



GWAS reveals two novel loci for photosynthesis-related traits in soybean

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Abstract

Photosynthesis plays an extremely important role throughout the life cycle of plants. Improving the photosynthetic rate is a major target for increasing crop productivity. This study was conducted to identify single nucleotide polymorphisms (SNPs) associated with the net photosynthetic rate (Pn), stomatal conductance (Cond), intercellular carbon dioxide concentration (Ci) and transpiration rate (Trmmol) through genome-wide association study (GWAS) and to inspect the relationships among these traits in soybean (*Glycine max* (L.) Merr.). A population of 219 soybean accessions was used in this research. A total of 12 quantitative trait loci (QTLs) associated with Pn, Cond, Ci and Trmmol were detected and distributed on chromosomes 1, 2, 6, 7, 9, 11, 12, 13, 15, 16, 18, and 19, and some of these QTL overlapped with previously reported QTLs. Furthermore, four candidate genes were identified, and there were significantly different expression levels between the high-light-efficiency accessions and low-light-efficiency accessions. These putative genes may participate in the regulation of photosynthesis through different metabolic pathways. Therefore, the associated novel QTLs and candidate genes detected in this study will provide a theoretical basis for genetic studies of photosynthesis and provide new avenues for crop improvement.

Keywords Photosynthesis · GWAS · SNPs · Candidate gene · Soybean

Introduction

Photosynthesis is the basis of plant survival. Crop yield relies on photosynthesis. However, in the past 50 years, a significant proportion of the global population has experienced malnutrition due to a lack of access to sufficient food (Long et al. 2015). As the population continues to increase and the area of arable land decreases, food production may

not meet demand. It is expected that the world will experience a food shortage crisis by 2050 (Tilman et al. 2011; Alexandratos and Bruinsma 2012; Grundy et al. 2016). Resolving this imminent problem will be one of the most challenging goals of scientific research in the next several decades (Ort et al. 2015). Unless the productivity of major crops doubles by 2050, there is little chance of reversing this situation (Long and Ort 2010; Ray et al. 2012).

At present, research on improving the yield of major food crops is stagnant. Since the Green Revolution began in 1960, rice production has undergone revolutionary changes through the introduction of high-yield semidwarf varieties and the use of synthetic fertilizers and pesticides (Reeves et al. 2016). Rice production grew steadily at a rate of 2.3–2.5% per year from 1970s to 1980s, but the growth rate declined gradually in the twenty-first century, failing to meet the needs for human survival (Dogara and Jumare 2014). Due to the influence of climate deterioration, pests and diseases, the yield of wheat in many regions is also declining annually (Hernandez-Ochoa et al. 2018; Wang et al. 2018). In Mexico, the production of wheat fell by 2–3% per decade between 1980 and 2010 (Asseng et al. 2015). In addition, the status quo of corn production is not

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optimistic. Under the general trend of global warming, maize production will gradually decline and become more variable (Tigchelaar et al. 2018). As an important source of high-quality plant protein and edible oil, soybean is undoubtedly extremely significant for human production and life (Yang et al. 2017). Although China is the origin of soybean, the production of domestic soybean is far from meeting the needs of Chinese people. The country still needs to import a large amount of soybeans from other countries every year (Huang et al. 2017). In the past few decades, increases in crop production were mainly due to improvement of the portion of biomass partitioned into the grain, which is now close to its theoretical upper limit. In contrast, there is still much room to increase yields by improving the efficiency of plant light energy use (Zhu et al. 2008; Long et al. 2006). As the core of the second green revolution, improving photosynthetic efficiency is a conspicuously important strategy with which to address the food crisis (Xu and Shen 2002).

