Planta (2021) 254:110
https://doi.org/10.1007/s00425-021-03760-8

ORIGINAL ARTICLE

Identification of soybean phosphorous efficiency QTLs and genes using chlorophyll fluorescence parameters through GWAS and RNA-seq

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Received: 30 August 2021 / Accepted: 14 October 2021 / Published online: 30 October 2021

Abstract

Main conclusion **Soybean phosphorous efficiency QTLs were identified and candidate genes were predicted using chlorophyll fluorescence parameters through GWAS and RNA-seq.**

Abstract Phosphorus (P) is an essential nutrient element for crop growth and development, lack of P uptake seriously affects yield in various crops. Photosynthesis is the basis of crop production, while it is very sensitive to P deficiency. It is of great importance to study the genetic relationship between photosynthesis and P efficiency to provide genetic insight for soybean improvement. In this study, a genome-wide association study (GWAS) was performed using 292,035 SNPs and the ratios of four main chlorophyll fluorescence parameters of 219 diverse soybean accessions under P deficiency and normal P across three experiments. In total, 52 SNPs in 12 genomic regions were detected in association with the four main chlorophyll fluorescence parameters under sufficient or deficient P levels. Combined it with RNA-seq analysis, we predicted three candidate genes for the significant genomic regions. For example, the expression level of the candidate gene (*Glyma.18g092900*) in P deficiency tolerant accession was three times higher than that of P deficiency sensitive one under phosphorous deficiency condition. This study provides insight into genetic links between photosynthetic and phosphorous efficiency and further functional analysis will provide valuable information for understanding the underlying genetic mechanism to facilitate marker-assisted breeding in soybean.

Keywords Candidate genes · Chlorophyll fluorescence parameters · Genome-wide association study (GWAS) · *Glycine max* · Low-phosphorus · RNA-seq

Communicated by Dorothea Bartels.

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Abbreviations

DEGs	Differential expression genes
Fv/Fm	Maximum quantum efficiency of photosystem II
GWAS	Genome-wide association study
NPQ	Non-photochemical quenching
qP	Photochemical quenching
ΦPSII	Quantum efficiency of photosystem II
QTL	Quantitative trait locus
SNP	Single nucleotide polymorphism
W82	Soybean accession Williams 82

Introduction

As an essential nutrient element required for plant growth and development, P plays an irreplaceable role in energy acquisition, storage and utilization, regulation of enzyme

activities, and yield formation (Cong et al. 2020). Adequate P supplies can significantly increase crop production, such as wheat, rice, finger millet, maize and soybean (Wissuwa and Ae 2001; Galindo-Castañeda et al. 2018; Mallarino et al. 2018; Ceasar et al. 2020). However, P shortages constraining crop productivity are widespread worldwide and may be more sever in the future because of the increasing food demand worldwide and the pursuit of high crop yield (Veneklaas et al. 2012). P is a non-renewable resource. Increaing the ability of P uptake in plants would be important for sustainable agricultural development.

Soybean is rich in protein and oil, which has been an important source for animal feedstocks and food industry (Liu et al. 2020). Being a C3 crop, the photosynthetic efficiency is significantly lower than that of C4 crops like corn and sorghum, which is a main factor that restricts soybean yield to increase (Bassham 1977). Studying the regulation mechanism of photosynthesis is of great significance to the increase of soybean yield. However, photosynthesis is a complex biochemical reaction process and can be inhibited by many internal and environmental factors (Pedersen et al. 2018; Qian et al. 2018). For example, the effective quantum yield of photosystem II (PSII) photochemistry was significantly reduced in leaves deficient in P (Yan et al. 2015), and the electron transport at photosystem I (PSI) was also affected (Frydenvang et al. 2015). In wheat and cotton, stomatal conductance positively correlated with P concentration in certain conditions (Rao and Terry 1989; Abbas et al. 2018; Wang et al. 2018). It can be assumed that P is an important source for ATP synthesis, and ATP is involved in regulating the opening and closing of stomata. Under P restriction condition, the stomatal conductance of peas was higher than that under the adequate P condition (Jin et al. 2014). These results suggest that the relationships between the P efficiency and photosynthetic efficiency are complex. Identification of the key genes that can enhance P efficiently and photosynthesis efficiency will lay a foundation for breeding soybean varieties with low P tolerance and high light efficiency.

Chlorophyll fluorescence, an nonintrusive indicator for rapid evaluation of photosynthesis in vivo without damage of the leaves, is an effective method being widely used for studying the effects of different environmental conditions on plant photosynthesis (Maxwell and Johnson 2000; Sayed 2003; Kalaji et al. 2016). Changes in chlorophyll fluorescence parameters reflect the process of light energy absorption, utilization, transfer and dissipation of PSI and PSII (mainly PSII) (Schreiber et al. 1995). It is one of the most effective technologies to study the changes in photosynthesis when exposing to external stresses (Sayed 2003). Among the parameters of chlorophyll fluorescence, the ratio of variable fluorescence (Fv) to maximum fluorescence (Fm) is an important index to evaluate the maximum efficiency of PS II under abiotic stress conditions, and it can be used as a powerful tool to screen the ability of PS II to resist abiotic stress (Prasad et al. 2007), such as low temperature in wheat (Rapacz et al. 2015), height temperature in wheat (i Azam et al. 2015), salinity in ryegrass (Dabrowski et al. 2016), waterlogging in barley (Bertholdsson et al. 2015), drought in *Festuca arundinace* (Kosmala et al. 2012), magnesium deficiency in radish (Samborska et al. 2018), nitrogen deficiency stress in radish (Cetner et al. 2017).

Although physiological and biochemical research on chlorophyll fluorescence have been made in various plants, there are rare reports investigating genetic mechanisms affecting the natural variation of chlorophyll fluorescence in soybean challenged by low P availability. Our previous genetic studies have demonstrated that there was a significantly positive correlation between P efficiency and photosynthetic-related traits in soybean, particularly under P deficiency. The follow-up gentic analyses revealed several major QTLs and the underlying candidate genes with potentials in improving both P efficiency and photosynthesis were also identified (Li et al. 2016; Lü et al. 2018; Yang et al. 2020). However, because of its complexity and the small effect of genetic factors, researches on the genetic mechanism of soybean P efficiency and photosynthesis-related traits are far from enough, and more indicators should be used to unravel genetic mechanisms.

A genome-wide association study (GWAS) has been recognized as a useful tool for genetic analysis of complex traits in diverse plant species (Zhu et al. 2016). Here, we adopted this approach intending to identify new QTLs for low P and high photosynthetic efficiency and explore related key genes. Four main chlorophyll fluorescence parameters were used to index the changes of photosynthetic efficiency, including maximum quantum efficiency of photosystem II (Fv/Fm), quantum efficiency of PSII (ΦPSII), photochemical quenching (qP) and non-photochemical quenching (NPQ) of soybean leaves during the V2 seedling stage under normal or low P conditions. In total, 12 QTLs were identified for relative chlorophyll fluorescence parameters in 1–3 experiments. Among them, 4 QTLs were physically overlapped with the previously reported P efficiency and photosynthesis QTLs, and the other 8 were novel. Three QTLs were identified in two or three experiments and the promising candidate genes were indicated based on transcriptome analysis. These results provide gene source for a better knowledge for breeding soybean varieties with better P efficiency and photosynthesis efficiency.

Materials and methods

Plant materials and experiments

A natural population containing 219 soybean [*Glycine max* (L.) Merrill] accessions was used in the study, of

which 204 accessions originated from China, 10 from other countries (one from Brazil, two from Japan and seven from America) and there remaining five were unknown. Plant growing was carried out in hydroponics experiments in an artificial climate chamber (28–33/20–25 °C day/night temperature, 14 h light/ 10 h dark photoperiod). Briefly, seeds were disinfected by exposing them to chlorine for 4–5 h, then planted in vermiculite to germinate. After about 4–5 days, six seedlings with the same growth status were selected and moved into a normal P and P deficiency hydroponic environment (each contains three seedlings), respectively.

The composition method of low and normal P nutrient solution was referred to the description of Zhang et al. (2016). One-half Hoagland solution [2.5 mM $Ca(NO_3)_2$, 2.5 mM KNO_3, 1.0 mM $MgSO_4$, 0.5 mM KH_2PO_4, 10 µM EDTA Na_2, 10 µM $FeSO_4$, 23 µM H_3BO_3, 4.5 µM $MnCl_2$, 0.15 µM $CuSO_4$, 0.4 µM $ZnSO_4$, 0.05 µM Na_2MoO_4] was used as normal P nutrient solution, and the low P nutrient solution (with 0.005 mM KH_2PO_4, the lack of KH_2PO_4) was replaced by equal concentration of KCl). The hydroponic tanks (60 × 40 × 16, length × width × height, cm) used contained 60 holes for cultivating seedlings. Randomized blocks design was used and the nutrient solution (pH 5.8) was changed every three days to ensuring adequate nutrients. The experiment was conducted from May to July, 2017 as environment 1 (E1), June to August, 2018 as environment 2 (E2) and May to June 2019 as environment 2 (E3), respectively. The seeds used in the three experiments were harvested in the field from the previous year.

