Characterization on the P-Associated and Agronomic Traits as Well as Associated Molecular Processes in Wheat Under Pi Deprivation Condition

Yingjia Zhao
Hebei Agricultural University
Yanyang Zhang
Hebei University of Science and Technology
Ruize Lin
Hebei Agricultural University
Xinyang Bai
Hebei Agricultural University
Pingping Du
Hebei Agricultural University
Kai Xiao (kaixiao1112@163.com)
Hebei Agricultural University

Research Article

Keywords: wheat (Triticum astivum L.), P deprivation, P-associated and growth traits, differentially expressed gene, biochemical pathways

Posted Date: March 18th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-262154/v1

Abstract

Phosphorus (P) acts as one of essential macronutrients and plays critical roles in regulating plant growth, development, and the yield formation capacity of crops. Elucidating the physiological and molecular processes underlying plant P deprivation responses benefit the crop cultivation with high P use efficiency (PUE). In this study, the P-associated and agronomic traits as well as the transcriptome profile were investigated under contrasting P levels combined with deficit irrigation, in two wheat cultivars (i.e., high PUE Shixin 828 and P deprivation sensitive Jimai 518). The deficient-P (DP) treatment decreased the P accumulative amounts, photosynthetic function, and biomass of the plants at various growth stages and reduced the yields with respect to sufficient-P (SP) condition. Although the two cultivars were comparable on growth and P-associated traits as well as yields under SP, Shixin 828 was better on above growth traits and P accumulation and higher on yields than Jimai 518 under DP, suggesting that the high PUE cultivars under DP display enhanced P uptake that positively affects photosynthetic function, biomass production, and productivity of plants under P deprivation conditions. A large quantity of genes with differential expression patterns, including 2948 to be upregulated and 1844 downregulated, were identified based on RNA-seq analysis in the Shixin 828 plants treated with P deprivation. The DE genes are associated with biological process (i.e., metabolic process and cellular process etc.), cellular components (cell body and organelle etc.), and molecular function (binding and catalytic activity), and phytohormone signaling pathways. Transgene analysis on *TaZFP1*, a gene in ZFP transcription factor family exhibited upregulated expression in the P-deprived plants, validated its role in enhancing P accumulation and plant P starvation adaptation. Our results suggested that plant P deprivation response is underlying modulation of various physiological processes via modification of gene transcription at global level.

Introduction

Phosphorus (P) acts as one of essential inorganic nutrients for plant growth and development, impacting largely on the productivities of crops. Due to the nature of P nutrition to be prone to immobilization in growth media, application of the P fertilizers has been an effective technique for improving the plant biomass production and yield formation capacity for cereal crops. However, unsuitable management on P fertilizer has resulted in lowered crop P use efficiency (PUE) in North China as well as other regions with similar ecology, leading to deteriorated environmental quality aside from the increased production cost (Holford et al. 1997; Hinsinger et al. 2001). Therefore, further improvement for P nutrition management benefits elevation of crop PUE and promotion of the sustainable agriculture worldwide.

The P uptake and internal translocation across tissues and needed to be further determinedtation derstanding on wheat ZFP functions, city to are accomplished through mediation of complicate biological processes, which are associated with P acquisition, cellular P remobilization, and the P nutrition recycling during late growth stage. Under Pi deprivation, psre acheasedzer hasannualy lants are acclimated to the stress condition via a subset of evolved mechanisms at molecular level. For example, a quantity of genes functional in modulating diverse physiological processes, such as signaling transduction, transcriptional regulation, primary and secondary metabolisms, protein synthesis, cellular

growth processes, and stress defensiveness, modify transcription efficiency under P starvation and are involved in plant Pi deprivation response (Rausch et al. 2002; Raghothama et al. 2005; Jain et al. 2007; Wang et al. 2011; Zhang et al. 2019). Elucidation of the molecular processes can help dissection of mechanisms underlying plant Pi starvation acclimation and benefit breeding of high PUE crop cultivars.

Winter wheat and summer maize cultivation over whole year acts as an important cropping system in North China, contributing greatly to the crop productivity and regional food security. Currently, enhancement of water and nutrient use efficiencies for crops cultivated has been an urgent issue to improve the regional sustainable agriculture, given the limitation of water resource and the increase of inorganic fertilizer invest (Xue et al. 2003; 2006; Zhao et al. 2006). Thus far, the investigations concentrated on elucidation of plant P uptake, P translocation across tissues, agronomic traits under P treatments and the related physiological processes and biochemical pathways have been extensively investigated in wheat (Leikam et al. 1983; Fiedler et al. 1989; Luther et al. 2010). However, the growth and P-associated traits in contrasting PUE wheat cultivars and the molecular processes associated with high PUE behavior in wheat plants under P deprivation combined with water-saving condition, are needed to be further characterized. In this study, two wheat cultivars sharing contrasting P deprivation response (i.e., high PUE Shixin 828 and P deprivation sensitive Jimai 518) were used as materials to aim at addressing following issues: (i) elucidation of varietal variation on plant P-associated and growth traits under Pi deprivation combined by deficit irrigation; (ii) characterization of the transcriptome profile underlying high PUE and the associated biochemical pathways in wheat treated with P- and water-saving condition; (iii) functional characterization of *TaZFP1*, a gene in the transcription factor (TF) family of zinc finger protein (ZFP), in mediating plant tolerance to low-Pi stress. Our investigation provides insights into plant P deprivation response and PUE-associated molecular processes, and benefits breeding for high PUE cultivars in wheat cultivated under P-saving and water-limited conditions.

Materials And Methods

Experimental design

Field experiments were conducted at the Experimental Station of Hebei Agricultural University, Xinji city, China, during the 2017-2018 and 2018-2019 growth seasons. The climate in this region is specified by a typical temperate continental monsoon, with precipitation concentrated at summer season. The meteorological factors during two spring growth seasons are shown in Table S1. The surface soil for experiments was loamy containing the following nutrients: organic matter 17.56 g/kg, available N 70.38 mg/kg, available P 20.41 mg/kg, and exchangeable K 121.04 mg/kg. The treatments were arranged based on split plot design with triplicates, with plot area of 35 square meter (7 m in length and 5 m in width). Among the treatments, P input level was set up as main plot whereas cultivar (Shixin 828, a high PUE cultivar and Jimai 518, a P deprivation sensitive one) as sub-plot. The P input treatments included two levels: sufficient-P (control, with P amount 150 kg P_2O_5/ha, SP) and deficient-P (P deprivation, with P amount 60 kg P_2O_5/ha, DP). SP and DP were established by basally applied the P fertilizer (with superphosphate as P source, containing 15% P_2O_5) with amount 1000 kg/ha and 400 kg/ha, respectively.

Additionally, in total of 225 kg N/ha (with urea as N source, half of which applied as basal and another half topdressed at jointing together with irrigation) and 120 kg/ha K_2O (with potassium chloride as K source, all of which used as basal) were applied for all of the plots. Deficit irrigation management with two irritations performed prior to seeds-sown (75 mm) and at jointing stage (60 mm, controlled by water amount analyzer) was conducted for all of the treatments. Seed sowing dates was arranged on October 12 and 14 during the 2017-2018 and 2018-2019 growth seasons, respectively, with seed amounts to generate a 3750-thousand seedling population per hectare. During the growth cycles, the cultivation practices conducted were similar to the conventional ones used by the local farmers.

Assay of P-associated traits, photosynthetic parameters, and agronomic traits

At growth stages of jointing, flowering (0 d), mid-filling (20 d after flowering), and maturity, the representative plants in each treatment were sampled and subjected to assay of plant biomass, P concentrations, and P accumulative amounts. Among them, plant biomass was obtained based on the oven-dried samples following conventional approach; P concentrations were assessed as the method described by Guo et al. (2011); the P accumulative amounts in plants were calculated by multiplying the plant biomass and P concentration. Several photosynthetic parameters, including photosynthetic rate (Pn), stomatal conductance (gs), intercellular CO_2 concentration (Ci), photosystem II photochemical efficiency (ΨPSII), and non photochemical-quenching coefficient (NPQ), were measured as those described by Guo et al. (2013). At maturity, the spikes in two square meters were counted in each plot to calculate the population spike numbers. The spike seeds were separately threshed from each plot at maturity (i.e., June 12 and 14 at 2018 and 2019 seasons, respectively) using a mini harvest machine. After air dried, the seeds were weighed and to calculate the grain yields. The grain weights were obtained by weighing the air-dried seeds at maturity. Spike seed numbers were calculated based on the total seeds counted from thirty representative spikes.