Crop yield relies on photosynthesis. Photosynthesis is a major target for improving crop productivity. However, photosynthesis is a complex quantitative trait controlled by multiple genes, and its mechanism is more difficult to explore than that of qualitative characteristics. With the rapid development of molecular biological techniques, molecular markers have been used to reveal phenotypic variation and genotype correlations, and some major quantitative trait loci (QTLs) for photosynthesis-related traits have been identified in rice, wheat, and other crops (Teng et al. 2004; Takai et al. 2010; Bhusal et al. 2018; Hervé et al. 2001). However, there are a few reports on QTLs related to photosynthesis-related traits in soybean. Yin et al. (2010) used the chlorophyll fluorescence parameters and photosynthetic rate of soybean as phenotypic data for linkage mapping analysis and found four major QTLs in the A2, C1, M, and O linkage groups. Moreover, Li et al. (2016) analyzed the genetic relationship between phosphorus efficiency and photosynthesis-related traits in soybean using a high-density genetic map and identified three main QTLs related to the phosphorus utilization rate and photosynthesis. In recent years, with the continuous improvement of sequencing technology, increasing numbers of molecular markers have been developed. Genome-wide association studies (GWASs) using high-quality single nucleotide polymorphisms (SNPs) have proven to be advantageous in explaining the genetic basis of complex quantitative traits (Huang et al. 2010). GWASs consider more historical recombination events, more alleles and more abundant genetic variation than traditional linkage mapping methods, making GWAS superior (Yu and Buckler 2006; Huang et al. 2012). In previous studies, several phosphorus utilization efficiency- and photosynthesis-related multi effect QTLs were identified by GWASs under hydroponic conditions (Lü et al. 2018). Although these studies have helped

us understand the genetic basis of soybean photosynthesis, much more research is needed.

To explore the genetic variation in photosynthesis-related traits in soybean, four photosynthesis-related traits (including the net photosynthetic rate (Pn), stomatal conductance (Cond), intercellular carbon dioxide concentration (Ci) and the transpiration rate (Trmmol)) of natural populations composed of 219 soybean accessions were measured under greenhouse conditions, and GWAS was performed using 292,035 high-quality SNPs from the NJAU 355K Soy SNP array described by Wang et al. (2016). A total of 12 QTLs were identified, and several candidate genes were predicted in two stable QTLs, which could provide a theoretical basis for the study of photosynthesis and improving the photosynthetic rate for breeding crops with high light efficiency.

Materials and methods

Plant materials and field experiments

A set of 219 soybean accessions were used for the GWAS, which were derived from 26 different provinces and six ecological regions of soybean cultivation in China and included 24 elite varieties and 195 landraces with diverse geographic origins and different phenotypes (Wang and Gai 2002).

Plants were grown in the greenhouse of the Jiangpu Experimental Station of Nanjing Agricultural University (32.1° N 118.4° E), Nanjing, China, in plastic pots (240 × 185 mm in size) filled with sand from the Yangtze River. The experiments were performed in June 2015, April 2016, and June 2016 (designated E1, E2 and E3, respectively) and followed a randomized block design. Three replicates of each accession were used, and Hoagland's nutrient solution was supplied appropriately during soybean growth. When the soybean plants grew to the V4 stage, the photosynthetic data, including the Pn ($\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$), Cond ($\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$), Ci ($\mu\text{mol}\cdot\text{mol}^{-1}$), and Tr ($\text{mmol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$), were measured by the LI-6400 portable photosynthesis system (LI-COR 6400XT, LI-COR, Lincoln, NE, USA) on sunny days from 9:00 to 11:00 am. The third leaf from the top was measured three times per plant, the average of the three data points was taken as an independent phenotypic value, and a total of three plants were measured for each accession.

Phenotypic data analysis

We performed analyses of variance (ANOVA) and calculated the broad-sense heritability (h^2) of the phenotypic data using R software (RStudio Team 2015). ANOVA was performed according to the formula $Y_{ijk} = \mu + G_i + E_j + (\text{GE})_{ij} + \varepsilon_{ij}$, where Y_{ijk} represents the phenotypic 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 the residual error. The h^2 was calculated as $h^2 = \sigma_g^2 / (\sigma_g^2 + \sigma_{gy}^2/n + \sigma_e^2/nr)$, where σ_g^2 is the genetic variance, σ_{gy}^2 is the interaction of genotype with the environment, σ_e^2 is the residual error, n is the number of environments, and r is the number of replicates (Knapp et al. 1985).

