi S (2008) Gibberellin metabolism and its regulation. *Annu Rev Plant Biol* 59:225-251
96. Yang JJ, Lia GJ, Hua SQ, Bishopp A, Heenatigala PPM, Kumar S, Duan PF, Yao LG, Hou HW (2018) A protocol for efficient callus induction and stable transformation of *Spirodela polyrhiza* (L.) Schleiden using *Agrobacterium tumefaciens*. *Aquat Bot* 151:80-86
97. Yang TR, Hao L, Yao SF, Zhao YY, Lu WJ, 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 Physiol Biochem* 104:99-113
98. 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 Physiol* 138:2087-2096
99. Yoon J, Cho LH, Tun W, Jeon JS, An G (2021) Sucrose signaling in higher plants. *Plant Sci* 302:110703
100. Yu C, Zhao X, Qi G, Bai Z, Wang Y, Wang S, Ma Y, Liu Q, Hu R, Zhou G (2017) Integrated analysis of transcriptome and metabolites reveals an essential role of metabolic flux in starch accumulation under nitrogen starvation in duckweed. *Biotechnol Biofuels* 10:
101. Yu P, Gutjahr C, Li CJ, Hochholdinger F (2016) Genetic Control of Lateral Root Formation in Cereals. *Trends Plant Sci* 21:951-961
102. Yuan H, Liu D (2008) Signaling components involved in plant responses to phosphate starvation. *J Integr Plant Biol* 50:849-859
103. Zhang Q, Wang C, Tian J, Li K, Shou H (2011) Identification of rice purple acid phosphatases related to phosphate starvation signalling. *Plant Biol* 13:7-15
104. Zhao X, Li G, Sun Z, Chen Y, Guo W, Li Y, Chen Y, Yang J, Hou H (2021) Identification, structure analysis, and transcript profiling of phosphate transporters under Pi deficiency in duckweeds. *Int J Biol Macromol* 188:595-608
105. Zhao X, Yang J, Li G, Sun Z, Hu S, Chen Y, Guo W, 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* 113:1761-1777
106. 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 Physiol Bioch* 86:72-81

107. Zhou J, Jiao FC, Wu ZC, Li YY, Wang XM, He XW, Zhong WQ, Wu P (2008) OsPHR2 is involved in phosphate-starvation signaling and excessive phosphate accumulation in shoots of plants. *Plant Physiol* 146:1673-1686
108. Zhou YZ, Kishchenko O, Stepanenko A, Chen GM, Wang W, Zhou J, Pan CZ, Borisjuk N (2022) The Dynamics of NO_3^- and NH_4^+ Uptake in Duckweed Are Coordinated with the Expression of Major Nitrogen Assimilation Genes. *Plants-Basel* 11:11