Obtainment of the transcripts datasets upon P deprivation

At flowering stage, the representative flag leaves of Shixin 828 plants treated with SP and DP were collected and subjected to high-throughput RNA-seq analysis. Total RNA in the samples was extracted using the Plant RNA Extraction Kit (Invitrogen, USA) and high quality of RNA was confirmed based on an agarose gel electrophoresis. In total of 2 μg of RNA derived from the SP- and DP-treated leaves was separately subjected to RNA-seq analysis using an Illumina sequencing analyzer (Huanuo biotechnology Co. Ltd., China). The transcripts generated that shared high quality were obtained after removal of the adapter sequences, low-quality reads, and the reads less than 20 bp by using the TopHat tool (Kim et al. 2013). Datasets derived from the transcript reads were further assembled using paired end assembly method implemented in Cufflinks program (Trapnell et al. 2009). Among them, the reads mapped to the genomic regions of reference genome (cv. Chinese spring) were further subjected to calculation of expression levels.

The genes to be differentially expressed (DE) under DP were defined based on the transcript counts in the DP plants with respect to those in the SP ones, using the default parameters of Chi-square test as described by Benjamini and Yekutieli (2001). Of which, the thresholds with $P \leq 0.05$ and a |log 2 RPKM ratio| ≥ 1 were used in defining the statistical significance of the gene transcripts.

Expression analysis of the representative DE genes in RNA-seq datasets

Ten of the randomly selected DE genes, including five of upregualted and another five of downregulated, were evaluated for transcripts using qRT-PCR. The DE genes with upregulated expression pattern examined included: *TaZFP1*, zinc finger protein gene (Traes_5BL_92E9B7394); *TaAP*, ATP binding gene (Traes_7AL_54340D34F); *TaCS*, cytoskeleton gene (Traes_1DL_390BF3A1C); *TaGT*, glutathione S-transferase gene (Traes_3AS_6A756328A); and *TaKFM*, kinesin family gene (Traes_5AS_353BAD199). The DE genes with downregulated expression pattern included: *TaTP*, transporter gene (Traes_6AS_9477059DE); *TaCC*, cytochrome P450 gene (Traes_4AS_8527E5F3D); *TaHH*, histone H2A gene (Traes_4AS_B97A404E2); *TaHL*, helicase gene (Traes_4DL_B1BBC732F); and *TaHD*, hydrolase gene (Traes_2BL_3EDC425A7). qRT-PCR was performed similarly as described previously (Guo et al. 2013), using gene specific primers (Table S2). *Tatubulin*, a constitutive gene in *T. aestivum*, was used as an internal reference to normalize the target transcripts.

GO annotation and KEGG analysis for the DE genes

The DE genes identified in wheat plants under DP condition were subjected to GO annotation using the WEGO software. Moreover, the functional categorizations of them were defined as described previously (Ye et al. 2006). KEGG ontology analysis on the DE genes was conducted using the KOBAS 2.0 tool (KEGG Orthology Based Annotation System, v2.0) as reported by Xie et al. (2011).

Transgene analysis on *TaZFP1*

TaZFP1, a gene of transcription factor family of the zinc finger protein (ZFP) class that was upregulated in transcript dataset under DP, was selected and subjected to functional characterization for the role in mediating plant P deprivation acclimation. With this purpose, the open reading frame (ORF) of *TaZFP1* was amplified based on RT-PCR using gene specific primers (Table S2), then integrated into *Nco*I/*Bst*EII restriction sites in binary vector pCAMBIA3301 at position downstream of the CaMV35S promoter. The target gene genetically transformed into *T. aestivum* (cv. Shixin 828) based on an *A. tumefaciens*-mediated approach as described previously (Guo et al. 2013). Line 2 and Line 3, two T3 lines with more *TaZFP1* transcripts together with wild type (WT) were subjected to P input treatments by culturing in standard MS solution (1.2 mM Pi, SP) and modified MS solution containing low P (0.1 mM Pi, DP). Five weeks after the treatments, the P concentrations, biomass, P accumulative amounts, and the photosynthetic parameters in transgenic and WT plants were assessed similarly to those mentioned above.

Statistical analysis

Averages of plant biomass, P concentrations, photosynthetic parameters, P accumulative amounts, grain yields, RNA-seq datasets, and the qRT-PCR data were all derived from triplicate results. Standard errors on averages and significant differences among the averages were analyzed by using the Statistical Analysis System software (SAS Corporation).

Results

The plant growth and P-associated traits, photosynthetic parameters, and yields

The growth and P-associated traits as well as the photosynthetic parameters at various growth stages during two growth seasons are shown in Figs. 1a-1h. Compared with sufficient-P (SP), deficient-P treatment (DP) decreased the biomass, P concentrations, P accumulative amounts of plants (Figs. 1a-1c), deteriorated behaviors of Pn, gs, Ci, ΨPSII, and enhanced NPQ (Figs. 1d-1h) in tested cultivar plants managed by deficit irrigation. Although Shixin 828 (high NUE cultivar) was comparable on the above growth, P-associated and photosynthetic traits under SP conditions, it was more improved on the traits mentioned than Jimai 518 under DP (Figs. 1a-1h). The yields in the cultivars under contrasting P input treatments are consistent with above growth, P-associated, and the photosynthetic traits (Table 1), with higher shown in Shixin 828 than Jimai 518 under DP. These results suggested that the high PUE cultivars possess enhanced yield formation capacity under P deprivation condition, which is associated with the improved P accumulation, photosynthetic function, and plant biomass production.

The transcript datasets generated under P deprivation condition

The transcript datasets in Shixin 828 treated with both SP and DP were established based on RNA-seq analyses. Among the raw base pairs yielded over 58 millons each, more than 51 million were identified to be clean reads. Of which, 88.36-90.22% were mapped to the reference genome (c.v. Chinese spring), with 75.33-76.42% were suggested to be unique and 14.75-15.08% to share a nature of multi mapping characterization (Table 2). The transcript datasets obtained in this study suggested the feasibility using RNA-seq approach for detection of transcriptome in wheat under modified P input conditions.

The DE genes identified under P deprivation conditions

A large quantity of genes in transcript datasets derived from the contrasting P input treatments were differentially expressed (DE) under DP, including 2948 of upregulated and 1844 of downregulated (Datasets 1 and 2). The scatter plot showing expression patterns of the DE genes are shown in Fig. 2. The DE genes detected by RNA-seq analysis indicates that the plant P deprivation response is comprehensively determined by modified transcription of genes at global level.

The expression patterns of randomly selected DE genes

Ten of DE genes were randomly selected from the modified transcript datasets under DP and subjected to expression level evaluation. As expected, the five genes with upregulated expression pattern elevated the transcripts under DP with respect to SP, with comparable folds of modified expression as shown in

transcript datasets (Figs. 3a-3e). Likewise, the five genes with downregulated pattern lowered the expression levels in plants treated with DP compared with those with SP, all of them being similar on modified transcripts as shown in the RNA-seq analysis (Figs. 3f-3j). These results together validated the reproducibility of the transcriptome results generated under high throughput RNA-seq evaluation.

The functional classifications of DE genes

The DE genes were categorized into three functional groups based on GO assignment analysis, including '*biological process*', '*cellular components*', and '*molecular function*' (Fig. 4a). Among them, the genes in the '*biological process*' group are enriched by following processes: metabolic process, cellular process, single-organism process, response to stimulus, cellular component organization or biogenesis, biological regulation, regulation of biological process, localization, developmental process, and multicellular organisamal process; the genes in the '*cellular components*' group are overrepresented by following constituents: cell, cell part, organelle, organelle part, membrane, macromolecular complex, membrane part, and extracellular region; the genes in '*molecular function*' group are closely associated with molecule binding and catalytic activity (Fig. 4a). These results suggested that plant P deprivation acclimation is underlying modulation of numerous modified biological processes regulated by the DE genes, which act in coordination to mediate plant P starvation response.