Measurements of the chlorophyll fluorescence parameters

After 15 days of hydroponic treatment, chlorophyll fluorescence parameters were measured with a MINI-PAM portable chlorophyll fluorometer (Walz) as described elsewhere (Munekage et al. 2002) with some modifications as for soybean seedlings. Firstly, plants were dark acclimated for 20 min, then a light pulse of 3000 µmol photons m^{-2} s^{-2} for 800 ms and actinic light of 120 µmol photons m^{-2} s^{-2} for 5 min was used to determine initial fluorescence (F_0), the maximal fluorescence (Fm) and maximum fluorescence (Fm′), steady-state fluorescence (Ft) and initial fluorescence (F_0') under light, respectively. Finally, calculate the maximum quantum efficiency of photosystem II (Fv/Fm), quantum efficiency of PSII (ΦPSII), photochemical quenching (qP), non-photochemical quenching (NPQ) according to the formula as described in the handbook of operation of MINI-PAM (www.walz.com) were calculated.

Analysis of the chlorophyll fluorescence parameters

The analyses of variance and broad heritability of the phenotypic data were carried out using the R software (Team 2015). The formula "$Y_{ijk} = \mu + G_i + E_j + (GE)_{ij} + \varepsilon_{ijr}$" was used to analysis the variance, of which Y_{ijk} is the phenotype value of the *i*th genotype in the *j*th environment and the *k*th block, μ is the population mean, G_i is the effect of the *i*th genotype, E_j is the effect of the *j*th environment, $(GE)_{ij}$ is the genotype-by-environment interaction effect, and ε_{ij} is residual error. The broad-sense heritability (H^2) rate was calculated by the following formula: $H^2 = \sigma^2_g/(\sigma^2_g + \sigma^2_{gy}/n + \sigma^2/nr)$, σ^2_g is the genetic variance, σ^2_{gy} is the interaction of genotype with environment, σ^2 is the residual error, n is the number of environments, and r is the number of replications (Knapp et al. 1985).

Genome wide association mapping (GWAS)

The 219 soybean accessions were genotyped by 292,035 SNPs derived from 355 K SoySNP array described by Wang et al. (2016). GWAS were performed based on the compressed mixed linear model (Zhang et al. 2010) using the GAPIT package (Lipka et al. 2012). The relative chlorophyll fluorescence parameters (ratio of the chlorophyll fluorometer parameters in low P vs normal P conditions) were used as phenotypic data, which was a benefit for conditional genetic effects analysis (Zhu 1995). SNPs with a minor allele frequency (MAF) ≥ 0.05 were used. The population structure was accounted by principle component analysis (PCA), and the relatedness was calculated by the VanRaden method (VanRaden 2008). The threshold for significant association was set to $1/n$ (n is the marker numbers, $P < 1/n$ ($P < 4.82 \times 10^{-6}$)) (Yang et al. 2014). Manhattan plots and QQ plots were generated using the R package qqman (Turner 2014). The linkage disequilibrium (LD) decay rate of these 219 cultivated soybeans was 130 kb (Wang et al. 2016). The upstream and downstream 130 kb interval of the significant SNPs were taken as significant QTLs.

RNA sequencing and data analysis

According to the hydroponic experiments, two soybean accessions were selected for RNA-sequencing. One was tolerant to low P accessions, Williams82 (W82), the other was the low P sensitive accession Jack. The leaves were harvested after 15 days of hydroponics (normal and low P treated conditions), each with three biological replicates. All samples were quick-frozen with liquid nitrogen and then stored at − 80 °C for RNA isolation and RNA sequencing. The RNA-seq experiment data analysis was commissioned

by Shanghai Ling 'en Biotechnology Company. Genes with a false discovery rate < 0.005 and $\log_2$ (fold change) ≥ 1 were declared differential expression genes (DEGs).

Candidate genes prediction and expression analysis

According to the annotation of the soybean reference genome (Wm82.a2.v1) in Phytozome v.12 (https://phytozome.jgi.doe.gov), genes among the main QTLs were analyzed. Based on transcriptome analysis results, we selected genes that were significantly differentially expressed in transcriptome and fell within the QTL region as candidate genes. For tissue expression analysis, the candidate genes expression date containing six tissues with different development stages was downloaded from the Soy Base (https://www.soybase.org/soyseq).

Quantitative real-time PCR

Total RNA isolation from leaves of soybean lines W82 and Jack under normal and low P conditions were extracted and each had three biological replicates. About 50–100 mg leaves were used for the isolation of RNA according to the manufacturer's instructions with the use of Plant RNA Extract Kit (TianGen, Beijing, China). Then about 1 μg RNA was used to configure to synthesize cDNA with the application of Hifair® II 1st Strand cDNA Synthesis SuperMix for qRT-PCR (gDNA digester plus) (Yeasen, Shanghai, China). The soybean constitutive expression gene *Tublin* (GenBank accession number: AY907703) was used as a reference gene for qRT-PCR. The qRT-PCR was conducted on a CFX96 Touch real-time PCR system (Bio-Rad Laboratories, Hercules, CA,USA) with the use of SYBR Green Realtime Master Mix (Yeasen). Data were analyzed with a standard curve-based method calculated with CFX Manager™ software.

Results

Chlorophyll fluorescence exhibited significant variation in different P levels

Chlorophyll fluorescence parameters is an effective index to indicate the effects of different environmental conditions on plant photosynthesis. To comprehensively investigate the genetic variation of photosynthesis associated with P supply, a total of 219 diverse soybean accessions were used and four main chlorophyll fluorescence parameters including Fv/Fm, ΦPSII, qP and NPQ were measured in plants under sufficient or deficient P supplies. The descriptive statistics analysis showed that there were large variations among the four main chlorophyll fluorescence parameters (Table 1). The coefficients of variation of Fv/Fm, ΦPSII and qP varied greatly in both conditions, ranging from 2.44 to 10.1% and 3.34–15.0% under normal P and low P conditions, respectively. In contrast, the value of NPQ shows the largest coefficients of variation ranging from 30.7 to 37.5% and 32.2 to 34.9% under normal P and low P conditions, respectively.

Under normal P condition, the mean values of Fv/Fm in E1, E2 and E3 were 0.816, 0.819 and 0.813, respectively; for ΦPSII, the means were 0.682, 0.40 and 0.705, respectively; mean values of qP were 0.805, 0.759 and 0.807, respectively; the NPQ mean values were 0.368, 0.466 and 0.469, respectively. However, under low P condition, all the the chlorophyll fluorescence parameters significantly decreased except for NPQ (Table 1). These suggested that, when plants suffered P deficiency, photosynthesis in plants was suppressed and further revealed the molecular association with P deficiency with the photosynthesis process. The solar energy assimilated by leaves used for non-photochemical quenching increased, and the light quantum efficiency decreased.

Variance analysis showed significant variations for the four chlorophyll fluorescence parameters among genotypes, environments and genotype × environmental interaction effects in either normal P or low P conditions (Table 1), suggesting a rich varation in the gentic association between P response with photosynthesis. These chlorophyll fluorescence parameters have overall high broad-sense heritabilities raging from 59.1 to 88.2% (Table 1), indicating that genetic effects play major roles in the phenotypic variation (These traits are to a large extent controlled genetically with varying effects by environments or genotype × environments). On the other hand, the four traits exhibit normal or nearly normal distribution (Fig. 1), strongly indicating that these traits are quantitatively inherited and controlled by multiple genes.

Correlation analysis of chlorophyll fluorescence parameters

To investigate the correlation of the four chlorophyll fluorescence parameters, correlation analysis was performed based on the average values of the three experiments (Table 2). The results showed that NPQ was significantly negatively correlated with ΦPSII and qP in normal P condition, but what was interesting was that the NPQ in low P condition was significantly positive correlated with ΦPSII. NPQ is an indicator for estimating changes in the apparent rate constant for excitation decay by heat loss (Baker 2008). Under P deficiency stress, an increase in the amount of captured light energy used for heat loss may alleviate the damage to the photosystem caused by P deficiency. This suggested that NPQ was an important physiological change for plants to adapt to low phosphorus stress and could be used as an index of P efficiency material screening.