Figures

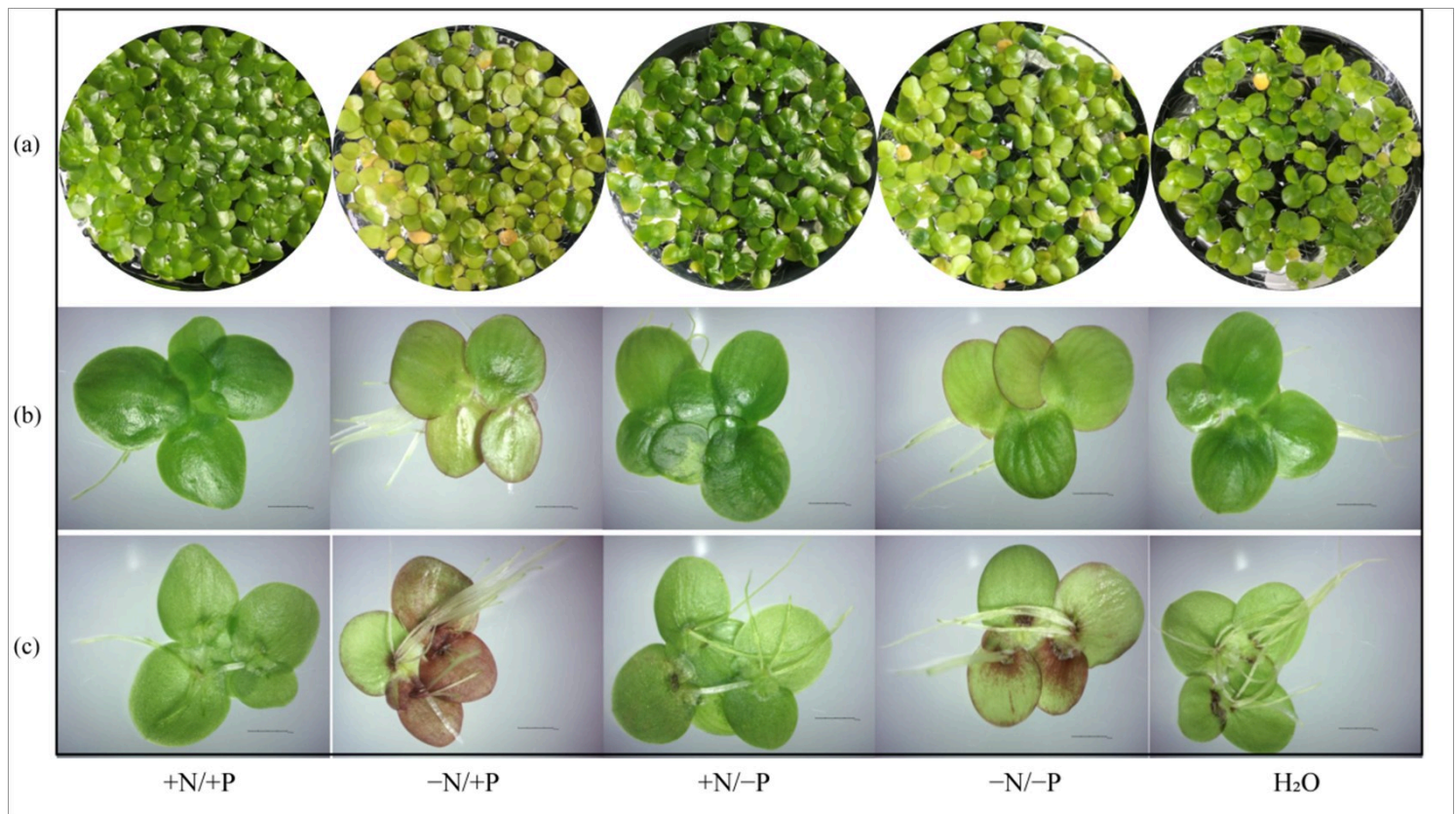


Figure 1

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 *S. polyrhiza*.

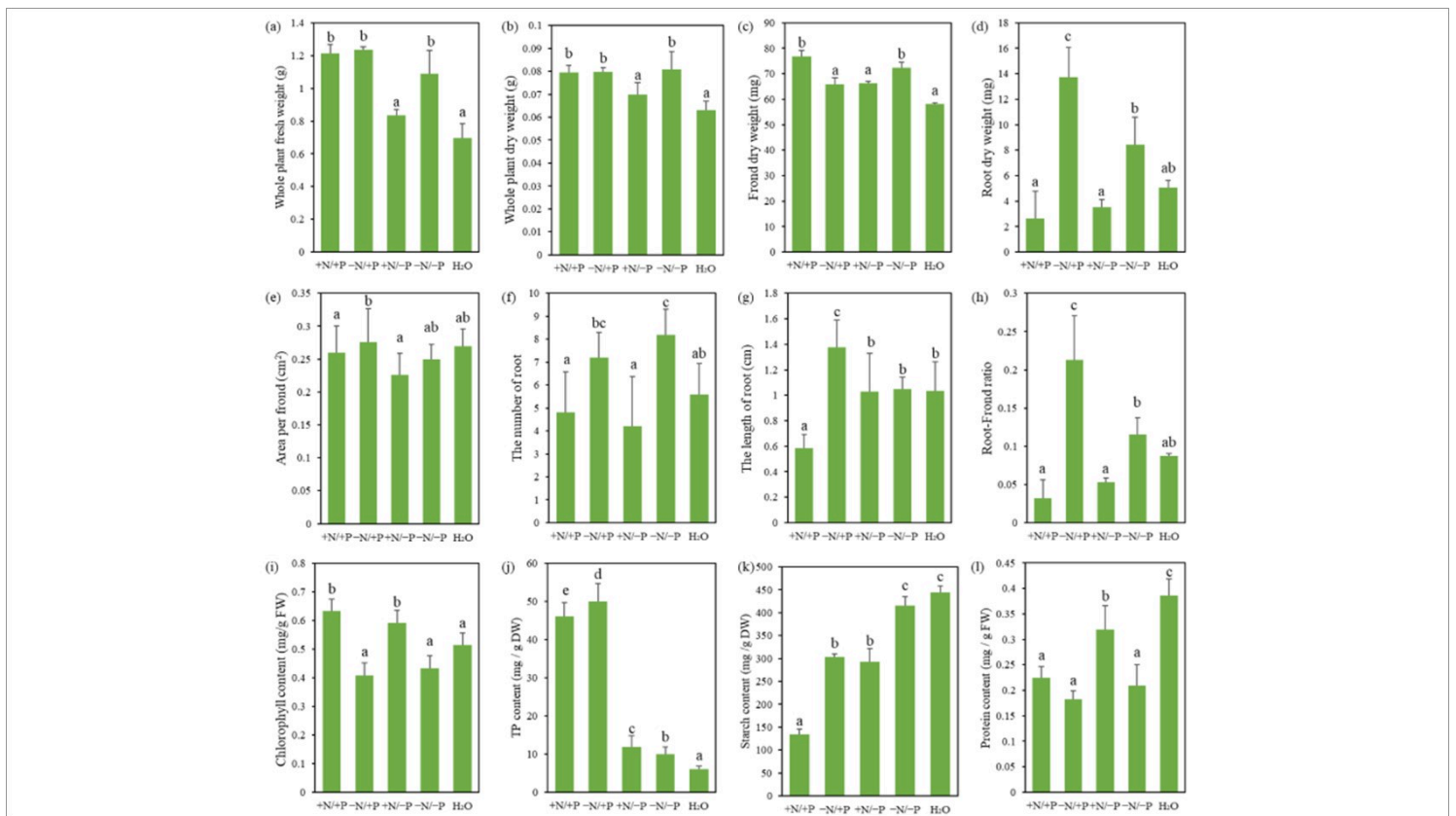


Figure 2

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).