The Pi deprivation-associated biological processes overrepresented by the DE genes were defined based on KEGG analysis, which included those associated with photosynthesis-antenna proteins, carbon fixation in photosynthetic organisms, glyoxylate and dicarboxylate metabolism, phenylalanine metabolism, porphyrin and chlorophyll metabolism, alanine, aspartate and glutamate metabolism, cysteine and methionine metabolism, arginine biosynthetsis, DNA replication, taurine and hypotaurine metabolism, and fructose and mannose metabolism, etc. (Fig. 4b). Therefore, these processes and related biochemical pathways were suggested to exert essential roles in modulating plant P starvation adaptation in high PUE wheat cultivar, through improving P uptake, internal P translocation across tissues, photosynthetic function, and biomass production.

The phytohormone signaling-associated genes from DE genes

The phytohormones, such as auxin, cytokinin, gibberellin, abscisic acid, ethylene, salicylic acid, and jasmonic acid, are critical regulators and involved in modulating wide ranges of physiological processes. Among the DE genes identified, a large set of them were shown to be phytohormone signaling-associated ones, including those to be associated with responses to auxin, cytokinin, gibberellin, abscisic acid (ABA), ethylene, salicylic acid (SA), and jasmonic acid (Table 2). These results suggested the crucial roles of the phytohormone signals in plant P deprivation response through the cooperate mediation of diverse stress-associated biological processes.

The function of *TaZFP1* in regulating P starvation tolerance

Two lines overexpressing *TaZFP1* (i.e., Line 2 and Line 3) with more target transcripts (Fig. S1) together with wild type (WT) were cultivated under two P contrasting treatments, to address the function of target genes in regulating Pi deprivation response. Under SP condition, Line 2 and Line 3 were comparable on biomass, P concentrations, P accumulative amounts, and photosynthetic traits with the WT plants (Figs. 5a-5h). Under DP, however, the transgenic lines displayed more improved biomass, P concentrations, P accumulative amounts, and photosynthetic traits (i.e., Pn, gs, Ci, ΨPSII, and NPQ) of plants than WT (Figs. 5a-5h). These results suggested that *TaZFP1*, one of the significantly upregulated DE genes under DP, acts as a crucial regulator in plant P deprivation adaptation. The *TaZFP1*-improved low-P tolerance was associated with the gene function in enhancing P uptake in plants once challenged by low-P stress.

Discussion

It has been documented that the cereal cultivars display genetic variation on the P uptake, plant growth, and agronomic traits upon Pi deprivation, which impacts on the plant productivity under the stress conditions (Marschner et al. 2005; Seguel et al. 2017). In this study, the biomass, P-associated and photosynthetic traits, and yields in two contrasting PUE cultivars were in agreement with the previous results. Although the two cultivars (i.e., high PUE cultivar Shixin 828 and Jimai 518, a wheat cultivar to be P deprivation-sensitive) examined were similar on the growth and P-associated traits under sufficient-P condition (SP) and all of which were better than those shown under deficient-P treatment (DP), Shixin 828 was much better on these traits than Jimai 518 under the P deprivation combined deficit irrigation conditions. These results validated the previous reports indicating that the high PUE cultivars improve P uptake and yield formation under P deprivation treatment (Zhang et al. 2013). Therefore, adoption of high PUE cultivars is effective for improving winter wheat production managed with P- and water-saving practices.

Plant abiotic stress responses are accomplished through modulation of transcription efficiencies of various functional group genes (Sham et al. 2014; Matsui et al. 2017; Calixto et al. 2018; Zhang et al. 2019). In this study, large quantities of DE genes (i.e., 2848 upregulated and 1844 downregulated) were identified based on RNA-seq analyses in plants treated with DP. These results suggested the complicate nature underlying plant response to P deprivation condition. Previously, it has been confirmed that GO annotation and KEGG analyses on the DE genes can effectively dissect the molecular processes underlying plant stress acclimation (Rehman et al. 2018). In this study, the DE genes identified under DP were categorized into three functional groups based on GO characterization, including '*biological process*', '*cellular components*', and '*molecular function*'. The DE genes were overrepresented distinct biochemical pathways based on KEGG analysis, which are associated with photosynthesis-antenna proteins, carbon fixation in photosynthetic organisms, glyoxylate and dicarboxylate metabolism, phenylalanine metabolism, porphyrin and chlorophyll metabolism, alanine, aspartate and glutamate metabolism, cysteine and methionine metabolism, arginine biosynthetsis, DNA replication, taurine and hypotaurine metabolism, and fructose and mannose metabolism, etc. Therefore, the biochemical pathways related to photosynthesis (i.e., antenna proteins, carbon fixation, and chlorophyll metabolism etc.) affect the behaviors of photosynthetic function in wheat challenged by DP condition. In addition, the

pathways related to diverse primary and secondary mechanisms, such as glyoxylate and dicarboxylate metabolism, phenylalanine metabolism, porphyrin and chlorophyll metabolism, alanine, aspartate and glutamate metabolism, and those associated with N metabolism, including alanine, aspartate and glutamate metabolism, cysteine and methionine metabolism, arginine biosynthesis, were enriched in high PUE cultivars treated with DP. These results suggested that plant response to P deprivation is integrated covering various biochemical and molecular pathways.

Phytohormones such as auxin, gibberellin, and cytokinin, involves modulation of diverse physiological processes, to mediate plant growth and stress defensiveness upon environmental cues (Bhargava et al. 2013; Olatunji et al. 2017; Gras et al. 2018). Previously, it has been documented that phytohormones act with network mode to regulate the plant stress responses. For example, a set of genes involving auxin signaling (Jain et al. 2009; Wang et al. 2010; Blomster et al. 2011), cytokinin-mediated ROS homeostasis (Liu et al. 2002; Zavaleta-Mancera et al. 2007; Mýtinová et al. 2010), gibberellin metabolism, (Shan et al. 2014), and ABA signaling pathways (Zhang et al. 2014; Ma et al. 2016; Zang et al. 2016), play important roles in mediating responses of plant to abiotic stresses. In this study, a large set of the DE genes identified in high PUE cultivars treated by DP were related to the phytohormone signaling, associating with auxin response, cytokinin metabolism, gibberellin signaling, ABA response, cellular response to ethylene stimulus, SA signaling, and jasmonic acid signaling (Table 2). These results suggested the complicate cross-talk among the phyhormone signaling pathways modulated by DP to further impact plant P deprivation adaptation. Further characterization of the phyhormone signaling pathways can deepen understanding of the high PUE mechanisms in wheat cultivated under P-and water-saving conditions.

ZFP TFs constitute one of the large TF families in plant species (Miller et al. 1985; Klug 2010; Han et al. 2014). *ZFP3*, *ZAT10*, and *ZAT12*, three genes in ZFP family, were recorded for the roles in regulating plant responses to various abiotic stresses, such as high salinity (Han et al. 2014) and osmotic stress (Mittler et al. 2006) by enhancing osmolyte contents and alleviating electrolyte leakage via regulation of stress-responsive genes at transcriptional level. Additionally, *ZAT6* improves phosphate homeostasis due to regulation of root development and Pi uptake (Devaiah et al. 2007). In this study, *TaZFP1*, a gene with significantly upregulated in expression in RNA-seq datasets, was selected for functional characterization in mediating P starvation tolerance. Results indicated that overexpression of the target gene conferred plants elevated P concentrations, biomass, and P accumulative amounts under DP. Therefore, *TaZFP1* is suggested to be one of the valuable indices for PUE evaluation in wheat cultivars and a useful target for molecular breeding of the high PUE cultivars in wheat cultivated under P deprivation and deficit irrigation.

Conclusion

The wheat cultivars examined (i.e., Shixin 828 and Jimai 518) were varied on the growth and P-associated traits, photosynthetic function, and yields under P deprivation and deficit irrigation conditions. High PUE cultivar Shixin 828 displayed improved P accumulation, photosynthetic parameters, and agronomic traits with respect to Jimai 518 under deficient-P treatment (DP), suggesting its potential for

high PUE wheat cultivation managed by P- and water-saving practices. Large quantities of genes were identified to be differentially expressed in high PUE cultivar Shixin 828 challenged by P deprivation, which were associated with modulation of biological process (i.e., metabolic process and cellular process etc.), cellular components (cell body and organelle etc.), and molecular function (binding and catalytic activity). The DE genes were enriched in the phytohormone signaling pathways, suggesting the critical role of modified hormone signals under P deprivation in plant P deprivation response. *TaZFP1*, a transcription factor gene in the ZFP family, was functional in positively regulating plant P accumulation, biomass production under DP and conferred plants low-Pi stress tolerance. *TaZFP1* is a valuable index for evaluation of wheat PUE and for molecular breeding of high PUE cultivars in *T. aestivum* cultivated under low-P and deficit irrigation.