Table 1 Descriptive statistical results for chlorophyll fluorescence parameter of soybean under different P conditions

P level[a]	Year	Trait	Mean	Stdev	Kurtosis	Skewness	Min	Max	CV (%)[b]	G[c]	E[d]	G×E[e]	*H2* (%)[f]
NP	E1	Fv/Fm	0.816	0.025	1.539	1.19	0.714	0.864	3.06	***	***	***	75
	E2	Fv/Fm	0.819	0.02	2.36	1.44	0.738	0.856	2.44				
	E3	Fv/Fm	0.813	0.017	1.265	1.14	0.734	0.855	2.09				
	E1	ΦPSII	0.682	0.069	1.032	0.788	0.352	0.852	10.1	***	**	***	78.3
	E2	ΦPSII	0.74	0.027	1.628	0.611	0.598	0.865	3.64				
	E3	ΦPSII	0.705	0.039	0.141	0.74	0.5165	0.819	5.53				
	E1	qP	0.805	0.076	1.053	0.736	0.463	0.977	9.44	***	***	**	76.5
	E2	qP	0.759	0.065	0.349	0.627	0.502	0.93	8.56				
	E3	qP	0.807	0.039	1.335	0.695	0.614	0.953	4.83				
	E1	NPQ	0.368	0.138	0.694	0.648	0.029	0.892	37.5	***	***	**	67.5
	E2	NPQ	0.466	0.15	0.959	0.245	0.048	0.934	32.1				
	E3	NPQ	0.469	0.144	0.743	0.412	0.104	0.909	30.7				
LP	E1	Fv/Fm	0.683	0.026	1.539	1.038	0.572	0.735	3.79	***	***	**	88.2
	E2	Fv/Fm	0.688	0.03	2.358	0.987	0.365	0.745	4.32				
	E3	Fv/Fm	0.684	0.023	1.265	0.986	0.595	0.734	3.34				
	E1	ΦPSII	0.583	0.07	1.032	0.756	0.282	0.715	12	***	**	**	59.1
	E2	ΦPSII	0.703	0.049	1.628	0.677	0.342	0.815	6.92				
	E3	ΦPSII	0.477	0.071	0.141	0.249	0.283	0.685	15				
	E1	qP	0.821	0.066	1.053	1.079	0.565	0.963	8.08	***	***	***	75.8
	E2	qP	0.685	0.064	0.349	0.738	0.487	0.852	9.4				
	E3	qP	0.652	0.058	1.335	0.61	0.405	0.84	8.94				
	E1	NPQ	0.512	0.179	0.694	0.313	0.153	0.976	34.9	***	**	***	62.2
	E2	NPQ	0.553	0.18	0.959	0.184	0.149	0.983	32.6				
	E3	NPQ	0.548	0.177	0.743	0.229	0.135	0.979	32.2				

**Significant at $P < 0.01$

***Significant at $P < 0.001$

[a]P level:LP and NP represent low P and normal P conditions, respectively

[b]Coefficient of variation

[c]Genotype

[d]Environments

[e]Genotype × environments

[f]Broad-sense heritability

Genome-wide association study identified loci associated with P efficiency by chlorophyll fluorescence parameters

Facilitated by sequencing technology, GWAS has become a bridge between the natural variations of phenotypic parameters and the associated genetic variation (Hamdani et al. 2019). To detect SNPs significantly associated with P efficiency, the 201,994 SNPs with MAF ≥ 0.05 were selected from 292,053 high-quality SNPs and four chlorophyll fluorescence parameters across different environments (ratio of the values in low P and normal P conditions) from the soybean panel were used for GWAS. A total of 52 significant SNPs were detected (Fig. 2, Table S1 and Table 3). These SNPs are distributed on seven chromosomes including chromosomes 7, 9, 11, 12, 16, 18, and 20. Among these significant SNPs, 11 SNPs were detected in E1 for Fv/Fm, ΦPSII and NPQ, and distributed on chromosomes 11 and 18, respectively. In E2, 39 SNPs were detected and distributed on chromosomes 9, 11, 12 and 18, 20 for Fv/Fm, ΦPSII, qP and NPQ; 16 SNPs were detected for Fv/Fm, ΦPSII, qP and NPQ in E3. Notably, 38 SNPs distributed on chromosomes 18 and 20 were significantly detected in more than two environments and involved three regions. Other SNPs were detected in a single environment, which may be because of environmental factors. The interpretation rate of these SNPs for phenotypic variation ranged from 11.02 to 16.92%, suggesting that some QTLs with important functions in regulating photosynthesis under P deficiency stress might exist around these SNPs.

Based on the linkage disequilibrium (LD) decay calculated previously (Wang et al. 2016) the regions within

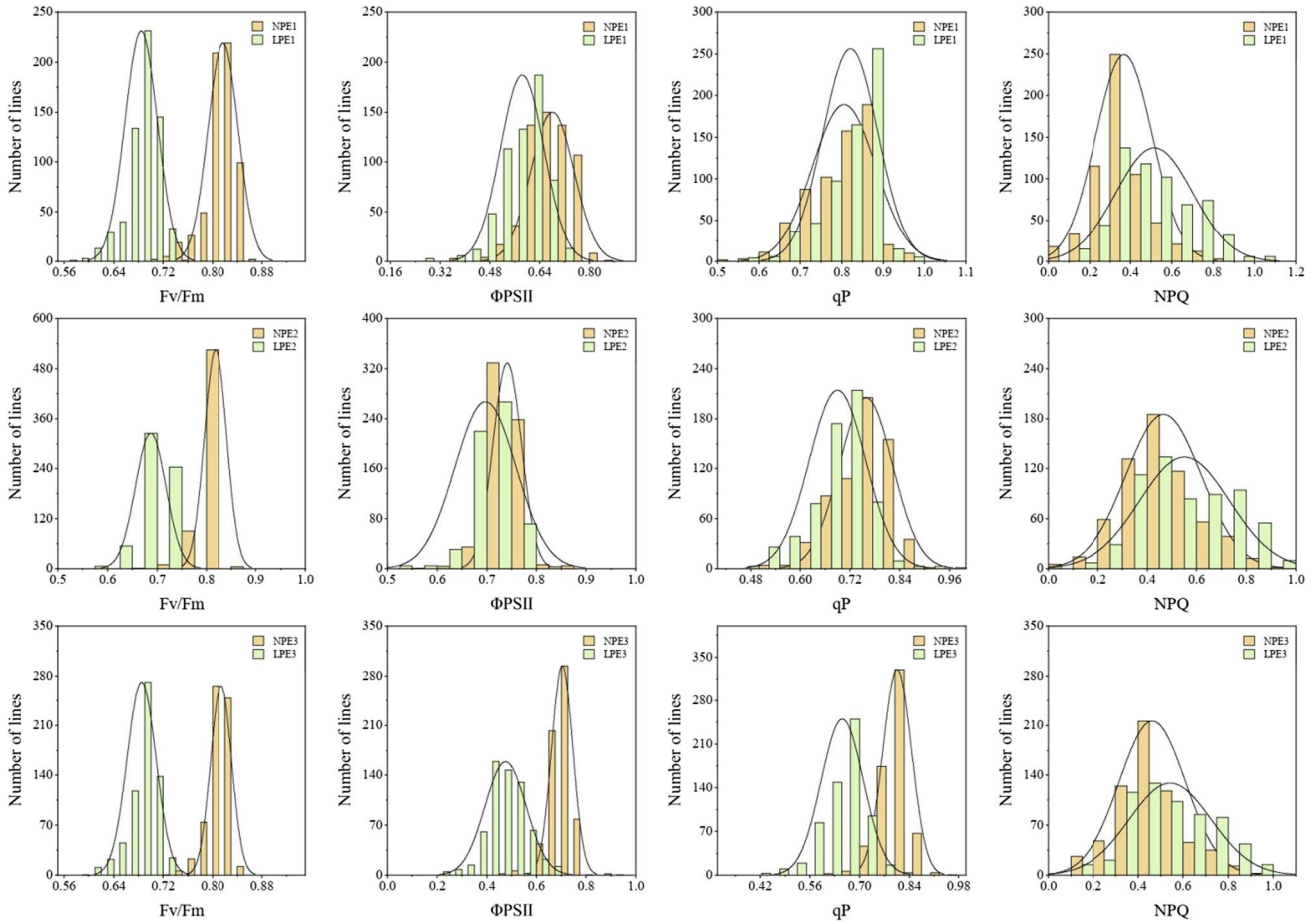

Fig. 1 Frequency distribution of four chlorophyll fluorescence parameters under low P and normal P conditions across three experiments. E1, E2 and E3 represent the three experiments, respectively; NP, normal phosphorus concentration; LP, low phosphorus concentration; Fv/Fm, maximum photochemical efficiency of photosystem II; ΦPSII, actual yield of photosystem II; qP, photochemical quenching coefficient; NPQ, non-photochemical quenching coefficient