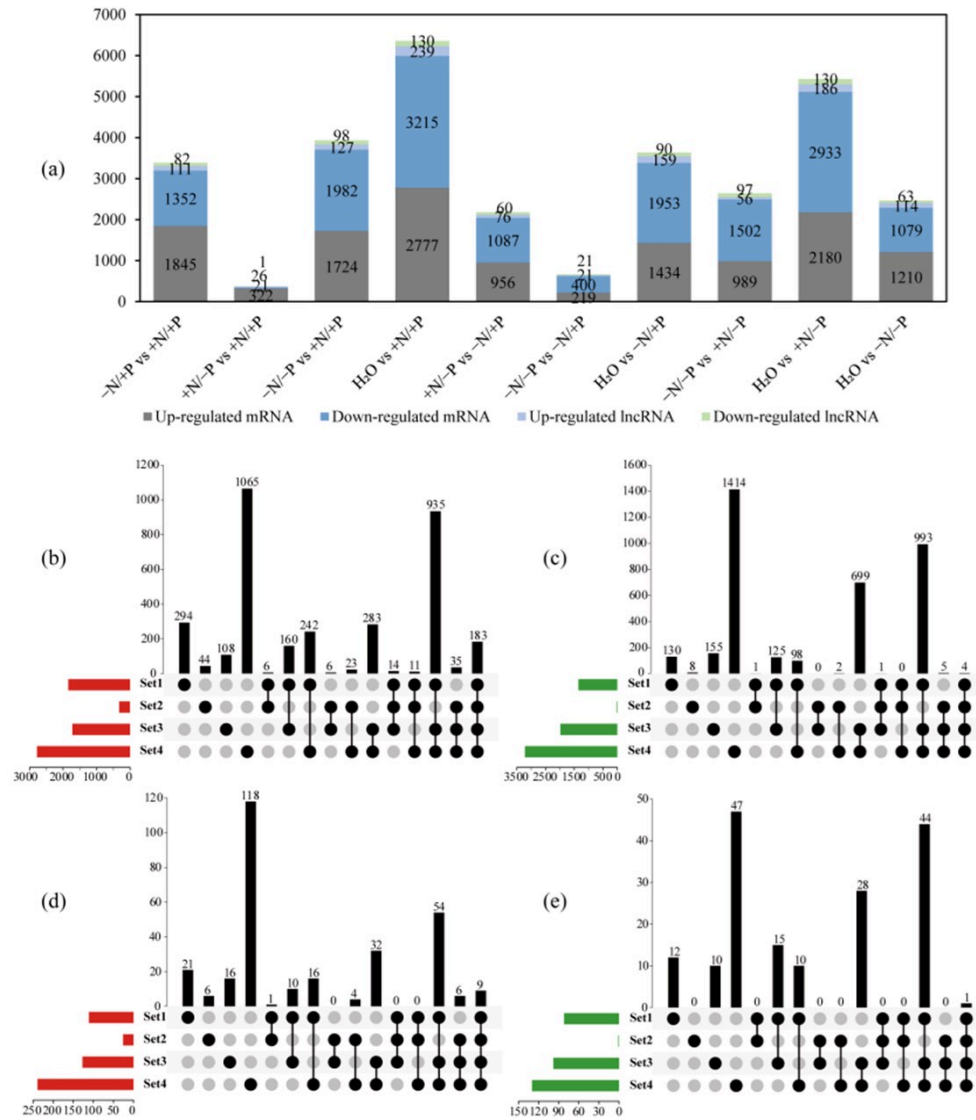


Figure 3

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: H₂O vs +N/+P.