Declarations

Acknowledgments

This work was financially supported by Chinese National Key Research and Development Project on Science and Technology (2018YFD0300503).

Author contributions

Kai Xiao designed the research. Yingjia Zhao, Yanyang Zhang, Ruize Lin, and Fangfang Li conducted the experiment and performed data analysis. Kai Xiao wrote the paper.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Benjamini Y, Yekutieli D (2001) The control of the false discovery rate in multiple testing under dependency. Annals Statistics 9:1165–1188
2. Bhargava A, Clabaugh I, To JP, Maxwell BB, Chiang Y, Schaller GE, Loraine A, Kieber JJ (2013) Identification of cytokinin-responsive genes using microarray meta-analysis and RNA-seq in Arabidopsis. Plant Physiol 162:272–294
3. Blomster T, Salojarvi J, Sipari N, Brosche M, Ahlfors R, Keinanen M, Overmyer K, Kangasjarvi J (2011) Apoplastic Reactive Oxygen Species Transiently Decrease Auxin Signaling and Cause Stress-Induced Morphogenic Response in Arabidopsis. Plant Physiol 157:1866–1883
4. Broeck LV, Dubois M, Vermeersch M, Storme V, Matsui M (2017) Inzé Dirk From network to phenotype: the dynamic wiring of an Arabidopsis transcriptional network induced by osmotic stress. Mol Syst Biol 13(12):961

5. Calixto CPG, Guo W, James AB, Tzioutziou NA, Entizne JC, Panter PE, Knight H, Nimmo HG, Zhang R. Brown JWS (2018) Rapid and dynamic alternative Splicing impacts the Arabidopsis cold response transcriptome. Plant Cell 30(7):1424–1444
6. Devaiah N, Nagarajan VK, Raghothama K (2007) Phosphate homeostasis and root development in Arabidopsis are synchronized by the zinc figer transcription factor ZAT6. Plant Physiol 145(1):147–159
7. Fiedler RJ, Sander DH, Peterson GA (1989) Fertilizer *phosphorus* recommendations for *winter wheat* in terms of method of *phosphorus application*, soil ph, and yield goal. Soil Sci Soc Am J, 53: 1282–1287
8. Gras DE, Vidal EA, Undurraga SF, Riveras E, Moreno S, Dominguez-Figueroa J, Alabadi D, Blázquez MA, Medina J, Gutiérrez RA (2018) SMZ/SNZ and gibberellin signaling are required for nitrate-elicited delay of flowering time in *Arabidopsis thaliana*. J Exp Bot 69(3):619–631
9. Guo C, Chang W, Zhang L, Cui X, Li S, Xiao K (2011) Effects of chromosome substitution on the utilization efficiency of nitrogen, phosphorus, and potassium in wheat. Front Agriculture China 5:253–261
10. Guo C, Zhao X, Liu X, Zhang L, Gu J, Li X, Lu W, Xiao K (2013) Function of wheat phosphate transporter gene *TaPHT2;1* in Pi translocation and plant growth regulation under replete and limited Pi supply conditions. Planta 237:1163–1178
11. Ha CV, Le DT, Nishiyama R, Watanabe Y, Sulieman S, Tran UT, Mochida K, Dong NV, Yamaguchi-Shinozaki K, Shinozaki K et al (2013) The auxin response factor transcription factor family in soybean: Genome-wide identification and expression analyses during development and water stress. DNA Res 20:511–524
12. Han G, Wang M, Yuan F, Sui N, Song J, Wang B (2014) The CCCH zinc finger protein gene AtZFP1 improves salt resistance in Arabidopsis thaliana. Plant Mol Biol 86:237–253
13. He P, Yang Y, Wang Z, Zhao P, Yuan Y, Zhang L, Ma Y, Pang C, Yu J, Xiao G (2019) Comprehensive analsyes of ZFP gene family and characterization of expression profiles during plant hormone response in coton. BMC Plant Bio 19:329
14. Hinsinger P (2001) Bioavailability of soil inorganic P in the rhizosphere as affected by root-induced chemical changes: a review. Plant Soil 237:173–195
15. Holford ICR (1997) Soil phosphorus: its measurement, and its uptake by plants. Soil Res 35:227–240
16. Jain A, Vasconcelos MJ, Raghothama KG, Sahi SV (2007) Molecular mechanisms of plant adaptation to phosphate deficiency. Plant Breed Rev 29:359
17. Jain M, Khurana JP (2009) Transcript profiling reveals diverse roles of auxin-responsive genes during reproductive development and abiotic stress in rice. FEBS J 276:3148–3162
18. Kiba T, Inaba J, Kudo T, Ueda N, Konishi M, Mitsuda N, Takiguchi Y, Kondou Y, Yoshizumi T, Ohme-Takagi M, Matsui M, Yano K, Yanagisawa S, Sakakibara H (2018) Repression of nitrogen starvation responses by members of the Arabidopsis GARP-type transcription factor NIGT1/HRS1 subfamily. Plant Cell 30(4):925–945

19. Kim D, Pertea G, Trapnell C, Kelley PR, Salzberg SL (2013) TopHat2: accurate alignment of transcriptomes in the presence of insertions, deletions and gene fusions. Genome Biol 14:R36
20. Klug A (2010) The discovery of zinc fingers and their applications in gene regulation and genome manipulation. Annu Rev Biochem 79:213–231
21. Leikam DF, Murphy LS, Kissel DE, Whitney DA, Moser HC (1983) Effects of nitrogen and *phosphorus application* method and nitrogen source on *winter wheat* grain yield and leaf tissue *phosphorus*. Soil Sci Soc Am J 47:530–535
22. Liu X, Huang B (2002) Cytokinin Effects on Creeping Bentgrass Response to Heat Stress. Crop Sci 42:466–472
23. Luther LK, Schillinger WF, Wuest SB, Christensen NW, Wysocki DJ (2010) Phosphorus fertilization of late-planted winter wheat into no-till fallow. Agron J 102(3):868–874
24. Ma X, Liang W, Gu P, Huang Z (2016) Salt tolerance function of the novel C2H2-type zinc finger protein TaZNF in wheat. Plant Physiol Biochem 106:129–140
25. Mao X, Chen S, Li A, Zhai C, Jing R (2014) Novel NAC transcription factor *TaNAC67* confers enhanced multi-abiotic stress tolerances in Arabidopsis. PLoS One 9(1):e84359
26. Marschner P, Solaiman Z, Rengel Z (2005) Growth, phosphorus uptake, and rhizosphere microbial community composition of a phosphorus-efficient wheat cultivar in soils differing in pH. J Plant Nutr Soil Sci 168(3):343–351
27. Miller J, Mclachlan A, Klug A (1985) Repetitive zinc-binding domains in the protein transcription factor IIIA from Xenopus oocytes. EMBO J 4:1609–1614
28. Mittal S, Mallikarjuna MG, Rao AR, Jain PA, Dash PK, Thirunavukkarasu N (2017) Comparative analysis of CDPK family in mize, Arabidopsis, rice, and sorghum revealed potential targets for drought tolerance improvement. Front Chem 5:115
29. Mittler R, Kim Y, Song L, Coutu J, Coutu A, Ciftci-Yilmaz S et al (2006) Gain-and loss-of-function mutations in Zat10 enhance the tolerance of plants to abiotic stress. FEBS Lett 580:6537–6542
30. Muhammad T, Zhang J, Ma Y, Li Y, Zhang F, Zhang Y, Liang Y (2019) Overexpression of a mitogen-activated protein kinase *SlMAPK3* positively regulates tomato tolerance to cadmium and drought stress. Molecules 24(3):556
31. Mýtinová Z, Motyka V, Haisel D, Gaudinová A, Lubovská Z, Wilhelmová N (2010) Effect of abiotic stresses on the activity of antioxidative enzymes and contents of phytohormones in wild type and AtCKX2 transgenic tobacco plants. Biol Plant 54:461–470
32. Olatunji D, Geelen D, Verstraeten I (2017) Control of endogenous auxin levels in plant root development. Int J Mol Sci 18(12):2587
33. Ostrowski M, Ciarkowska A, Jakubowska A (2016) The auxin conjugate indole-3-acetyl-aspartate affects responses to cadmium and salt stress in *Pisum sativum* L. J Plant Physiol 191:63–72
34. Pérez-Salamó I, Papdi C, Rigó G, Zsigmond L, Vilela B, Lumbreras V, Nagy I, Horváth B, Domoki M, Darula Z, Medzihradszky K, Bögre L, Koncz C, Szabados L (2014) The heat shock factor A4A confers

salt tolerance and is regulated by oxidative stress and the mitogen-activated protein kinases MPK3 and MPK6. Plant Physiol 165(1):319–334