Table 2 Correlations between four chlorophyll fluorescence parameters under different P conditions

NP\LP	Fv/Fm	ΦPSII	qP	NPQ
Fv/Fm	1	−0.0502	−0.0071	0.151*
ΦPSII	0.0043	1	−0.1253	0.3205***
qP	−0.0197	−0.0156	1	−0.3093***
NPQ	0.07	−0.5368***	−0.1609*	1

NP normal P condition, *LP* low P condition

*Significant at $P < 0.05$

**Significant at $P < 0.01$

***Significant at $P < 0.001$

130 kb around the significant SNPs that were used as the QTL in regulating photosynthesis under P deficiency stress. Further analysis showed that the 52 SNPs were involved 12 regions in total. Among the 12 regions, three regions were significantly detected in more than two experiments. Two (*qPNPQ18-2* and *qPQP18-3*) were on chromosome 18, and one (*qPQP20-1*) was on chromosome 20. The QTLs *qPNPQ18-2* and *qPQP18-3* which included 9 significant SNPs extended 384 kb and 668 kb, respectively, and overlapped with three reported QTLs *Leaf carotenoid content 1-g13.1* (Bandillo et al. 2015), *Leaf carotenoid content 1-g13.6* (Bandillo et al. 2015), and *q18-2*. The *q18-2* was reported to be related to photosynthesis under low P condition (Lü et al. 2018). The *qPQP20-1* including 10 significant SNPs extended 303 kb, was overlapped with two reported QTLs *Seed protein 3-g11* and *Seed oil 4-g8* (Bandillo et al. 2015). Furthermore, *qPNPQ11-1* and *qPPSII12* were overlapped with the reported P efficiency QTLs (Lü et al. 2018; Yang et al. 2020); *qPPSII9*, *qPF12* and *qPNPQ18-1* were overlapped with the reported QTLs that were related to the quality of soybean seeds and so on. Other four QTLs (*qPF7*, *qPNPQ11-2*, *qPPSII16* and *qPF20-2*) were novel loci for P efficiency. These results showed that the QTLs

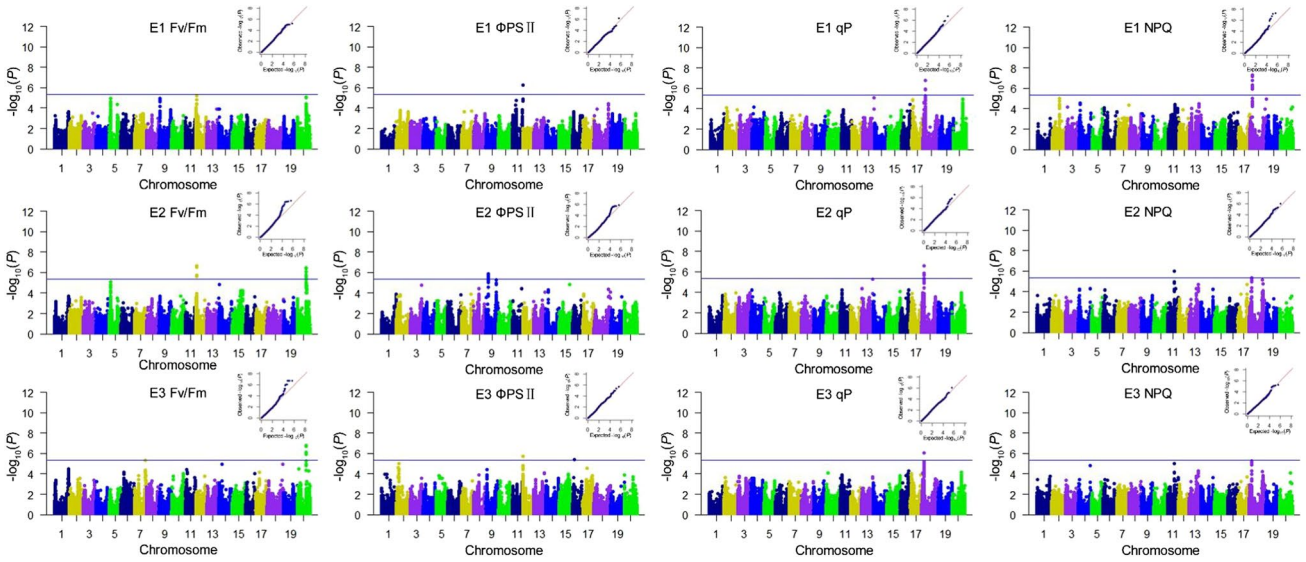

Fig. 2 Manhattan plots and quantile–quantile plots of the GWAS results for the ratio of four chlorophyll fluorescence parameters under different phosphorus levels (low P/normal P) in 219 soybean accessions (MLM, Q + K, P < 4.82 × 10–6). E1, E2 and E3 represent the three environments, respectively; Fv/Fm, maximum photochemical efficiency of photosystem II; ΦPSII, actual yield of photosystem II; qP, photochemical quenching coefficient; NPQ, non-photochemical quenching coefficient

significantly associated with P deficiency stress might also be related to other traits.

Differentially expressed genes within the associated QTLs for P efficiency

To identify candidate genes among these QTLs, the low P resistant soybean line (W82) and low P sensitive line (Jack) were selected, and RNA-sequence analysis of leaves under different P levels were performed. The results are shown in Fig. 3. Totally, 90 genes in W82 were significantly upregulated and 70 genes were significantly downregulated in low P vs normal P comparison group, while in Jack, 232 genes were significantly upregulated and 80 genes were significantly downregulated, respectively. There were 142 fewer genes upregulated and 10 fewer genes downregulated in W82 than in Jack when the plants suffered P deficiency stress. These DEGs were classified in four main categories as follows: (1) the DEGs (73 downregulated and 221 upregulated) only in low P vs normal P comparison group of Jack; (2) the DEGs (57 downregulated and 85 upregulated) only in low P vs normal P comparison group of W82; (3) the DEGs (6 downregulated and 4 upregulated) both significantly differential expressing in low P vs normal P comparison group of W82 and Jack; (4) the DEGs (7 and 1 genes) which had the opposite mode of expression in low P vs normal P comparison group. These data suggested that different soybean materials were involved in different genes in response to P deficiency stress.

To further determine the candidate genes, we specifically focused on the DEGs that located in QTLs identified by GWAS. Notably, there were three DEGs located in the three stable major QTLs (*qPNPQ18-2*, *qPNPQ18-3* and *qPQP20-1*). One was *Glyma.18g092900*, one was *Glyma.18g098800* and another was *Glyma.20g089400*. These three genes were took as candidate genes of photosynthetic efficiency under P deficiency stress.

Functional prediction of candidate genes

To further understand the function of these genes, the soybean public database (https://www.soybase.org) was searched based on the annotation of the soybean reference genome W82.a2.v1. BLASTP were took against *Arabidopsis* genome according to soybean genome annotation information to annotate the candidate genes (Table 4). Among the three genes, *Glyma.18g092900* (in *qPNPQ18-2*) was annotated to encode a mannose-1-phosphate guanylyltransferases (GDP) and mainly involved in the glucose metabolic process in the cytoplasm, which was homologous to *VCT2* in *Arabidopsis*. In plants, *VCTs* have been proven to be implicated in many processes including programmed cell death (De Pinto et al. 2006), plant growth (Pignocchi and Foyer 2003) and protection against environmental stresses like UV radiation (Gao and Zhang 2008), high light intensity (Müller-Moulé et al. 2004) and high temperatures (Larkindale et al. 2005). The *vtc2vtc5* double-mutant seedlings stopped growing after the initial expansion of the cotyledons, then bleached and could not photosynthesize (Dowdle et al. 2007).