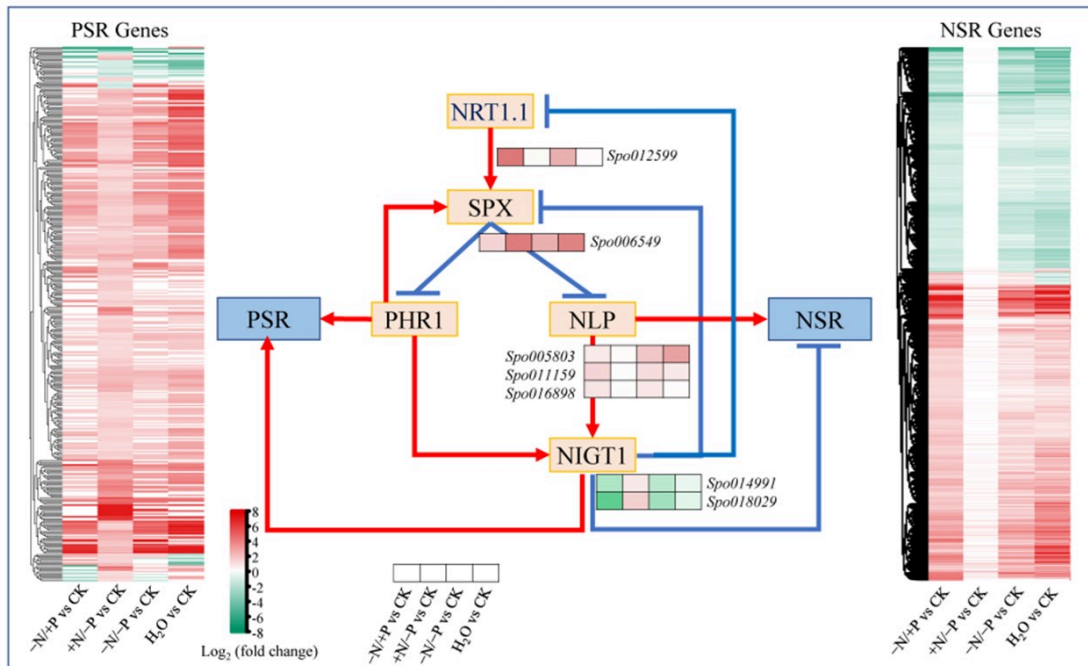


Figure 4

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

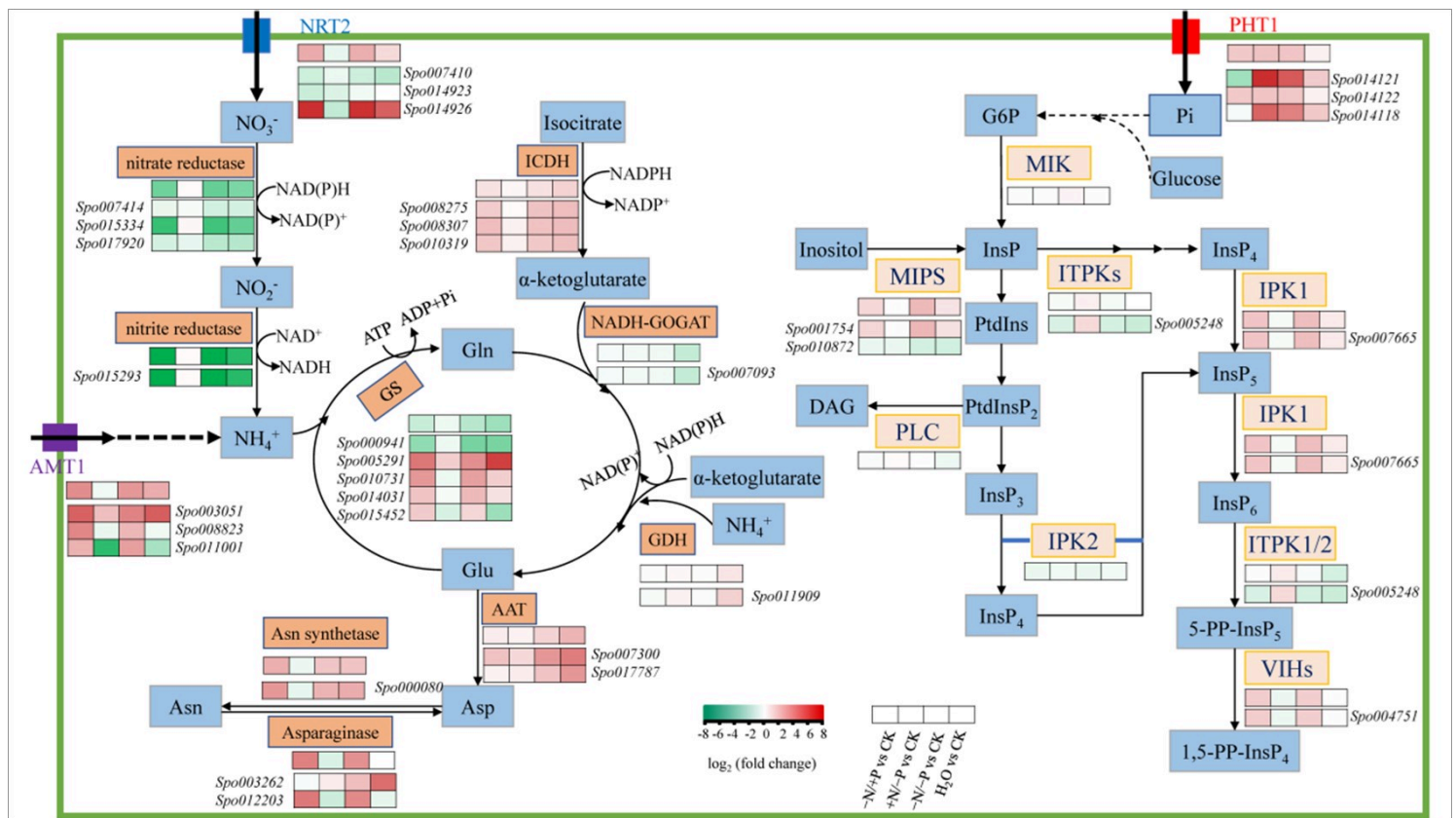


Figure 5