35. Raghothama KG, Karthikeyan AS (2005) Phosphate acquisition. Plant Soil 274:37–49
36. Rausch C, Bucher M (2002) Molecular mechanisms of phosphate transport in plants. Planta 216:23–37
37. Rehman HM, Nawaz MA, Shah ZH, Ludwig-Müller J, Chung G, Ahmad MQ, Yang S, Lee SI (2018) Comparative genomic and transcriptomic analyses of Family-1 UDP glycosyltransferase in three Brassica species and Arabidopsis indicates stress-responsive regulation. Sci Rep 8:1875
38. Schroeder JI, Kwak JM, Allen GJ (2001) Guard cell abscisic acid signalling and engineering drought hardiness in plants. Nature 410:327–330
39. Seguel A, Cornejo P, Ramos A (2017) Phosphorus acquisition by three wheat cultivars contrasting in aluminium tolerance growing in an aluminium-rich volcanic soil. Crop Past Sci 68(4):305–316
40. Sham A, Al-Azzawi A, Al-Ameri S, Al-Mahmoud B, Awwad F, Al-Rawashdeh A, Iratni R, AbuQamar S (2014) Transcriptome analysis reveals genes commonly induced by Botrytis cinerea infection, cold, drought and oxidative stresses in Arabidopsis. PLoS One 9(11):e113718
41. Shan C, Mei Z, Duan J, Chen H, Feng H, Cai (2014) OsGA2ox5, a gibberellin metabolism enzyme, is involved in plant growth, the root gravity response and salt stress. PLoS One 9(1):e87110
42. Shinozaki K, Yamaguchi-Shinozaki K (2000) Molecular responses to dehydration and low temperature: differences and cross-talk between two stress signaling pathways. Curr Opin Plant Biol 3:217–223
43. Trapnell C, Pachter L, Salzberg SL (2009) TopHat: discovering splice junctions with RNA-SEq. Bioinform 25:1105–1111
44. Virk N, Li D, Tian L, Huang L, Hong Y, Li X, Zhang Y, Liu B, Zhang H, Song F (2015) Arabidopsis Raf-like mitogen-activated protein kinase kinase kinase gene *Raf43* is required for tolerance to multiple abiotic stresses. PLoS One 10(7):e0133975
45. Wang H, Xu Q, Kong Y, Chen Y, Duan J, Wu W, Chen Y (2014) Arabidopsis WRKY45 transcription factor activates *PHOSPHATE TRANSPORTER1;1* expression in response to phosphate starvation. Plant Physiol 164:2020–2029
46. Wang J, Liu S, Li C, Wang T, Zhang P, Chen K (2017) PnLRR-RLK27, a novel leucine-rich repeats receptor-like protein kinase from the Antarctic moss Pohlia nutans, positively regulates salinity and oxidation-stress tolerance. PLoS One 12(2):e0172869
47. Wang L, Li Z, Qian W et al (2011) The Arabidopsis purple acid phosphatase AtPAP10 is predominantly associated with the root surface and plays an important role in plant tolerance to phosphate limitation. Plant Physiol 157:1283–1299
48. Wang S, Bai Y, Shen C, Wu Y, Zhang S, Jiang D, Guilfoyle TJ, Chen M, Qi Y (2010) Auxin-related gene families in abiotic stress response in Sorghum bicolor. Funct Integr Genom 10:533–546

49. Wang X, Hao L, Zhu B, Jiang Z (2018) Plant calcium signaling in response to potassium deficiency. Int J Mol Sci 19(11):3456
50. Xie C, Mao X, Huang J, Ding Y, Wu J, Dong S, Kong L, Gao G, Li C, Wei L (2011) KOBAS 2.0: a web server for annotation and identification of enriched pathways and diseases. Nucleic Acid Res 39:316–332
51. Xue Q, Zhu Z, Musick JT, Stewart BA, Dusek DA (2006) Physiological mechanisms contributing to the increased water-use efficiency in winter wheat under deficit irrigation. J Plant Physiol 163(2):154–164
52. Xue Q, Zhu Z, Musick JT (2003) Root growth and water uptake in winter wheat under deficit Irrigation. Plant Soil 257:151–161
53. Yang WT, Baek D, Yun D, Lee KS, Hong S, Bae K, Chung YS, Kwon YS, Kim DH, Jung KH, Kim DH (2018) Rice OsMYB5P improves plant phosphate acquisition by regulation of phosphate transporter. PLoS One 13(3):e0194628
54. Ye J, Fang L, Zheng H, Zhang Y, Chen J, Zhang Z, Wang J, Li S, Li R, Bolund L (2006) WEGO: a web tool for plotting GO annotations. Nucleic Acid Res, 34: 293–297
55. Ye J, Fang L, Zheng H, Zhang Y, Chen J, Zhang Z, Wang J, Li S, Li R, Bolund L (2006) WEGO: a web tool for plotting. GO annotations Nucleic Acid Res 34:293–297
56. Zang D, Li H, Xu H, Zhang W, Zhang Y, Shi X et al (2016) An Arabidopsis zinc finger protein increases abiotic stress tolerance by regulating sodium and potassium homeostasis, reactive oxygen species scavenging and osmotic potential. Front Plant Sci 7:1272
57. Zavaleta-Mancera HA, López-Delgado H, Loza-Tavera H, Mora-Herrera M, Trevilla-García C, Vargas-Suárez M, Ougham H (2007) Cytokinin promotes catalase and ascorbate peroxidase activities and preserves the chloroplast integrity during dark-senescence. J Plant Physiol 164:1572–1582
58. Zhang H, Liu Y, Wen F, Yao D, Wang L, Guo J et al (2014) A novel rice C2H2-type zinc finger protein, ZFP36, is a key player involved in abscisic acid-induced antioxidant defence and oxidative stress tolerance in rice. J Exp Bot 65:5795–5809
59. Zhang J, Jiang F, Shen Y, Zhan Q, Bai B, Chen W, Chi Y (2019) Transcriptome analysis reveals candidate genes related to phosphorus starvation tolerance in sorghum. BMC Plant Biol 19:306
60. Zhang Y, Zhou Z, Yang Q (2013) Genetic variations in root morphology and phosphorus efficiency of *Pinus massoniana* under heterogeneous and homogeneous low phosphorus conditions. Plant Soil 364:93–104
61. Zhao R, Chen X, Zhang F, Zhang H, Schroder J, Rmheld V. 2006. Fertilization and nitrogen balance in a wheat–maize rotation system in north china. *Agronomy Journal*, 98(**4**), 938.

Tables

Table 1 Grain yields and the yield components of the tested cultivars under various P input treatments

Growth season	P input treatment	Cultivar	Spike number (10^4 ha^{-1})	Kernel numbers	Grain weight (mg)	Yield (kg ha^{-1})
2017-2018	Sufficient-P	Shixin 828	723.56 a	33.48 a	40.98 b	8.472 a
		Jimai 518 518Shimai 120	698.37 a	32.96 a	42.54 a	8.337 a
	Deficient-P	Shixin 828	650.27 b	31.22 ab	39.83 c	6.913 b
		Jimai 518	618.06 c	30.43 b	41.12 bc	6.584 c
2018-2019	Sufficient-P	Shixin 828	738.42 a	33.09 a	42.14 a	8.742 a
		Jimai 518	730.29 a	32.84 a	42.38 a	8.685 a
	Deficient-P	Shixin 828	666.87 b	31.60 b	40.36 c	7.234 b
		Jimai 518	624.12 c	30.52 c	41.66 b	6.782 c

Data are shown by averages from triplicates and different lowercase letters indicate to be statistical significance across the cultivar and P input treatments at each growth season.