Genome-wide association study

Using the GAPIT package (Zhang et al. 2010), a mixed linear model (MLM) (Lipka et al. 2012) was used to perform the GWAS of different photosynthetic parameters in different years, and we used the SNPs with a minor allele frequency (MAF) of at least 0.05. We used the GAPIT package in R software to interpret population structure using principal component analysis (PCA) and calculate relatedness using the VanRaden method (VanRaden 2008). The threshold for significant associations was set to $1/n$ [where n is the marker number and the P value was $< 1/n$ (the threshold set in this study was $P < 4.82 \times 10^{-6}$)] (Yang et al. 2014). Manhattan plots were generated using the qqman package in R software (Turner 2014).

Prediction of candidate genes

Within the linkage disequilibrium (LD) decay distance of significant SNPs, the candidate genes related to photosynthesis were predicted using the SoyBase website (<https://www.soybase.org>) based on all the genes annotated in Glyma1.1, Glyma2.0 and NCBI RefSeq gene models. We also referred to the annotation of homologous genes in *Arabidopsis thaliana*.

qRT-PCR analysis

The 12 high-light-efficiency accession and 12 low-light-efficiency accessions were selected as materials for analyzing the expression level of candidate genes. When the soybean plants had grown to the V4 stage, young leaves were selected and stored in a -80°C refrigerator after being flash-frozen in liquid nitrogen. Total RNA was extracted from leaves using a Plant RNA Extraction Kit (TIANGEN, Beijing, China), and the first strand of cDNA was synthesized using a TaKaRa Prime Script™ RT reagent kit according to the manufacturer's instructions. The expression level of genes in the leaves was determined by qRT-PCR assays using an ABI 7500 system (Applied Biosystems, Foster City, CA, United States) with SYBR Green Real-time Master Mix (Toyobo). The qRT-PCR system (primer sequences are listed in Supplemental Table 1 and reaction procedure were performed

according to the specifications of the Real Universal Color Premix (SYBR Green) kit (TIANGEN, Beijing, China). The soybean *Tubulin* gene (GenBank: AY907703.1) was selected as the control, and three technical replicates were used for each accession. The relative expression of genes was calculated according to the formula described by Livak and Schmittgen (2001).

Results

Phenotypic characteristics of photosynthesis-related traits

To investigate the genetic variation in photosynthesis-related traits, 219 soybean accessions were used in this study. The Pn, Cond, Ci and Trmmol at the soybean seedling stage (V4) were measured in three different environments. Descriptive statistics revealed high variation among the panel of accessions in the experiments, as shown in Table 1. In the E1, E2 and E3 environments, the coefficient of variation of the four photosynthetic traits ranged from 13.77 to 66.67%. ANOVA revealed significant differences in the four traits due to the genotype, environment, or genotype and environment interaction. All traits showed a large amount of variation, especially Cond, where a difference of approximately 29 times was observed between the minimum and maximum, and most other traits (such as Pn and Trmmol) showed a difference of approximately 4–7 times. The h^2 of the four traits ranged from 50.05% for Ci to 87.22% for Pn, indicating that although the environment had a certain influence on the phenotypic variation, genetic effects played a predominant role (Table 1). The frequency distribution of each of the four phenotypes approached a normal distribution (Fig. 1). These results indicated that photosynthesis-related phenotypes are environmentally sensitive complex traits controlled by multiple genes and are suitable for GWASs.