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

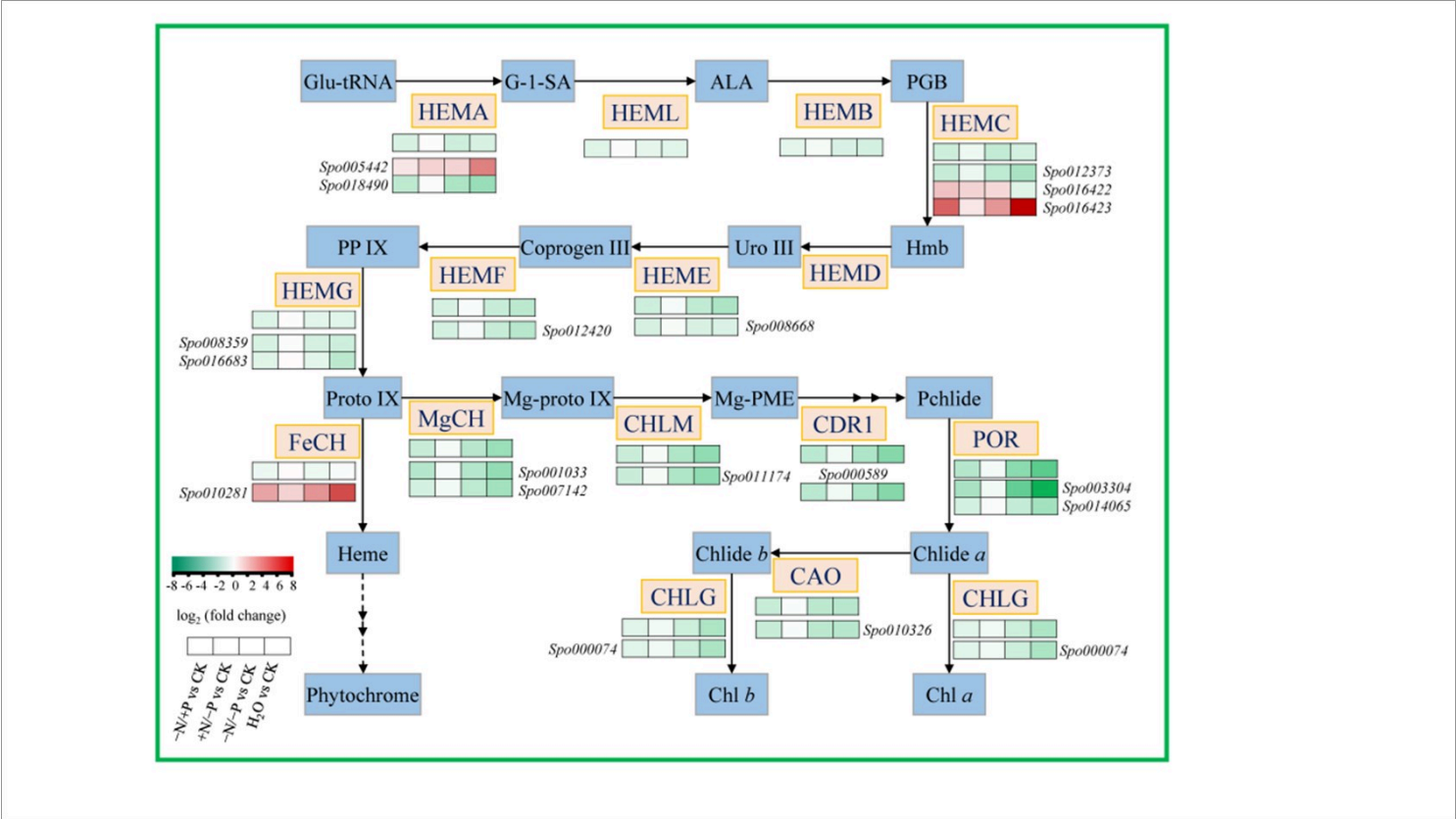


Figure 6

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

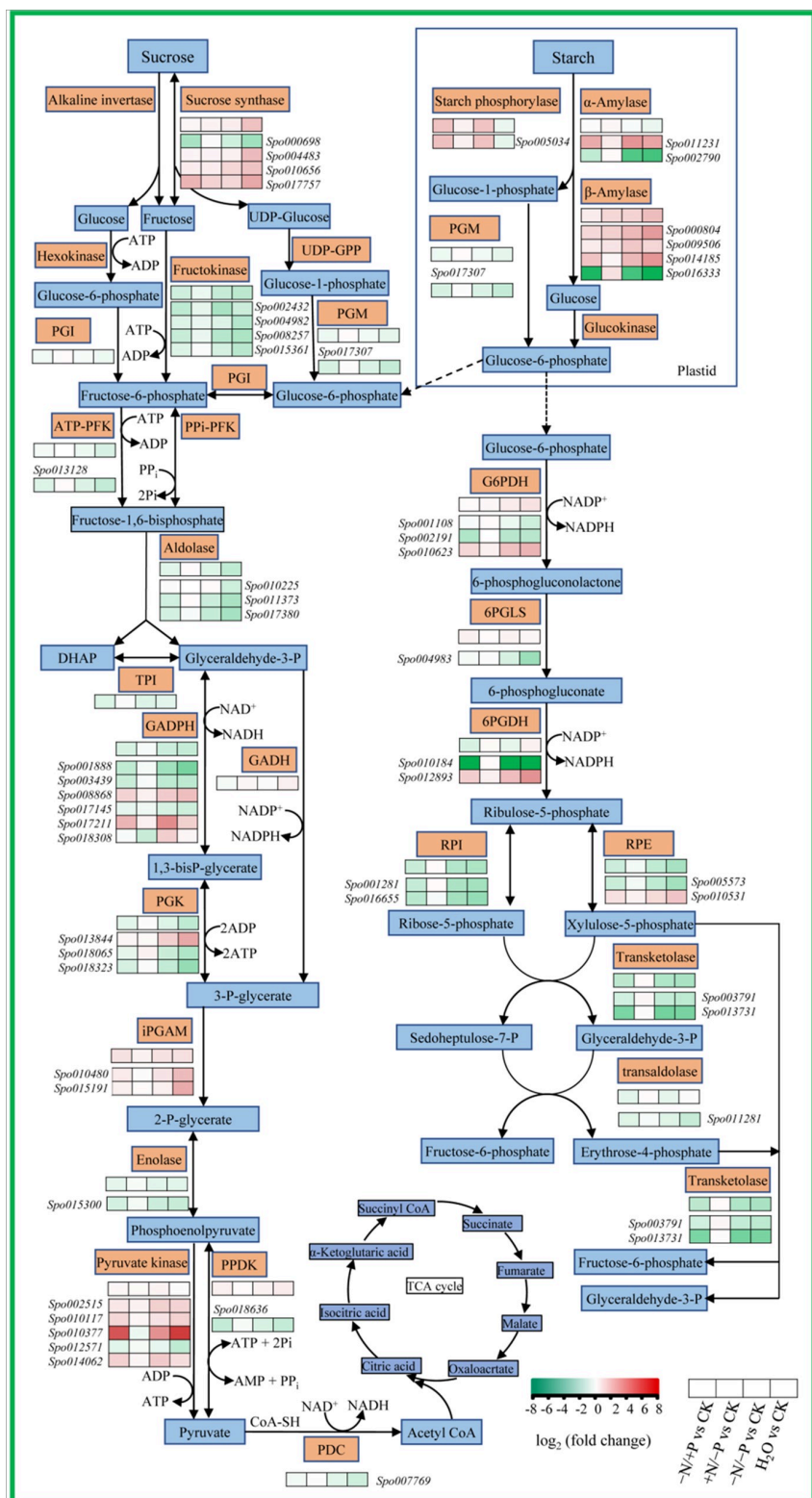