Table 2 The transcript reads derived from the RNA-seq analysis in wheat plants under P input treatments

Read type	Sufficient-P (SN)	Deficient-P (DN)
Valid reads	59127488	58062356
Mapped reads	53344820 (90.22%)	51303898 (88.36%)
Unique mapped reads	44540737 (75.33%)	44371252 (76.42%)
Multi mapped reads	8916425 (15.08%)	8564198 (14.75%)

Table 3 The biological roles of the DE genes involving the phytohormone signaling

Phytohormone	Gene accession number	GO annotation	Biological function	log2(fc) value
Auxin	Traes_1AL_46F40DCDD	GO:0009733	response to auxin	3.000
	Traes_3B_BDF4FBBCE		response to auxin	2.807
	Traes_6DL_AC45C7E28		response to auxin	2.322
	Traes_4DL_B39DDFB61		response to auxin	2.000
	Traes_7BS_514F94003		response to auxin	1.678
	Traes_5BL_8A463E577		response to auxin	1.585
	Traes_7DL_61466B22F		response to auxin	1.503
	Traes_6AL_CD95299CC		response to auxin	1.354
	Traes_6BS_F34130139		response to auxin	1.024
	Traes_3B_5DAEC6587	GO:0009734	auxin-activated signaling pathway	3.000
	Traes_7AS_FB4D5B81E		auxin-activated signaling pathway	2.585
	Traes_3AS_9594B1341		auxin-activated signaling pathway	2.000
	Traes_5DL_FA51A1F01		auxin-activated signaling pathway	2.000
	Traes_7BL_20B08C649		auxin-activated signaling pathway	1.585
	Traes_3DL_6F92C3370		auxin-activated signaling pathway	1.585
	Traes_2DL_36A19AD5A		auxin-activated signaling pathway	1.485
	Traes_6AS_967D58FB4		auxin-activated signaling pathway	1.096
	Traes_1BL_54CD82AC3		auxin-activated signaling pathway	1.062
	Traes_2DL_E566C63F1	GO:0010329	auxin efflux transmembrane transporter activity	1.278
	Traes_2DL_4B09052CE	GO:0009926	auxin polar transport	1.415
	Traes_1DL_E04699042	GO:0071365	cellular response to auxin stimulus	1.361
Cytokinin	Traes_7DL_6A6BEA0C5	GO:0019139	cytokinin	3.907

			dehydrogenase activity	
	Traes_2AL_D12464ADB		cytokinin dehydrogenase activity	2.459
	Traes_2DL_C141AAB8D	GO:0009690	cytokinin metabolic process	3.585
	Traes_4DL_89A2EBEA3		cytokinin metabolic process	3.585
	TRAES3BF186800020CFD_g		cytokinin metabolic process	2.000
	Traes_4AL_1159F9192	GO:0009691	cytokinin biosynthetic process	1.322
	Traes_4DS_A0680809B	GO:0019955	cytokinin binding	1.268
	Traes_5BL_E63627BC8	GO:0009735	response to cytokinin	1.091
Gibberellin	Traes_4DL_3C29B5BE0	GO:0009739	response to gibberellin	2.322
	Traes_7DS_E3D069829	GO:0071370	cellular response to gibberellin stimulus	1.322
Abscisic acid	Traes_2AL_C0670BBDD	GO:0009737	response to abscisic acid	2.700
	Traes_2AS_FA5CACFDD		response to abscisic acid	2.585
	Traes_7DL_B0F61B0B6		response to abscisic acid	2.322
	Traes_2AL_CF862CF2D		response to abscisic acid	1.748
	Traes_2DL_6EA103A58		response to abscisic acid	1.585
	Traes_2BL_1F96D8DB3		response to abscisic acid	1.585
	Traes_2BS_CE0B2A211		response to abscisic acid	1.585
	Traes_7AL_FA77CC1F41		response to abscisic acid	1.485
	Traes_2DL_574F25C6E		response to abscisic acid	1.459
	Traes_7DS_8AA003CE9		response to abscisic acid	1.379
	Traes_2DL_607AD3522		response to abscisic	1.244

			acid	
	Traes_2AL_8EE7F34F6		response to abscisic acid	1.239
	Traes_7BS_5427AAAA9		response to abscisic acid	1.222
	Traes_4AS_9662391A9	GO:0010427	abscisic acid binding	2.322
	Traes_4AS_72BEF89AC		abscisic acid binding	1.959
	Traes_5BL_C1D02590D	GO:0009788	abscisic acid-activated signaling pathway	1.222
Ethylene	Traes_2BL_B877F41B8	GO:0071369	cellular response to ethylene stimulus	2.722
	Traes_2BL_051AAAA05		cellular response to ethylene stimulus	2.585
	Traes_2BL_F442E87DD		cellular response to ethylene stimulus	2.322
	Traes_1DL_21AC9E85F	GO:0009723	response to ethylene	2.237
	Traes_5BL_F8A34CF5A		response to ethylene	1.206
	Traes_2AS_93E61F00D	GO:0009873	ethylene-activated signaling pathway	2.000
	Traes_4BL_39B08F4B9	GO:0010364	regulation of ethylene biosynthetic process	1.874
	Traes_3DL_B1B3D756E	GO:0010105	regulation of ethylene-activated signaling pathway	1.222
	Traes_1BS_79EF5B41A	GO:0051740	ethylene binding	1.051
Salicylic acid	Traes_7BL_EE72D8712	GO:0009862	salicylic acid mediated signaling pathway	3.322
	Traes_7AL_BF3EA280F	GO:0009751	response to salicylic acid signaling pathway	2.687
	Traes_2DL_8FFB2DC5D		response to salicylic acid signaling	1.848
	Traes_3AS_9FFB376AF	GO:0080142	regulation of salicylic acid biosynthesis process	1.970
	TRAES3BF022700010CFD_g	GO:0009863	salicylic acid mediated signaling pathway	1.432
	TRAES3BF022100050CFD_g		salicylic acid mediated signaling pathway	1.432

Jasmonic acid	Traes_3AL_532CA9EE7	GO:0009753	response to jasmonic acid	2.000
	Traes_4BS_F7359FA2E		response to jasmonic acid	1.846
	Traes_5DL_702D641A3		response to jasmonic acid	1.429
	Traes_1BS_4A4C11587		response to jasmonic acid	1.427
	Traes_4DS_94005294B		response to jasmonic acid	1.332
	Traes_7AL_E58674B35		response to jasmonic acid	1.264
	Traes_6DS_7CA5A8F12		response to jasmonic acid	1.191