GWAS of photosynthesis-related traits

GWAS has facilitated the study of complex traits controlled by multiple genes and is developing rapidly. In this study, to detect significant photosynthesis-related SNPs, 207,608 high-quality SNPs with a MAF > 0.05 were selected from 292,053 SNPs derived from the NJAU 355K Soy SNP array as molecular genetic markers. Meanwhile, association analysis was conducted at the genome-wide level with the data of the four photosynthesis-related phenotypes from 219 soybean accessions. GWAS was performed with a MLM, which greatly reduced the false positive rate.

The results of the GWAS are shown in Fig. 2 and Table 2. A total of 24 SNPs were significantly associated ($-\log_{10}(P) > 5.3$) with photosynthesis-related traits on

Table 1 Descriptive statistical results for photosynthesis-related traits of soybean

Trait	Year	Mean	Stdev	Kurtosis	Skewness	Minimum	Maximum	CV (%) ^a	<i>G</i> ^b	<i>E</i> ^c	<i>G</i> * <i>E</i> ^d	<i>H</i> ² (%) ^e
Pn	E1	20.02	4.52	0.22	0.31	8.44	32.75	22.56	***	***	**	87.22
	E2	20.83	3.17	1.08	0.12	7.02	30.76	15.22				
	E3	19.98	5.59	0.63	0.33	6.42	34.40	27.98				
Ci	E1	183.67	43.58	6.43	1.14	67.32	446.65	25.07	***	**	**	50.05
	E2	255.81	38.64	2.30	1.23	99.90	333.10	13.77				
	E3	285.76	51.83	0.32	0.13	132.97	444.09	18.14				
Cond	E1	0.20	0.06	0.38	0.44	0.05	0.34	30.00	***	**	**	52.17
	E2	0.21	0.14	0.83	0.97	0.03	0.86	66.67				
	E3	0.36	0.11	0.44	0.26	0.11	0.66	30.56				
Trmmol	E1	6.61	1.81	0.51	0.23	2.19	11.39	27.38	***	**	**	66.45
	E2	5.85	1.39	0.04	0.27	2.28	9.89	23.76				
	E3	5.99	2.16	1.02	0.11	1.74	11.86	36.06				

Significant at $P < 0.01$ *Significant at $P < 0.001$ ^aCoefficient of variation^bGenotype^cEnvironment^dGenotype \times environment^eBroad-sense heritability

chromosomes 1, 2, 6, 7, 9, 11, 12, 13, 15, 16, 18 and 19. Among these SNPs, six were detected on chromosome 9 for Ci in E2; four on chromosome 18 for Pn in E3; three on chromosomes 13 and 16 for Ci in E3 and Pn in E1, E2 and E3, respectively; and only one on the other chromosomes (including one on chromosome 1 for both Trmmol in E1 and Cond in E2). The phenotypic variance explained ranged from 11.16 to 20.98%, indicating potential photosynthesis-related QTLs near the significant SNPs.

Furthermore, according to the LD decay value calculated by Wang et al. (2016), the 130 kb regions before and after significant SNPs were selected as QTLs. Therefore, a total of 12 chromosomal regions on 12 chromosomes were found to be associated with photosynthesis, which were named *qP1*, *qP2*, *qP6*, *qP7*, *qP9*, *qP11*, *qP12*, *qP13*, *qP15*, *qP16*, *qP18* and *qP19*. Two of them, *qP1* and *qP16*, were associated with different traits in distinct environments; by contrast, the other QTLs were associated with only a single trait in a single environment. Further analysis of the 12 QTLs showed that *qP2*, *qP6*, *qP13* and *qP19* were likely new photosynthesis-related QTLs that had not been reported before and were associated with Trmmol in E2, Pn in E1, Ci in E3 and Pn in E1, respectively. *qP9* was detected for Ci in E2. In addition, *qP7*, *qP11*, *qP15* and *qP18* were associated with Cond in E1, Trmmol in E3, Ci in E1 and Pn in E3, respectively. On chromosome 12, *qP12* was detected for Cond in E3. Moreover, the two QTLs located on chromosomes 1 and 16 were two important and interesting QTLs, not only because they were mapped in at least two environments but