Table 3 Details of loci associated with the ratio of four chlorophyll fluorescence parameters under different phosphorus levels via GWAS in soybean

Name[a]	Chr[b]	Main SNP position[c]	No.of SNP[d]	*P* value	R2 (%)[e]	Significant region (bp)	QTLs	References	Env-trait[f]
qPF7	7	40597356	1	4.78E−06	14.23	40467356–40727356	–	–	E3-Fv/Fm
qPPSII9	9	7947277	11	1.28E−06	13.9	7774328–8137828	*Seed Ala 2-g3*	Li et al. (2018)	E2-ΦPSII
qPNPQ11-1	11	24275784	1	9.65E−07	16.92	24145784–24405784	*q11-2*	Li et al. (2016)	E2-NPQ
qPNPQ11-2	11	39146659	1	5.98E−07	15.6	39016659–39276659	–	–	E1-ΦPSII
qPPSII12	12	1469028	1	1.91E−06	11.02	1339028–1599028	*q12*; *qP12-1*	Lv et al. (2018); Yang et al. (2020)	E3-ΦPSII
qPF12	12	2168167	5	2.26E−07	11.09	1985346–2302830	*Ureide content 1-g31.1; Ureide content 1-g31.2*	Ray et al. (2015)	E2-Fv/Fm
qPPSII16	16	6469551	1	3.68E−06	10.34	6339551–6599551	–	–	E3-ΦPSII
qPNPQ18-1	18	4696773	1	2.86E−06	10.84	4566773–4826773	*Seed linolenic 4-g8; Seed oil 3-g7*	Li et al. (2015); Priolli et al. (2015)	E3-NPQ
qPNPQ18-2	18	9304791	8	4.94E−08	16.08	9170102–9554067	*Leaf carotenoid content 1-g13.1; q18-2*	Bandillo et al. (2015; Lv et al. (2018)	E1-NPQ; E2-NPQ
qPQq18-3	18	9836035	6	2.53E−07	15.45	9631929–10300106	*Leaf carotenoid content 1-g13.6; q18-2*	Bandillo et al. (2015); Lv et al. (2018)	E1-qP; E2-qP; E3-qP
qPQP20-1	20	33172755	10	1.64E−07	17.58	33001835–33305196	Seed protein 3-g11; Seed *oil 4-g8*	Bandillo et al. (2015)	E2-Fv/Fm; E3-Fv/Fm
qPF20-2	20	33732837	6	4.17E−07	12.78	33598169–33873189	–	–	E2-Fv/Fm

[a]QTL named by chromosome

[b]Chromosome

[c]Most significant SNP position

[d]Number of significant SNPs

[e]The proportion of phenotypic variance explained by each QTL

[f]Environment and trait

For the other candidate genes, *Glyma.18g098800* (in *qPNPQ18-3*) was annotated heat shock protein 70, which was homologous to *HSP70-16* in Arabidopsis. A lot of studies had shown that *HSP70s* was important for the response to heat or other stress conditions. *HSP70-6/7* was essential for chloroplast development (Latijnhouwers et al. 2010). *HSP70-14/15* was associated with plant growth and stomatal opening and closing, which could directly affect the photosynthesis (Jungkunz et al. 2011). *Glyma.20g089400* (in *qPQP20-1*) was annotated proteasome component (PCI) domain protein, which could regulate development and signal transduction (Kim et al. 2001). There are three known PCI complexes: the regulatory lid of the 26S proteasome, the COP9 signalosome and the translation initiation factor eIF3. The proteasomes had been reported to play key roles not only in plants (Yu et al. 2020), but also in human (Shi et al. 2021) and animals (Lillethorup et al. 2018). This indicates that the three candidate genes may directly affect the regulation of photosynthesis under low P conditions through different physiological and biochemical processes.

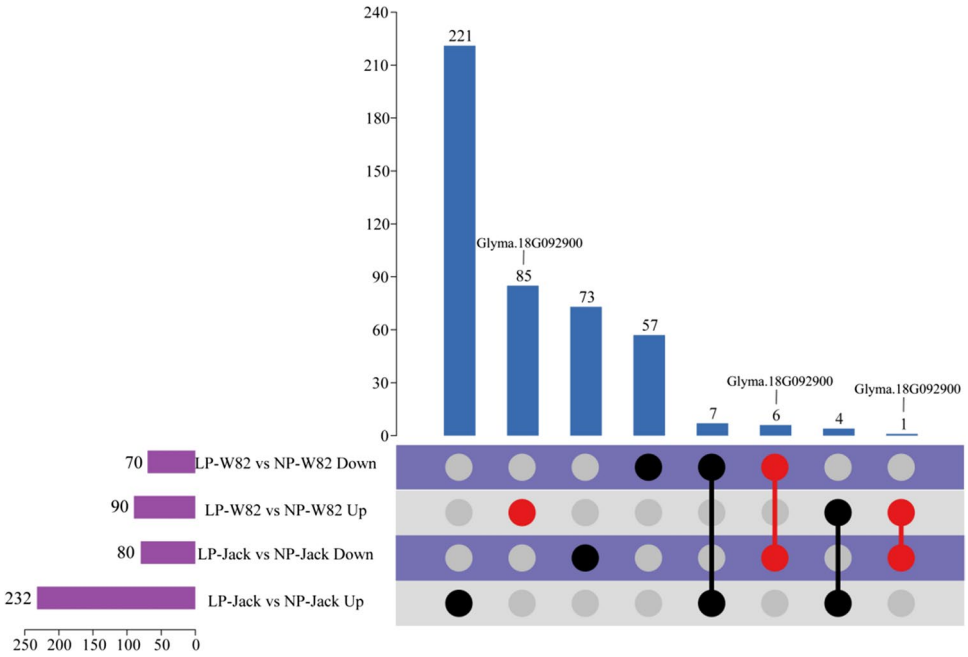

Fig. 3 DEGs identified by RAN-seq and distributed of unique and shared between W82 and Jack. The single black circle represent individually DEGs; the black circles connected by a black bar represent common DEGs; the red circles represent the groups candidate genes located; leftmost horizontal bars indicate the total number of DGEs for each type. LP, low P condition; NP, normal P condition. DEGs were classified into four main categories as follows: (1) the DEGs (73 downregulated and 221 upregulated) only in low P vs normal P comparison group of Jack; (2) the DEGs (57 downregulated and 85 upregulated) only in low P vs normal P comparison group of W82; (3) the DEGs (6 downregulated and 4 upregulated) both significantly differential expressing in low P vs normal P comparison group of W82 and Jack; (4) the DEGs (7 and 1 genes) which had the opposite mode of expression in low P vs normal P comparison group

Table 4 Putative genes associated with resistance to low phosphorus

QTL name	Candidate genes	W82 LP vs NP	Jack LP vs NP	Gene orthologs in Arabidopsis	Annotations
qPNPQ18-2	*Glyma.18g092900*	Up	Up	*AT4G26850* (*VTC2*)	Mannose-1-phosphate guanyltransferase (GDP)s
qPQq18-3	*Glyma.18g098800*	Down	Down	*AT1G11660* (*HSP70-16*)	Heat shock protein 70
qPQP20-1	*Glyma.20g089400*	Up	Down	*AT5G15610*	Proteasome component (PCI)

LP low P condition, *NP* normal P condition

Expression analysis of candidate genes

To understand the expression patterns of candidate genes in different soybean tissues, the RNA-seq data was downloaded from the Soybase database (https://www.soybase.org) and used for expressing analysis. The three genes expressed in all tissues and had high expression levels in roots and leaves (Fig. 4a). Then, the expression in leaves of W82 and Jack under different P levels were analysed by qRT-PCR (Fig. 4b). In leaves of W82, two candidate genes were upregulated by low P, and *Glyma.20G089400* was the most up-regulated expression. However, in leaves of Jack, *Glyma.18G092900* was upregulated, *Glyma.18G098800* and *Glyma.20G089400* were significantly downregulated. The data indicated that gene expressions were different for different genotypes soybean under P deficiency stress. The differences in the expression of these genes may be the cause of the different tolerance to P deficiency stress.

Discussion

As one of the main nutrient element, P can directly participate in carbon assimilation and photosynthetic phosphorylation of photosynthesis (Balemi and Negisho 2012). Therefore, photosynthesis is easily affected by P deficiency stress (Yang et al. 2020). Photosynthetic reduction of plants under low P availability is mainly through affecting photosynthetic electron transfer, ATP production, carbon dioxide fixation enzyme activity, etc. Chlorophyll fluorescence parameters