Figures

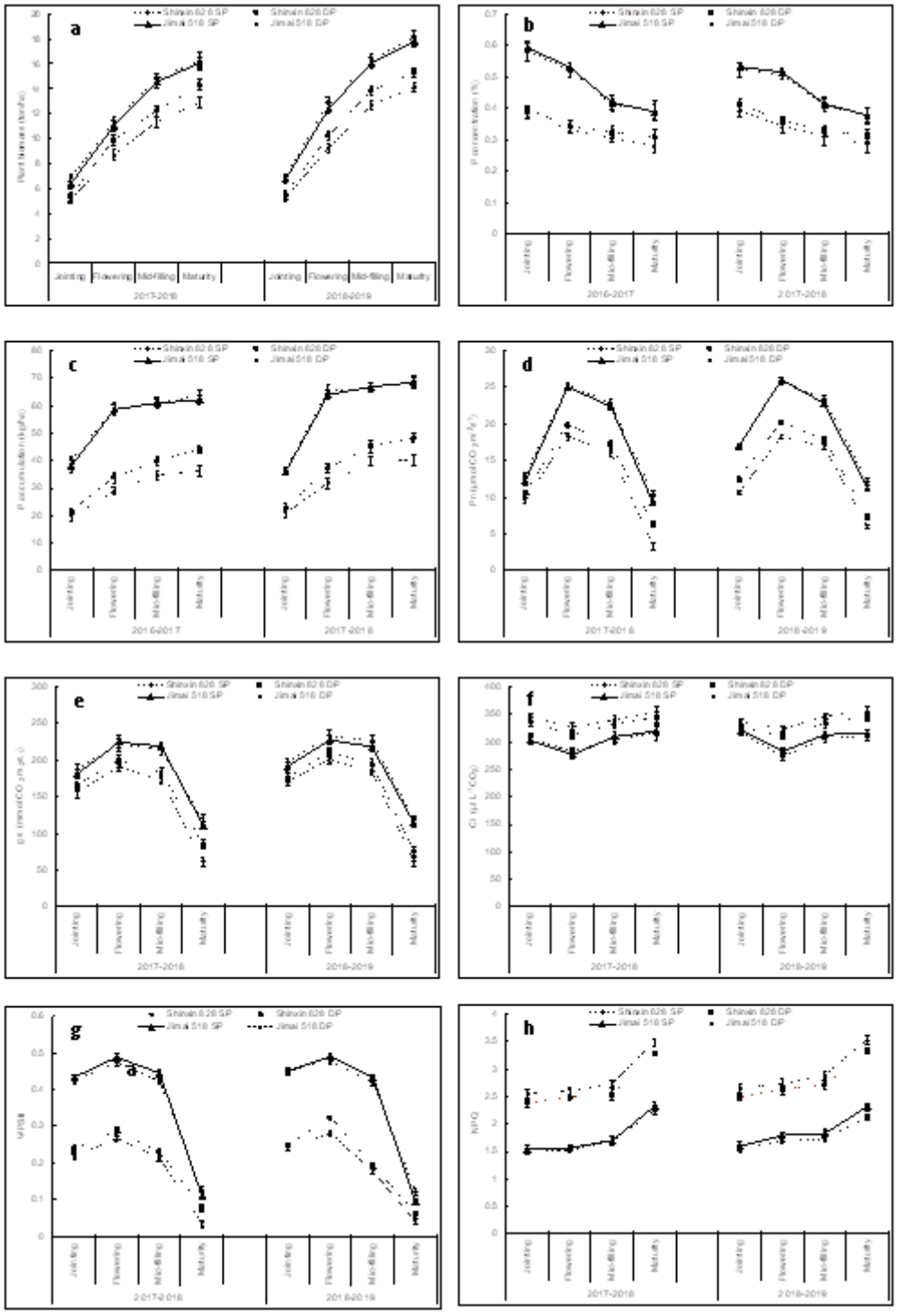

Figure 1

Plant biomass, P-associated traits, and photosynthetic parameters at various stages in tested wheat cultivars under P input treatments A, plant biomass; B, P concentrations; C, P accumulative amounts; D, Pn, E, gs; F, Ci, G, ΨPSII; H, NPQ. Data are shown by averages derived from triplicate results.

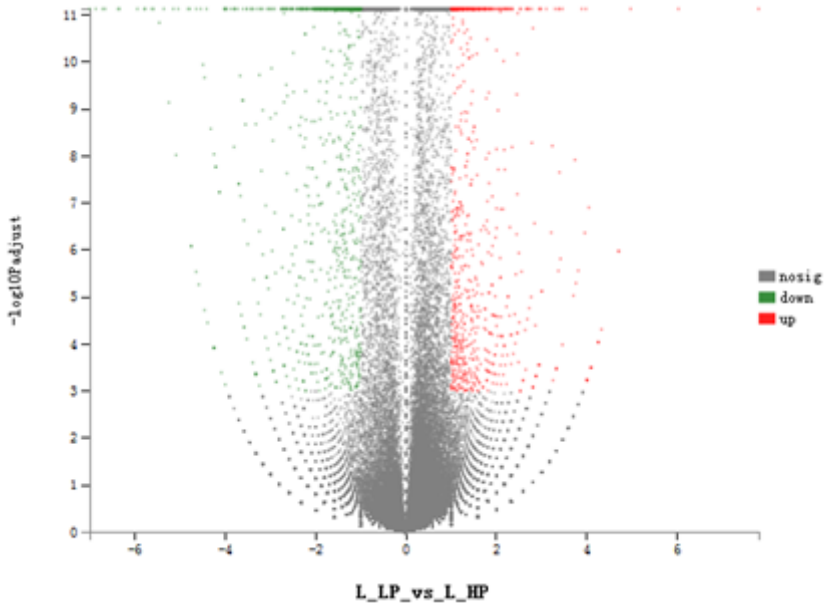

Figure 2

Scatter plot indicating the differentially expressed genes identified in the contrasting P input treatments managed by deficit irrigation L_LP, derived from leaves under low-P treatment. L_HP, derived from leaves under sufficient-P treatment.

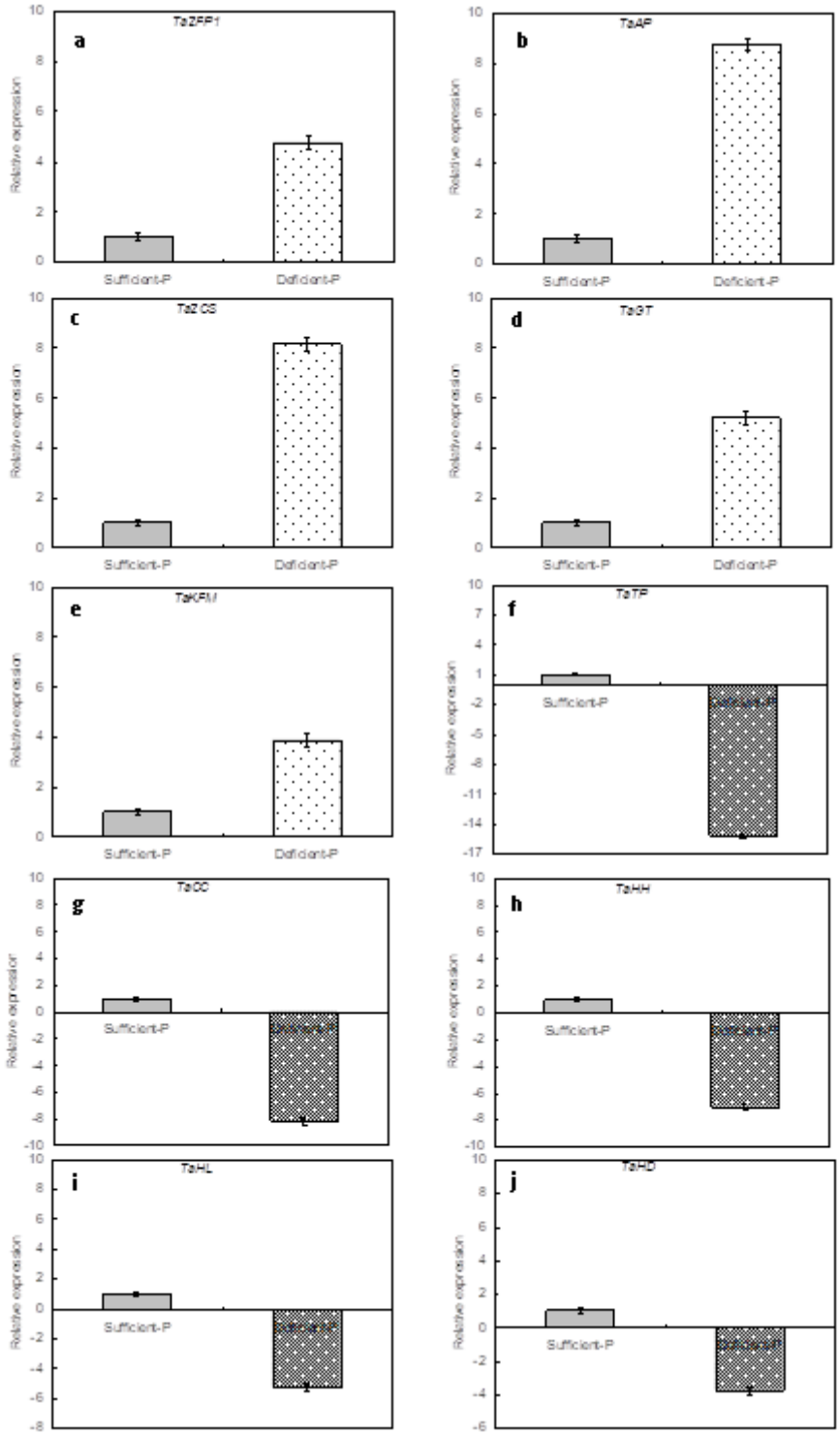

Figure 3

Expression patterns of randomly selected DE genes under P input treatments A, TaZFP1; B, TaAP; C, TaCS; D, TaGT; E, TaKFM; F, TaTP; G, TaCC; H, TaHH ; I, TaHL; J, TaHD. A-E, genes with upregulated expression pattern in transcript datasets. F-J, genes with downregulated expression pattern in transcript datasets. Data are averages derived from triplicate results. Expression levels of the DE genes under sufficient-P were set as value 1.