also because the amounts of phenotypic variation that they explained were relatively high compared with those of the other QTLs. *qP1* was identified for Trmmol in E1 and Cond in E2 and explained 16.89% of the phenotypic variation. Additionally, *qP16* was associated with Pn in E1, E2 and E3 and accounted for 16.3% of the phenotypic variation. Thus, *qP1* and *qP16* were identified as the two most representative QTLs associated with photosynthetic traits.

Prediction of candidate genes

According to the results of the GWAS, candidate genes related to photosynthesis were selected from the 130 kb flanking regions of significant representative SNPs detected in at least two environments. By referring to the annotation of genes on the SoyBase website and referring to the previously reported literature and functional studies of homologous genes in *Arabidopsis*, a total of four candidate genes possibly related to photosynthesis were identified, with two genes each in *qP1* and *qP16*. Information about the candidate genes is provided in Table 3. The two genes in *qP1* were *Glyma.01g158000* and *Glyma.01g159800*, and the other two genes were *Glyma.16g141100* and *Glyma.16g141700*, which were located in *qP16* (Table 3).

Among the genes, *Glyma.01g158000* is a member of the protein phosphatase 2Cs (PP2Cs), which may play an important role in regulating protein kinase pathways activated by diverse environmental stresses or developmental signaling cascades, especially in response to abscisic acid (ABA)