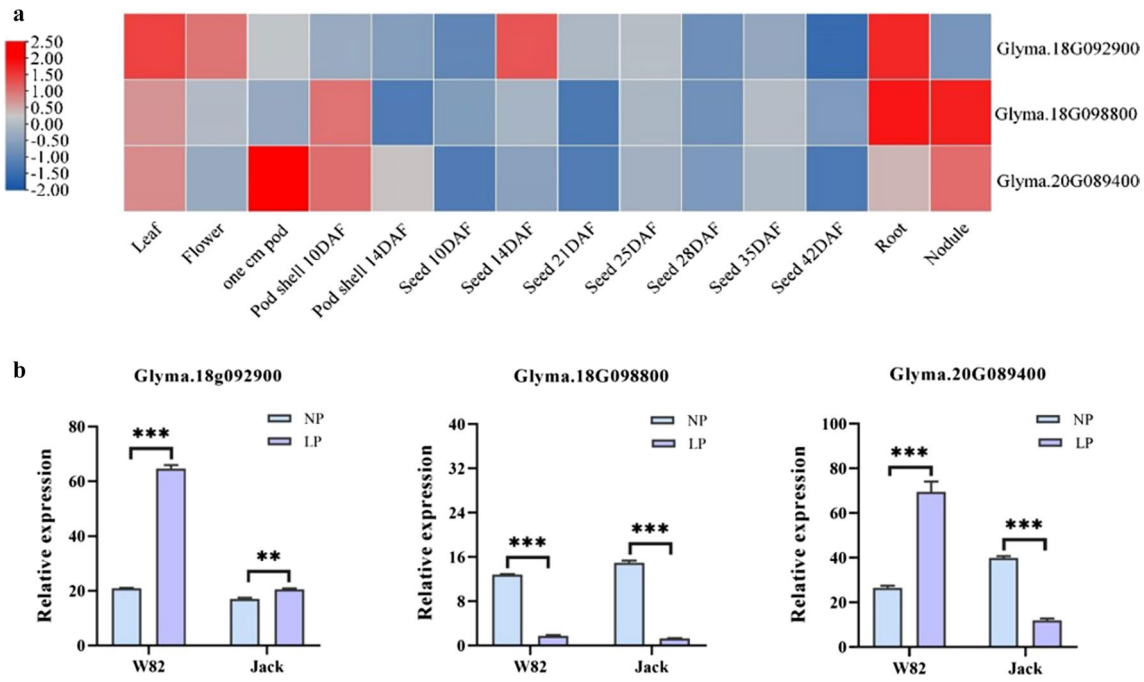

Fig. 4 Expression analysis of the three putative genes. **a** Digital expression levels of three genes in different tissues at different stages based on RNA-seq data. The values used in heatmaps from the microarray data were log2-transformed. **b** Expression analysis of the three putative genes in leaves of W82 and Jack under normal P and low conditions. *, ** and *** indicate significance at the 0.05, 0.01 and 0.001 levels; NP, normal P concentration; LP, low P concentration

are effective indicators of studying the effects of different environmental conditions on plant photosynthesis, which can reflect the process of light energy absorption, utilization, transmission and dissipation of PSI and PSII (Schreiber et al. 1995). Studies have shown that there is an important genetic relationship between photosynthesis and P efficiency (Li et al. 2016). In this study, GWAS were performed with the ratio of main chlorophyll fluorescence parameters in low P and normal P conditions and 12 QTLs were found in three experiments, which explained 10.34–16.92% of the phenotypic variation, respectively.

Novel loci for P efficiency

Cultivating low-P-tolerant soybean varieties and identifying new P efficiency locus and genes are necessary for improving P assimilation efficiency. Despite the widespread use of chlorophyll fluorescence parameters to measure the impact of abiotic stress factors, assessing genetic variation and/or mapping of genetic loci associated with chlorophyll fluorescence phenotypes in soybean were rather limited. We identified 52 SNPs that were significantly associated with P efficiency by chlorophyll fluorescence parameters. The 52 SNPs involved 12 regions, of which five regions were each only associated with a single SNP, other seven regions were each associated with more than five SNPs, respectively. Notably, eight regions were novel QTLs for P efficiency. These novel QTLs can provide a reference for the analysis of the regulatory mechanism of soybean P efficiency.

In addition, several important QTLs identified coincided with the reported QTLs for P efficiency or other traits. For example, the two QTLs on chromosome 18 detected in two or three separate experiments, all overlapped with *q18-2* (Lü et al. 2018), but our results divided this large interval into two small intervals, which can provide reference for fine mapping. On chromosome 20, *qPQP20-1* that was detected in E2 and E3 was overlapped with the *Seed protein 3-g11* and *Seed oil 4-g8* (Bandillo et al. 2015), which implied that *qPQP20-1* was a pleiotropic locus not only related to P efficiency, but also related to soybean seed protein and oil content. However, only 12 QTLs were identified in the three environments and 3 QTLs were identified repeatedly, which may due to the difference of seeds used in each experiment on the one hand and the factors that affecting photosynthesis were complicated on the other hand. More experiments were needed in different environments in the future.

GWAS combined with RNA-seq for candidate gene prediction

Although GWAS has been widely used to identify important candidate genes related to abiotic stress in plants, some problems still exist for finding potentially key genes, such as strong linkage disequilibrium (LD) and the

long LD decay distance, etc. (Hyten et al. 2007). RNA-seq has become an effective technology to detect gene expression at the whole genome level (Tai et al. 2016). However, a large amount of DEGs is usually obtained, which made it difficult to identify potential key candidate genes. Recently, combined GWAS with RNA-seq has been used to predict candidate genes in maize (Zhang et al. 2020), peanut (Zhang et al. 2021) and others. In our study, although many differentially expressed genes were identified by RNA-seq, the combination of GWAS greatly narrowed the target range, with only three genes to be considered as potential key candidate genes. Among the three genes, *Glyma.18G098800* and *Glyma.20G089400* were found to have different expression levels under low P in resistant and sensitive soybean lines; *Glyma.18g092900* was significantly up-regulated in both resistant and sensitive soybean lines after being induced by low P, but it was more than 2 times higher in resistant lines, so these genes were attractive candidate genes. This further indicated the reliability of the selected candidate genes.

In the present study, we contributed to understand the genetic control of P efficiency by chlorophyll fluorescence parameters of 219 soybean lines. Through GWAS, RNA-seq analysis, and validation via qRT-PCR, 12 QTLs were identified, and three candidate genes were found to have significantly different gene expression levels under low-P treatment in resistant and sensitive soybean lines. Further studies on the functions of these genes will help to elucidate the regulation mechanism of low P tolerance and high photosynthesis efficiency of soybean.

Author contribution statement DZ and YY designed the experiments. YY, RC, XZ and HL carried out the experiments. YY analyzed the data and wrote the manuscript. DZ, HC, JW and RW revised the manuscript. All authors read and approved the final manuscript.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00425-021-03760-8.

Acknowledgements This work was supported by National Natural Science Foundation of China (32072088), the Key Scientific Research Projects of Higher Education Institutions in Henan Province (20A210017), and the Henan agricultural university science and technology innovation fund (KJCX2019C02).

Data Availability The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Conflict of interests The authors declare no competing financial interests.

References

Abbas G, Chen Y, Khan FY, Feng Y, Palta JA, Siddique KH (2018) Salinity and low phosphorus differentially affect shoot and root traits in two wheat cultivars with contrasting tolerance to salt. Agronomy 8(8):155. https://doi.org/10.3390/agronomy8080155

Baker N (2008) Chlorophyll fluorescence: a probe of photosynthesis in vivo. Annu Rev Plant Biol 59:89–113. https://doi.org/10.1146/annurev.arplant.59.032607.092759

Balemi T, Negisho K (2012) Management of soil phosphorus and plant adaptation mechanisms to phosphorus stress for sustainable crop production: a review. J Soil Sci Plant Nutr 12(3):547–562. https://doi.org/10.4067/S0718-95162012005000015

Bandillo N, Jarquin D, Song Q, Nelson RL, Cregan P, Specht J, Lorenz A (2015) A population structure and genome-wide association analysis on the USDA soybean germplasm collection. Plant Genome. https://doi.org/10.3835/plantgenome2015.04.0024

Bassham JA (1977) Increasing crop production through more controlled photosynthesis. Science 197(4304):630–638. https://doi.org/10.1126/science.197.4304.630

Bertholdsson N-O, Holefors A, Macaulay M, Crespo-Herrera L (2015) QTL for chlorophyll fluorescence of barley plants grown at low oxygen concentration in hydroponics to simulate waterlogging. Euphytica 201(3):357–365. https://doi.org/10.1007/s10681-014-1215-0

Ceasar S, Ramakrishnan M, Vinod K, Roch G, Upadhyaya H, Baker A, Ignacimuthu S (2020) Phenotypic responses of foxtail millet (*Setaria italica*) genotypes to phosphate supply under greenhouse and natural field conditions. PLoS ONE 15(6):e0233896. https://doi.org/10.1371/journal.pone.0233896

Cetner M, Kalaji H, Goltsev V, Aleksandrov V, Kowalczyk K, Borucki W, Jajoo A (2017) Effects of nitrogen-deficiency on efficiency of light-harvesting apparatus in radish. Plant Physiol Biochem 119:81–92. https://doi.org/10.1016/j.plaphy.2017.08.016

Cong W, Suriyagoda L, Lambers H (2020) Tightening the phosphorus cycle through phosphorus-efficient crop genotypes. Trends Plant Sci 25(10):967–975. https://doi.org/10.1016/j.tplants.2020.04.013