Figure 7

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

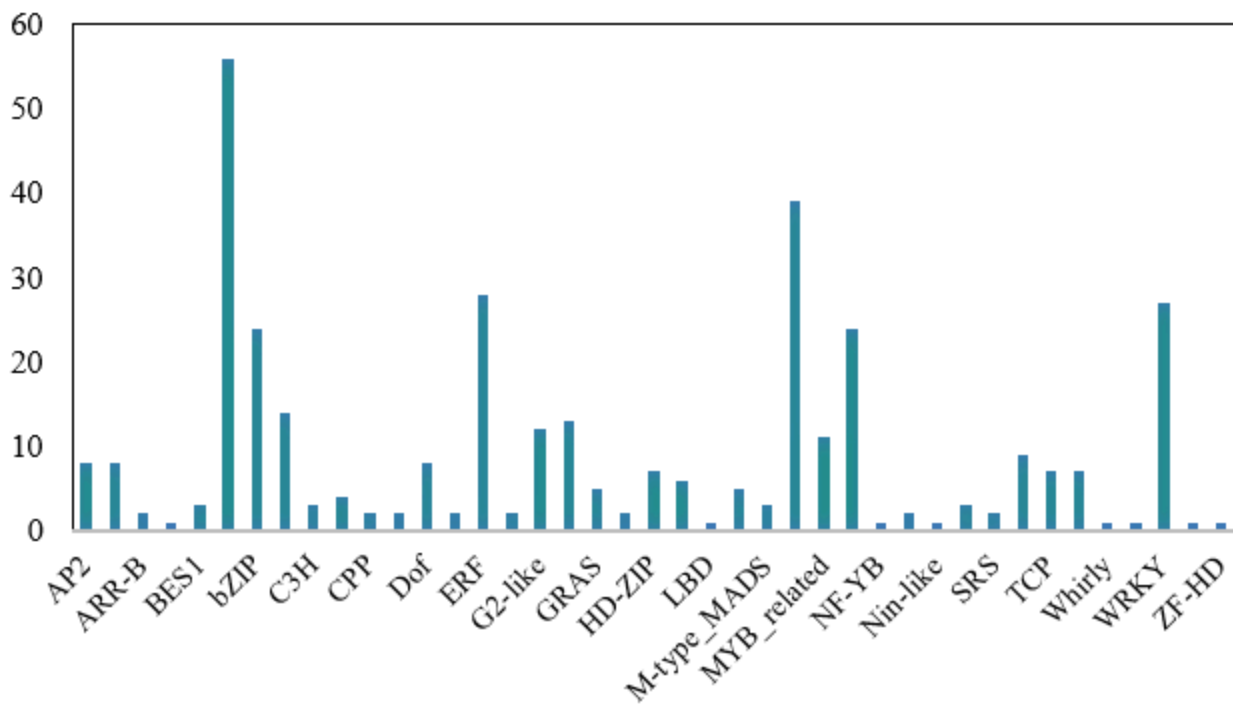


Figure 8

Distribution of DEGs in TF gene families.