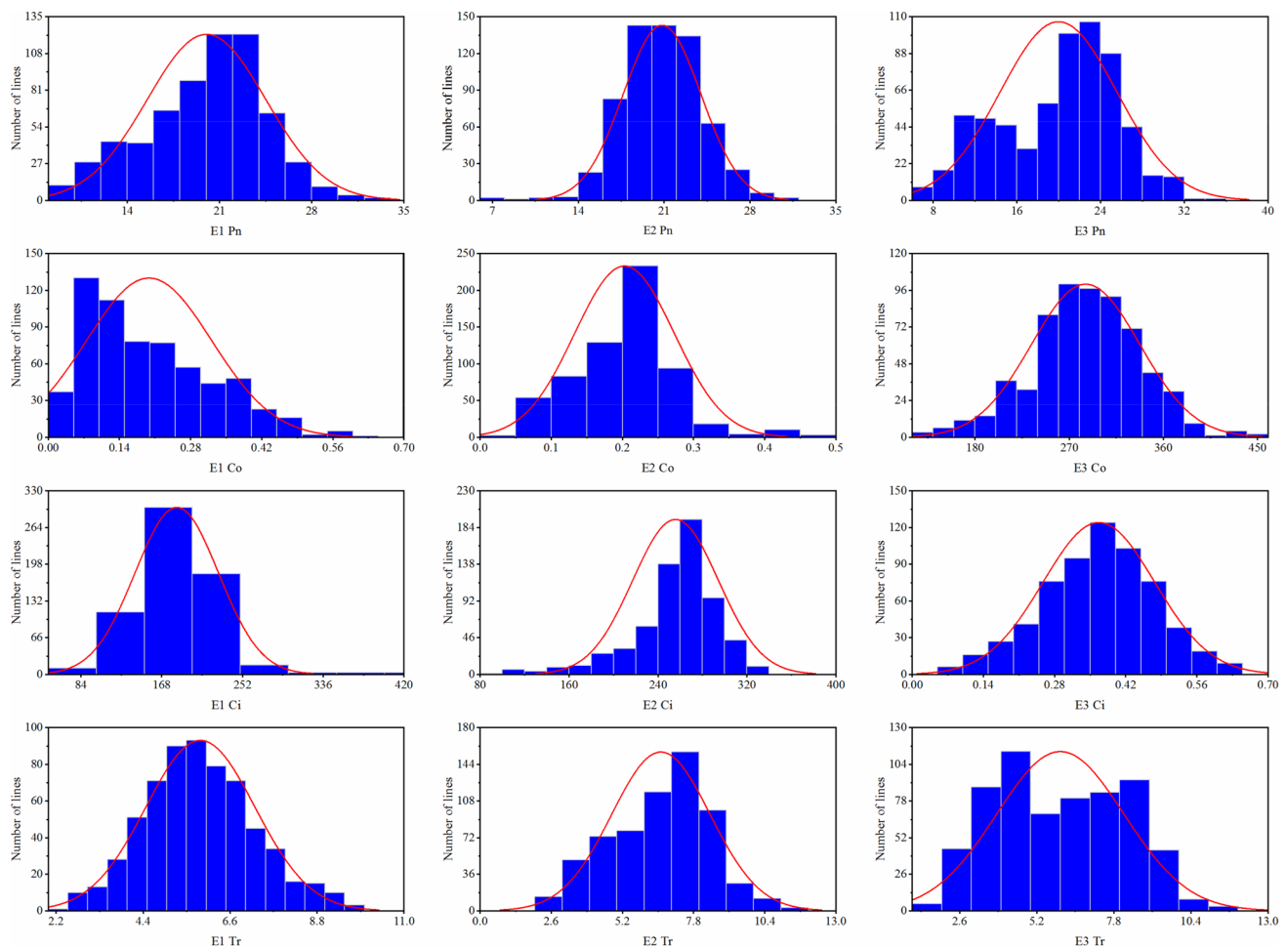


Fig. 1 Frequency distribution of different photosynthetic parameters (Pn, Co, Ci and Tr) in E1, E2 and E3. *Pn* net photosynthetic rate, *Co* stomatal conductance, *Ci* intercellular carbon dioxide concentration, *Tr* transpiration rate, *E* environment

signals. Another gene on chromosome 1, *Glyma.01g159800*, which is homologous to *PSAT1* in *Arabidopsis*, was annotated as a phosphoserine aminotransferase and has not been included in any relevant reports on soybean. In addition, *Glyma.16g141100*, a member of the zinc finger protein family, may play an important role in the germination and maturation of seeds and participate in the light morphogenesis of seeds, as well as the gibberellin (GA) and ABA signaling pathways. Another gene, *Glyma.16g141700*, which was annotated as geranylgeranyl pyrophosphate synthase 1, may also be related to photosynthesis. The genes described above are the candidate genes predicted in this study that may be related to photosynthesis.

Expression analysis of candidate genes in representative materials

Among the 219 soybean accessions used in this experiment, 12 materials with high photosynthetic efficiency

and 12 materials with low photosynthetic efficiency were selected as representative materials for the analysis of candidate gene expression. First, the total RNA of the leaves was sampled and extracted when the soybean plants were grown to the V4 stage, and then qRT-PCR was performed to analyze the expression level of the putative genes. The results are shown in Fig. 3. *Glyma.01g158000* and *Glyma.01g159800* on chromosome 1 were similarly expressed in representative materials. The expression levels of both genes were higher in materials with high photosynthetic efficiency than in materials with low photosynthetic efficiency, and the differences were extremely significant. Moreover, two candidate genes on chromosome 16 showed differential expression. The expression of *Glyma.16g141700* in materials with high photosynthetic efficiency was significantly greater than that in materials with low photosynthetic efficiency; in contrast, the expression level of *Glyma.16g141100* in materials with low photosynthetic efficiency was