Dąbrowski P, Baczewska A, Pawluśkiewicz B, Paunov M, Alexantrov V, Goltsev V, Kalaji M (2016) Prompt chlorophyll a fluorescence as a rapid tool for diagnostic changes in PSII structure inhibited by salt stress in Perennial ryegrass. J Photochem Photobiol B: Biolo 157:22–31. https://doi.org/10.1016/j.jphotobiol.2016.02.001

De Pinto MC, Paradiso A, Leonetti P, De Gara L (2006) Hydrogen peroxide, nitric oxide and cytosolic ascorbate peroxidase at the crossroad between defence and cell death. Plant J 48(5):784–795. https://doi.org/10.1111/j.1365-313X.2006.02919.x

Dowdle J, Ishikawa T, Gatzek S, Rolinski S, Smirnoff N (2007) Two genes in *Arabidopsis thaliana* encoding GDP-L-galactose phosphorylase are required for ascorbate biosynthesis and seedling viability. Plant J 52(4):673–689. https://doi.org/10.1111/j.1365-313X.2007.03266.x

Frydenvang J, van Maarschalkerweerd M, Carstensen A, Mundus S, Schmidt SB, Pedas PR, Laursen KH, Schjoerring JK, Husted S (2015) Sensitive detection of phosphorus deficiency in plants using chlorophyll a fluorescence. Plant Physiol 169(1):353–361. https://doi.org/10.1104/pp.15.00823

Galindo-Castañeda T, Brown K, Lynch J (2018) Reduced root cortical burden improves growth and grain yield under low phosphorus availability in maize. Plant Cell Environ 41(7):1579–1592. https://doi.org/10.1111/pce.13197

Gao Q, Zhang L (2008) Ultraviolet-B-induced oxidative stress and antioxidant defense system responses in ascorbate-deficient *vtc1* mutants of *Arabidopsis thaliana*. J Plant Physiol 165(2):138–148. https://doi.org/10.1016/j.jplph.2007.04.002

Hamdani S, Wang H, Zheng G, Perveen S, Qu M, Khan N, Khan W, Jiang J, Li M, Liu X, Zhu X, Govindjee ChuC, Zhu X (2019) Genome-wide association study identifies variation of glucosidase being linked to natural variation of the maximal quantum yield of photosystem II. Physiol Plant 166(1):105–119. https://doi.org/10.1111/ppl.12957

Hyten D, Choi I, Song Q, Shoemaker R, Nelson R, Costa J, Specht J, Cregan P (2007) Highly variable patterns of linkage disequilibrium in multiple soybean populations. Genetics 175(4):1937–1944. https://doi.org/10.1534/genetics.106.069740

i Azam F, Chang X, Jing R (2015) Mapping QTL for chlorophyll fluorescence kinetics parameters at seedling stage as indicators of heat tolerance in wheat. Euphytica 202(2):245–258. https://doi.org/10.1007/s10681-014-1283-1

Jin J, Lauricella D, Armstrong R, Sale P, Tang C (2014) Phosphorus application and elevated CO_2 enhance drought tolerance in field pea grown in a phosphorus-deficient vertisol. Ann Bot 116(6):975–985. https://doi.org/10.1093/aob/mcu209

Jungkunz I, Link K, Vogel F, Voll L, Sonnewald S, Sonnewald U (2011) AtHsp70-15-deficient Arabidopsis plants are characterized by reduced growth, a constitutive cytosolic protein response and enhanced resistance to TuMV. nPlant J 66(6):983–995. https://doi.org/10.1111/j.1365-313X.2011.04558.x

Kalaji HM, Jajoo A, Oukarroum A, Brestic M, Zivcak M, Samborska IA, Cetner MD, Łukasik I, Goltsev V, Ladle RJ (2016) Chlorophyll a fluorescence as a tool to monitor physiological status of plants under abiotic stress conditions. Acta Physiol Plant 38(4):102. https://doi.org/10.1007/s11738-016-2113-y

Kim T, Hofmann K, von Arnim A, Chamovitz D (2001) PCI complexes: pretty complex interactions in diverse signaling pathways. Trends Plant Sci 6(8):379–386. https://doi.org/10.1016/s1360-1385(01)02015-5

Knapp SJ, Stroup WW, Ross WM (1985) Exact confidence intervals for heritability on a progeny mean basis. Crop Sci 25(1):192–194. https://doi.org/10.2135/cropsci1985.0011183X002500010046x

Kosmala A, Perlikowski D, Pawłowicz I, Rapacz M (2012) Changes in the chloroplast proteome following water deficit and subsequent watering in a high- and a low-drought-tolerant genotype of *Festuca arundinacea*. J Exp Bot 63(17):6161–6172. https://doi.org/10.1093/jxb/ers265

Larkindale J, Hall JD, Knight MR, Vierling E (2005) Heat stress phenotypes of Arabidopsis mutants implicate multiple signaling pathways in the acquisition of thermotolerance. Plant Physiol 138(2):882–897. https://doi.org/10.1104/pp.105.062257

Latijnhouwers M, Xu X-M, Møller SG (2010) Arabidopsis stromal 70-kDa heat shock proteins are essential for chloroplast development. Planta 232(3):567–578. https://doi.org/10.1007/s00425-010-1192-z

Li H, Yang Y, Zhang H, Chu S, Zhang X, Yin D, Yu D, Zhang D (2016) A genetic relationship between phosphorus efficiency and photosynthetic traits in soybean as revealed by QTL analysis using a high-density genetic map. Front Plant Sci 7:924. https://doi.org/10.3389/fpls.2016.00924

Li X, Tian R, Kamala S, Du H, Li W, Kong Y, Zhang C (2018) Identification and verification of pleiotropic QTL controlling multiple amino acid contents in soybean seed. Euphytica 214(6):1–14. https://doi.org/10.1007/s10681-018-2170-y

Li Y, Reif J, Ma Y, Hong H, Liu Z, Chang R, Qiu L (2015) Targeted association mapping demonstrating the complex molecular genetics of fatty acid formation in soybean. BMC Genom 16(1):1–13. https://doi.org/10.1186/s12864-015-2049-4

Lillethorup TP, Glud AN, Alstrup AKO, Noer O, Nielsen EHT, Schacht AC, Landeck N, Kirik D, Orlowski D, Sørensen JCH, Doudet DJ, Landau A (2018) Longitudinal monoaminergic PET imaging of chronic proteasome inhibition in minipigs. Sci Rep 8(1):1–13. https://doi.org/10.1038/s41598-018-34084-5

Lipka AE, Tian F, Wang Q, Peiffer J, Li M, Bradbury PJ, Gore MA, Buckler ES, Zhang Z (2012) GAPIT: Genome association and prediction integrated tool. Bioinformatics 28(18):2397–2399. https://doi.org/10.1093/bioinformatics/bts444

Liu S, Zhang M, Feng F, Tian Z (2020) Toward a "Green Revolution" for soybean. Mol Plant 13(5):688–697. https://doi.org/10.1016/j.molp.2020.03.002

Lü H, Yang Y, Li H, Liu Q, Zhang J, Yin J, Chu S, Zhang X, Yu K, Lv L, Chen X, Zhang D (2018) Genome-wide association studies of photosynthetic traits related to phosphorus efficiency in soybean. Front Plant Sci 9:1226. https://doi.org/10.3389/fpls.2018.01226

Lv H, Yang Y, Li H, Liu Q, Zhang J, Yin J, Chu S, Zhang X, Yu K, Lv L, Chen X, Zhang D (2018) Genome-wide association studies of photosynthetic traits related to phosphorus efficiency in soybean. Frontiers in plant science 9:1226. https://doi.org/10.3389/fpls.2018.01226

Mallarino A, Jones J, Thompson L, Pecinovsky K (2018) Corn and soybean grain yield response to different phosphorus fertilization rates and soil-test phosphorus levels. Iowa State University Research and Demonstration Farms Progress Reports 2017(1)

Maxwell K, Johnson GN (2000) Chlorophyll fluorescence—a practical guide. J Exp Bot 51(345):659–668. https://doi.org/10.1093/jexbot/51.345.659

Müller-Moulé P, Golan T, Niyogi KK (2004) Ascorbate-deficient mutants of Arabidopsis grow in Hhigh light despite chronic photooxidative stress. Plant Physiol 134(3):1163–1172. https://doi.org/10.1104/pp.103.032375

Munekage Y, Hojo M, Meurer J, Endo T, Tasaka M, Shikanai T (2002) *PGR5* is involved in cyclic electron flow around photosystem I and is essential for photoprotection in *Arabidopsis*. Cell 110(3):361–371. https://doi.org/10.1016/s0092-8674(02)00867-x