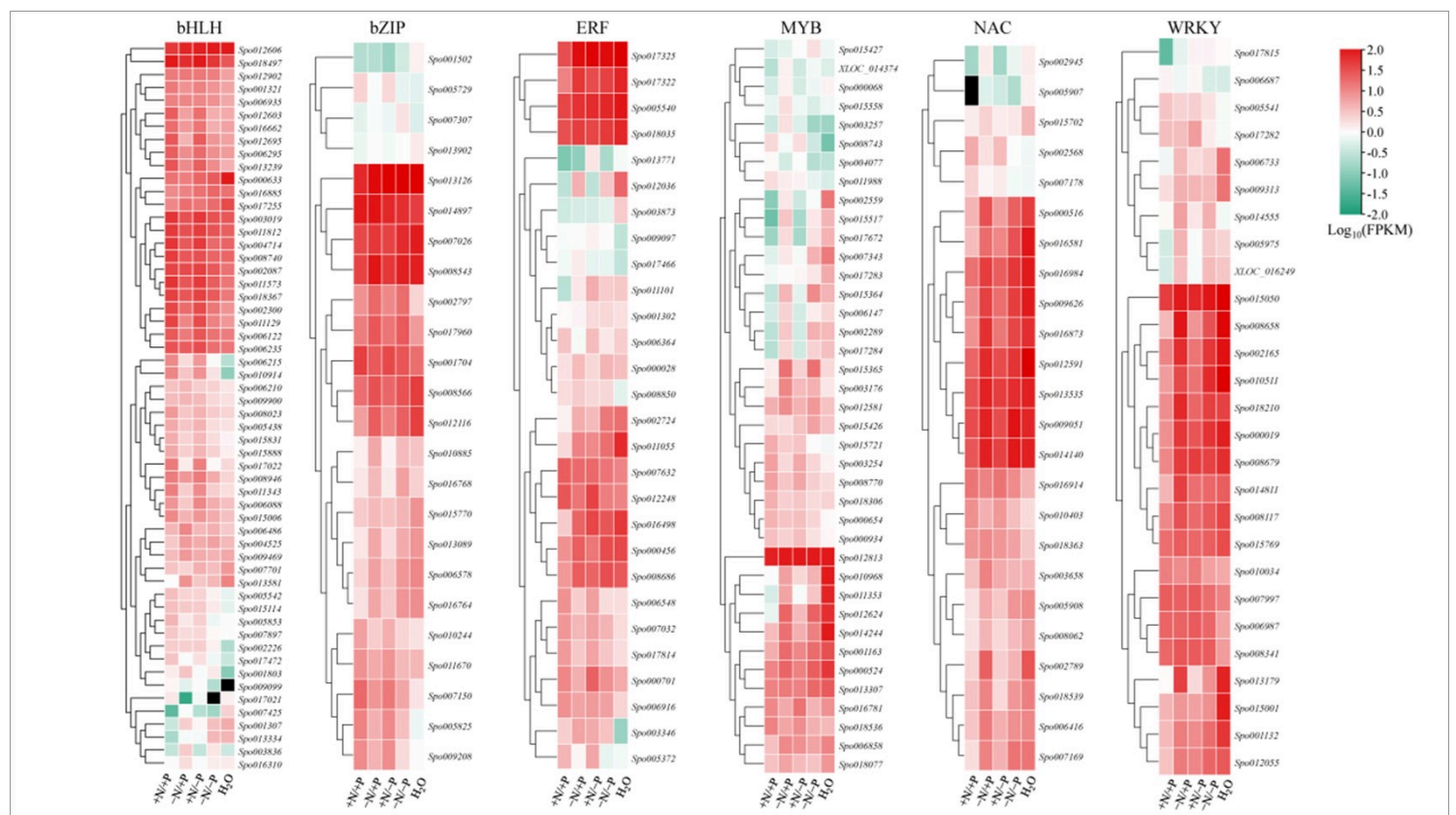


Figure 9

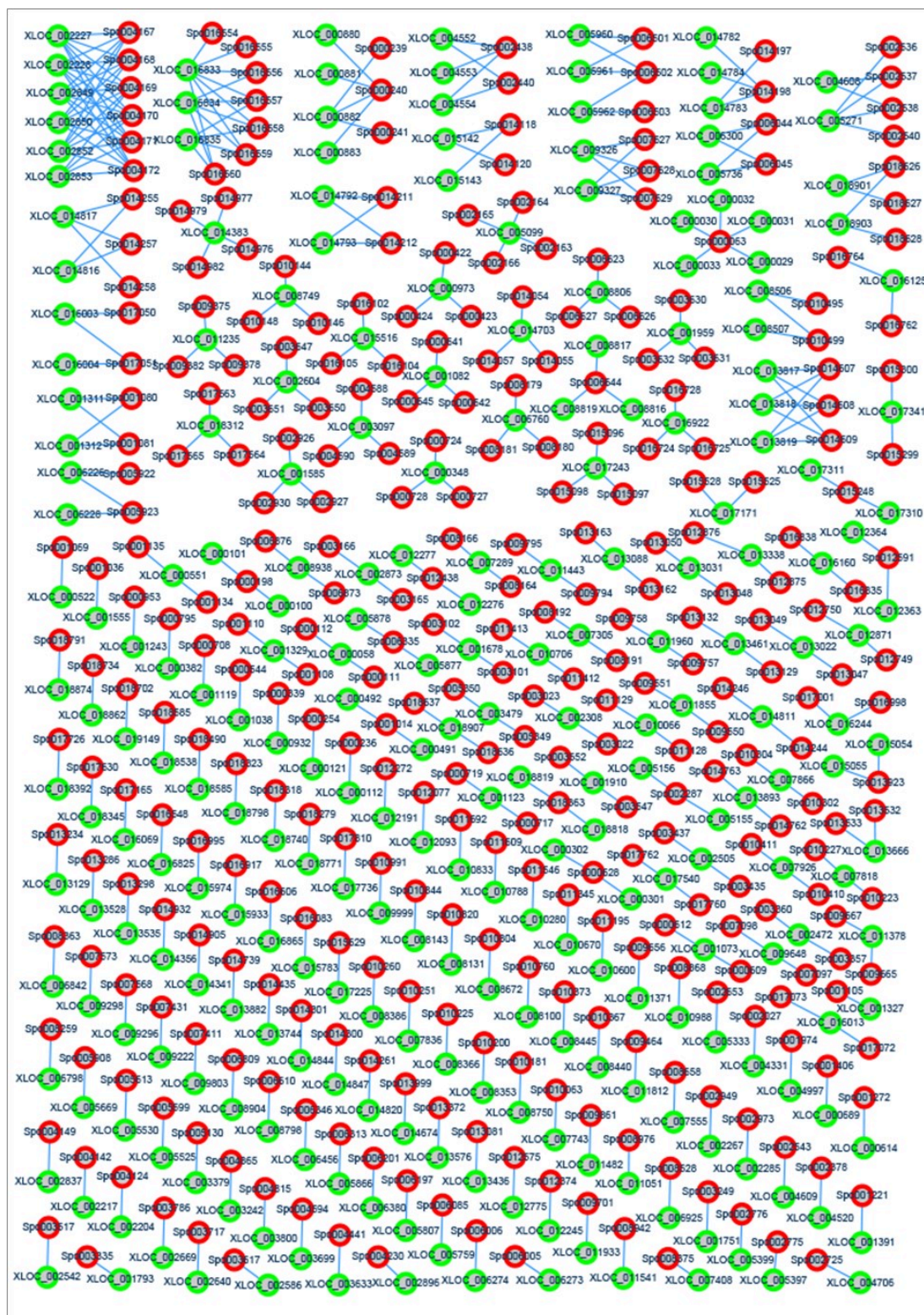


Figure 11

CeRNA networks analysis of mRNA-miRNA-lncRNA in *S. polyrhiza* under nutrient deficiencies, blue cycles represent miRNAs, red cycles represent coding genes, green cycles represent lncRNAs.