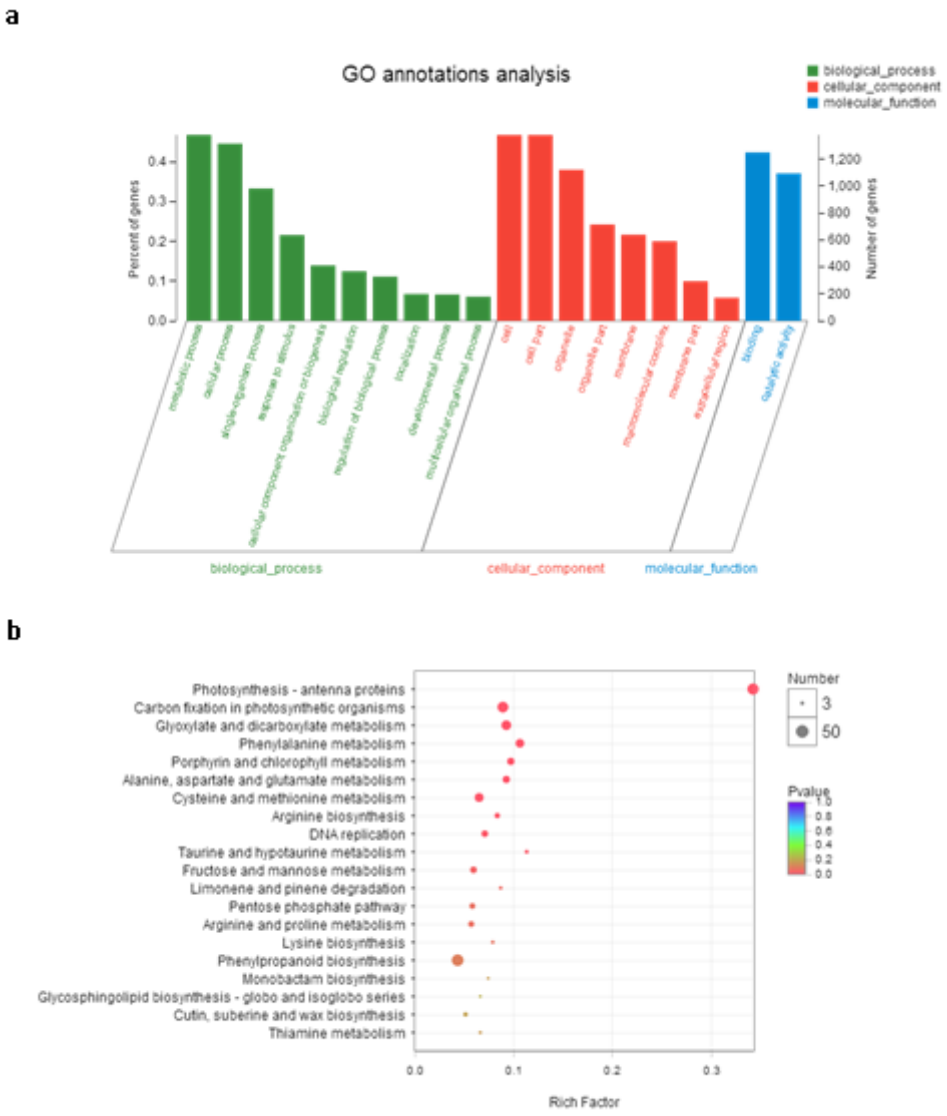

Figure 4

GO annotation analysis results and biochemical pathways enriched on the DE genes identified in the P-deprived wheat plants under deficit irrigation management A, GO annotation analysis results of the DE genes; B, biochemical pathways of the DE genes enriched.

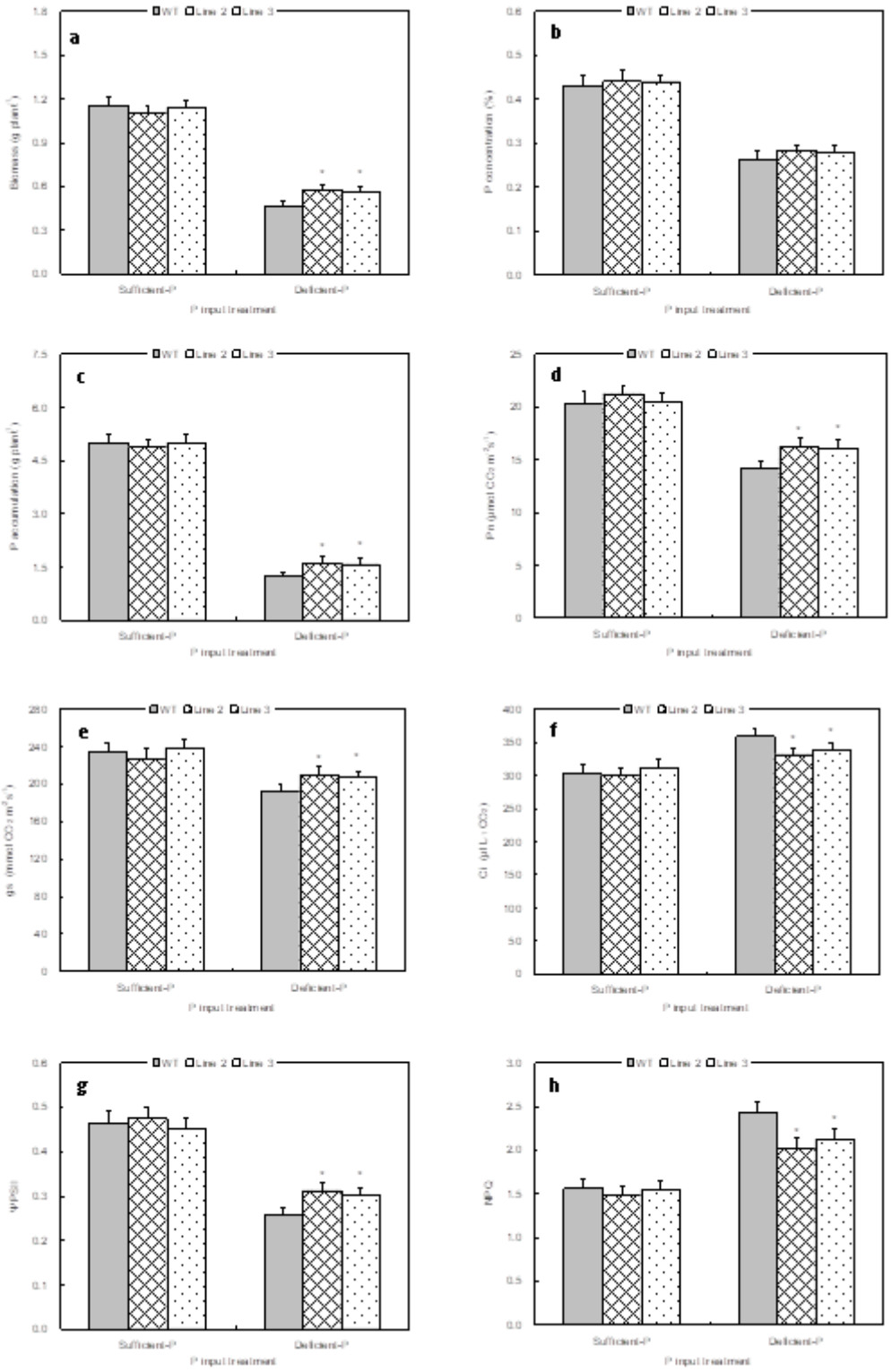

Figure 5

Plant growth, P-associated traits, and photosynthetic parameters in the transgenic lines overexpressing TaZFP1 under P input treatments. A, biomass, B, P concentrations. C, P accumulative amounts; D, Pn; E, gs; F, Ci; G, ΨPSII; H, NPQ. WT, wild type. Line 2 and Line 3, two TaZFP1 overexpression lines. Data are shown by averages derived from triplicate results and symbol * indicate to be significant between the transgenic lines and WT under same P treatment ($P<0.05$).

Supplementary Files

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

- Supplementaldata.doc