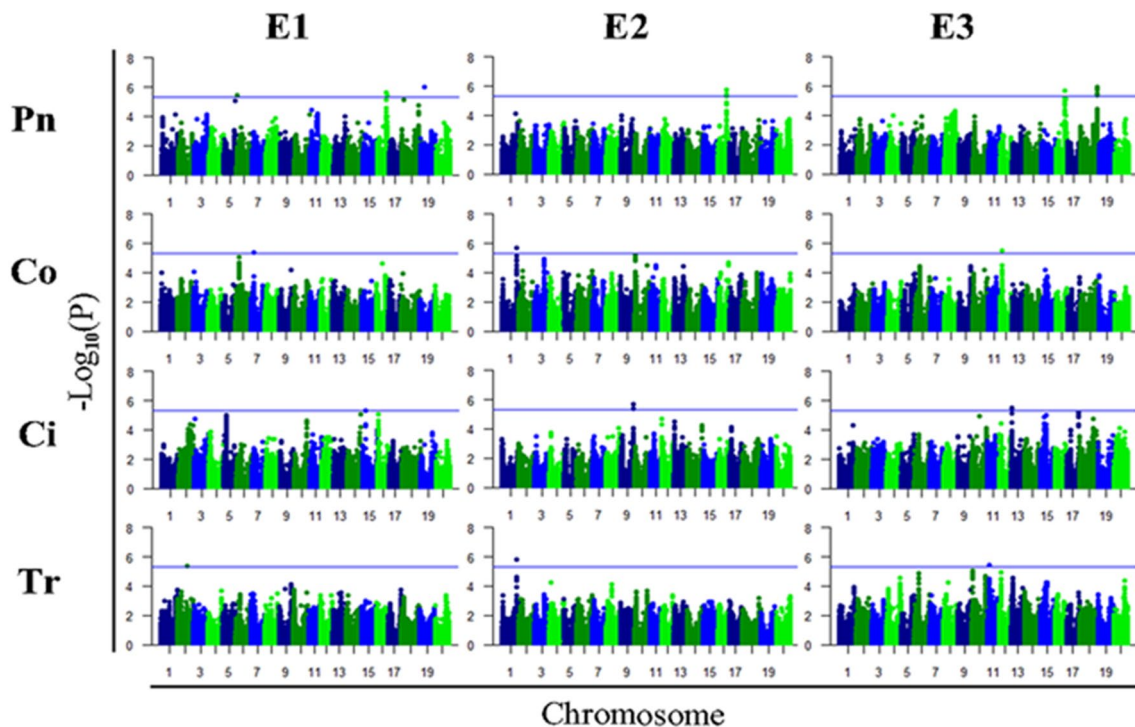


Fig. 2 GWAS of photosynthetic traits using an MLM (Q+K) across multiple environments. Manhattan plots for Pn, Co, Ci and Tr in different environments. The horizontal line depicts the significance threshold ($P < 4.82 \times 10^{-6}$); E1, E2, and E3 represent the three dif-

ferent environments respectively; Pn net photosynthetic rate, Co stomatal conductance, Ci intercellular carbon dioxide concentration, Tr transpiration rate, E environment

significantly greater than that in materials with high photosynthetic efficiency. Thus, *Glyma.16g141700* and *Glyma.16g141100* might play opposite roles in regulating photosynthesis. The results showed that the expression level of the candidate genes differed significantly between the representative materials with differences in photosynthesis. This finding provides an important basis for further study of the function of the target genes.

Discussion

Photosynthesis plays an extremely important role in plant growth and development. Crop yield relies on photosynthesis. Photosynthesis is a major target for improving crop productivity. However, little progress has been made in the analysis of natural variation in photosynthetic efficiency. In this study, GWAS was carried out with four photosynthesis parameters of 219 soybean accessions from different regions, 12 potential QTLs related to photosynthesis were identified, and four candidate genes involved in soybean photosynthesis were determined. It is possible to improve soybean overall productivity by modifying these genes.