Pedersen O, Colmer TD, Garcia-Robledo E, Revsbech NP (2018) CO_2 and O_2 dynamics in leaves of aquatic plants with C3 or CAM photosynthesis – application of a novel CO_2 microsensor. Ann Bot 122(4):605–615. https://doi.org/10.1093/aob/mcy095

Pignocchi C, Foyer CH (2003) Apoplastic ascorbate metabolism and its role in the regulation of cell signalling. Curr Opin Plant Biol 6(4):379–389. https://doi.org/10.1016/s1369-5266(03)00069-4

Prasad B, Carver BF, Stone ML, Babar MA, Raun WR, Klatt AR (2007) Genetic analysis of indirect selection for winter wheat grain yield using spectral reflectance indices. Crop Sci 47(4):1416–1425. https://doi.org/10.2135/cropsci2006.08.0546

Priolli R, Campos B, Stabellini N, Pinheiro J, Vello N (2015) Association mapping of oil content and fatty acid components in soybean. Euphytica 203(1):83–96. https://doi.org/10.1007/s10681-014-1264-4

Qian X, Zhang Y, Lun DS, Dismukes GC (2018) Rerouting of metabolism into desired cellular products by nutrient stress: fluxes reveal the selected pathways in cyanobacterial photosynthesis. ACS Synthetic Biol 7(5):1465–1476. https://doi.org/10.1021/acssynbio.8b00116

Rao IM, Terry N (1989) Leaf phosphate status, photosynthesis, and carbon partitioning in sugar beet: I. Changes in growth, gas exchange, and Calvin cycle enzymes. Plant Physiol 90(3):814–819. https://doi.org/10.1104/pp.90.3.814

Rapacz M, Sasal M, Kalaji HM, Kościelniak J (2015) Is the OJIP test a reliable indicator of winter hardiness and freezing tolerance of common wheat and triticale under variable winter environments? PLoS ONE 10(7):e0134820. https://doi.org/10.1371/journal.pone.0134820

Ray J, Dhanapal A, Singh S, Hoyos-Villegas V, Smith J, Purcell L, King C, Boykin D, Cregan P, Song Q, Fritschi F (2015) Genome-wide association study of ureide concentration in diverse maturity group IV soybean [Glycine max (L.) Merr.] accessions. G3: Genes, Genomes, Genetics 5(11):2391–2403. https://doi.org/10.1534/g3.115.021774

Samborska I, Kalaji H, Sieczko L, Goltsev V, Borucki W, Jajoo A (2018) Structural and functional disorder in the photosynthetic apparatus of radish plants under magnesium deficiency. Funct Plant Biol 45(6):668–679. https://doi.org/10.1071/FP17241

Sayed O (2003) Chlorophyll fluorescence as a tool in cereal crop research. Photosynthetica 41(3):321–330. https://doi.org/10.1023/B:PHOT.0000015454.36367.e2

Schreiber U, Bilger W, Neubauer C (1995) Chlorophyll fluorescence as a nonintrusive indicator for rapid assessment of in vivo photosynthesis. In: Schulze E-D, Caldwell MM (eds) Ecophysiology of photosynthesis. Springer, Berlin, pp 49–70

Shi L, Liu B, Shen D, Yan P, Zhang Y, Tian Y, Hou L, Jiang G, Zhu Y, Liang Y, Liang X, Shen B, Yu H, Zhang Y, Wang Y, Guo X, Cai X (2021) A tumor-suppressive circular RNA mediates uncanonical integrin degradation by the proteasome in liver cancer. Sci Adv. https://doi.org/10.1126/sciadv.abe5043

Tai H, Lu X, Opitz N, Marcon C, Paschold A, Lithio A, Nettleton D, Hochholdinger F (2016) Transcriptomic and anatomical complexity of primary, seminal, and crown roots highlight root type-specific functional diversity in maize (*Zea mays* L.). J Exp Bot 67(4):1123–1135. https://doi.org/10.1093/jxb/erv513

Team R (2015) RStudio: integrated development for R. RStudio Inc., Boston

Turner SD (2014) qqman: an R package for visualizing GWAS results using QQ and manhattan plots. Biorxiv. https://doi.org/10.1101/005165

VanRaden PM (2008) Efficient methods to compute genomic predictions. J Dairy Sci 91(11):4414–4423. https://doi.org/10.3168/jds.2007-0980

Veneklaas E, Lambers H, Bragg J, Finnegan P, Lovelock C, Plaxton W, Price C, Scheible W, Shane M, White P, Raven J (2012) Opportunities for improving phosphorus-use efficiency in crop plants. New Phytol 195(2):306–320. https://doi.org/10.1111/j.1469-8137.2012.04190.x

Wang J, Chu S, Zhang H, Zhu Y, Cheng H, Yu D (2016) Development and application of a novel genome-wide SNP array reveals domestication history in soybean. Sci Rep 6(1):20728. https://doi.org/10.1038/srep20728

Wang J, Chen Y, Wang P, Li Y, Wang G, Liu P, Khan A (2018) Leaf gas exchange, phosphorus uptake, growth and yield responses of cotton cultivars to different phosphorus rates. Photosynthetica 56(4):1414–1421. https://doi.org/10.1007/s11099-018-0845-1

Wissuwa M, Ae N (2001) Genotypic variation for tolerance to phosphorus deficiency in rice and the potential for its exploitation in rice improvement. Plant Breed 120(1):43–48. https://doi.org/10.1046/j.1439-0523.2001.00561.x

Yan N, Zhang Y-L, Xue H-M, Zhang X-H, Wang Z-D, Shi L-Y, Guo D-P (2015) Changes in plant growth and photosynthetic performance of *Zizania latifolia* exposed to different phosphorus concentrations under hydroponic condition. Photosynthetica 53(4):630–635. https://doi.org/10.1007/s11099-015-0149-7

Yang N, Lu Y, Yang X, Huang J, Zhou Y, Ali F, Wen W, Liu J, Li J, Yan J (2014) Genome wide association studies using a new nonparametric model reveal the genetic architecture of 17 agronomic traits in an enlarged maize association panel. PLoS Genet 10(9):e1004573. https://doi.org/10.1371/journal.pgen.1004573

Yang Y, Wang L, Zhang D, Cheng H, Wang Q, Yang H, Yu D (2020) GWAS identifies two novel loci for photosynthetic traits related to phosphorus efficiency in soybean. Mol Breed 40(3):29. https://doi.org/10.1007/s11032-020-01112-0

Yu F, Cao X, Liu G, Wang Q, Xia R, Zhang X, Xie Q (2020) ESCRT-I component VPS23A is targeted by E3 ubiquitin ligase XBAT35 for proteasome-mediated degradation in modulating ABA signaling. Mol Plant 13(11):1556–1569. https://doi.org/10.1016/j.molp.2020.09.008

Zhang Z, Ersoz E, Lai C-Q, Todhunter RJ, Tiwari HK, Gore MA, Bradbury PJ, Yu J, Arnett DK, Ordovas JM, Buckler ES (2010) Mixed linear model approach adapted for genome-wide association studies. Nat Genet 42(4):355–360. https://doi.org/10.1038/ng.546

Zhang D, Li H, Wang J, Zhang H, Hu Z, Chu S, Lv H, Yu D (2016) High-density genetic mapping identifies new major loci for tolerance to low-phosphorus stress in soybean. Front Plant Sci 7:372. https://doi.org/10.3389/fpls.2016.00372

Zhang H, Zhang J, Xu Q, Wang D, Di H, Huang J, Yang X, Wang Z, Zhang L, Dong L, Wang Z, Zhou Y (2020) Identification of candidate tolerance genes to low-temperature during maize germination by GWAS and RNA-seq approaches. BMC Plant Biol 20:333. https://doi.org/10.1186/s12870-020-02543-9

Zhang H, Wang ML, Dang P, Jiang T, Zhao S, Lamb M, Chen C (2021) Identification of potential QTLs and genes associated with seed composition traits in peanut (*Arachis hypogaea* L.) using GWAS and RNA-Seq analysis. Gene 769:145215. https://doi.org/10.1016/j.gene.2020.145215

Zhu J (1995) Analysis of conditional genetic effects and variance components in developmental genetics. Genetics 141(4):1633–1639. https://doi.org/10.1093/genetics/141.4.1633

Zhu Z, Zhang F, Hu H, Bakshi A, Robinson MR, Powell JE, Montgomery GW, Goddard ME, Wray NR, Visscher PM, Yang J (2016) Integration of summary data from GWAS and eQTL studies predicts complex trait gene targets. Nat Genet 48(5):481–487. https://doi.org/10.1038/ng.3538