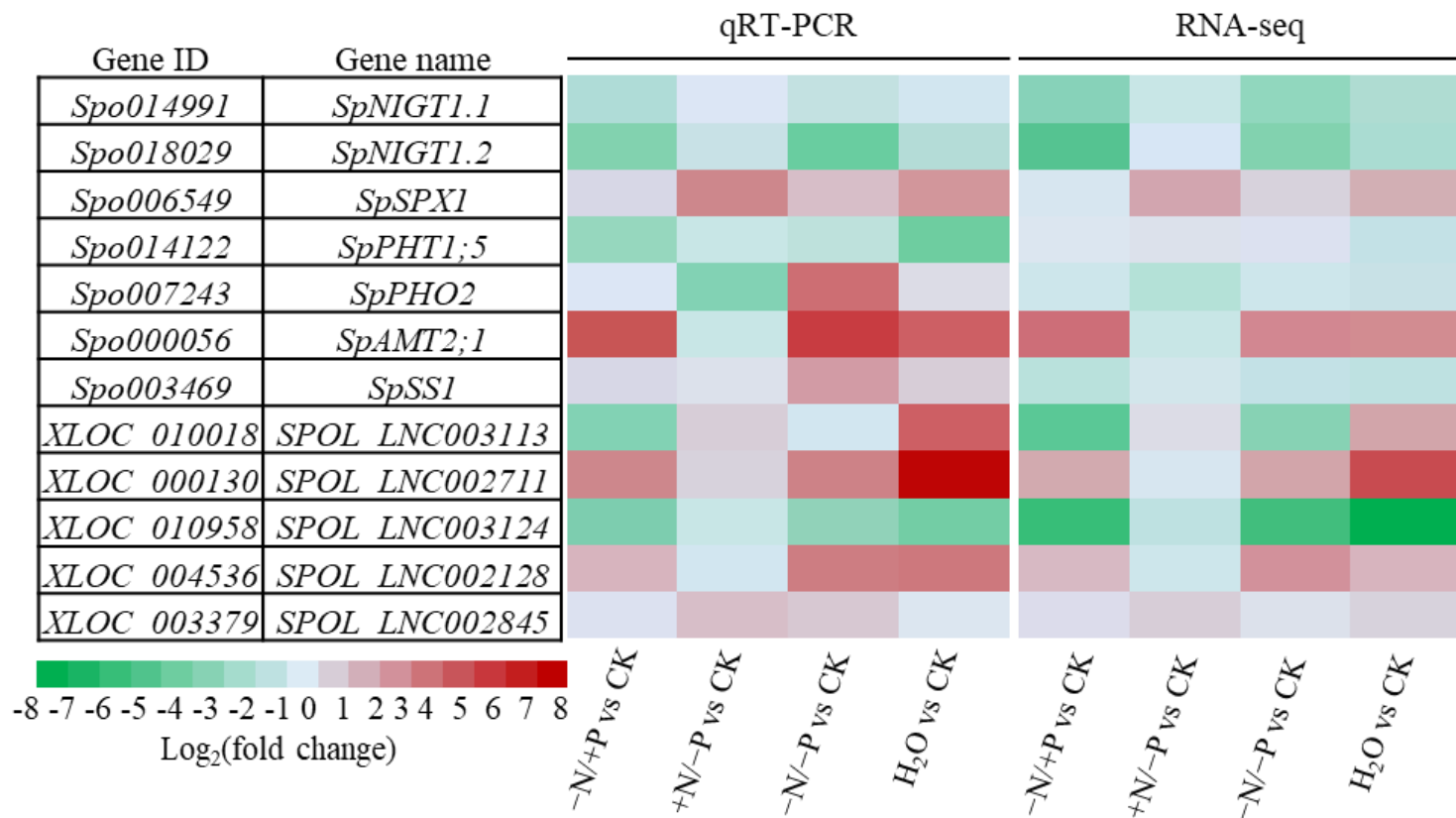


Figure 12

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

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [TableS1.docx](#)
- [TableS2.docx](#)
- [TableS3.xlsx](#)
- [TableS4.xlsx](#)