Photosynthesis-related traits

The photosynthesis of plants is the driving force of their growth, development and metabolism. It is the basis of plant material production and important for the global carbon cycle and other material cycles. Photosynthesis depends not only on the genetic characteristics of the plant itself but also on the influence and constraints of external environmental factors (light, temperature, CO₂, moisture, etc.). The photosynthesis of plants under natural conditions is a very sensitive physiological process influenced by multiple environmental factors that may interact with each other. Therefore, it is necessary to use a variety of photosynthetic parameters under different environmental conditions to more accurately assess the photosynthetic capacity of plants. The parameters for evaluating photosynthesis include the Pn, Cond, Ci, and Trmmol, among others. In addition, chlorophyll fluorescence parameters are also very important indicators. Chlorophyll fluorescence has been used to monitor photosynthetic performance since the Kautsky effect was first described in 1931 (Jee 1995). Chlorophyll fluorescence parameters can directly or indirectly reflect certain qualitative and quantitative information related to plant photosynthesis and have become important parameters for studying the

Table 2 Details of loci associated with photosynthesis-related traits via GWAS in soybean

Name ^a	Chr ^b	Main SNP position ^c	No. of SNP ^d	P value	R ² (%) ^e	Significant region (bp)	QTLs	References	Env-trait ^f
qP1	Chr.01	48,860,794	1	1.53E−06	16.89	48,730,794–48,990,794	P use efficiency 2-g5.2	Zhang et al. (2014)	E1-Trmmol, E2-Cond
qP2	Chr.02	29,818,344	1	4.35E−06	12.62	29,688,344–29,948,344	N	N	E2-Trmmol
qP6	Chr.06	840,768	1	3.90E−06	15.17	710,768–970,768	N	N	E1-Pn
qP7	Chr.07	9,483,659	1	4.06E−06	20.98	9,353,659–9,613,659	q7-2	Lü et al. (2018)	E1-Cond
qP9	Chr.09	43,857,037	6	1.42E−06	12.38	43,726,953–43,993,400	Shoot Fe 1-g12.1, Shoot Fe 1-g12.2; First flower 4-g29	Dhanapal et al. (2018), Mao et al. (2017)	E2-Ci
qP11	Chr.11	8,340,340	1	2.98E−06	15.97	8,210,340–8,470,340	q11-1	Lü et al. (2018)	E3-Trmmol
qP12	Chr.12	5,965,471	1	2.98E−06	12.39	5,835,471–6,095,471	Seed weight 3-g8; Seed yield 3-g2; Seed set 1-g19.2	Contreras-Soto et al. (2017), Fang et al. (2017)	E3-Cond
qP13	Chr.13	1,579,109	3	3.06E−06	11.16	1,365,373–1,709,109	N	N	E3-Ci
qP15	Chr.15	11,597,382	1	4.64E−06	12.12	11,467,382–11,727,382	Shoot Fe 1-g41.1; q12	Dhanapal et al. (2018), Lü et al. (2018)	E1-Ci
qP16	Chr.16	29,464,289	3	1.86E−06	16.30	29,321,701–29,782,826	Plant height 6-g18; Pod shattering 1-g1.1, Seed height 1-g3.1	Zhang et al. (2015), Fang et al. (2017)	E1,E2, and E3-Pn
qP18	Chr.18	55,526,432	4	1.09E−06	12.76	55,387,495–55,710,646	WUE 3-g30; Pod number 1-g1.3; q18-3	Dhanapal et al. (2015), Fang et al. (2017), Lü et al. (2018)	E3-Pn
qP19	Chr.19	12,487,588	1	1.04E−06	16.50	12,357,588–12,617,588	N	N	E1-Pn

^aQTL named by chromosome^bChromosome^cMost significant SNP position^dNumber of significant SNPs^eThe proportion of phenotypic variance explained by each QTL^fEnvironment and trait**Table 3** Putative genes associated with photosynthesis-related traits

Chromosome	Reported QTL	Candidate genes	Orthologous genes in <i>Arabidopsis</i>	Annotations
Chr.01	P use efficiency 2-g5.2	<i>Glyma.01g158000</i> <i>Glyma.01g159800</i>	<i>AtPP2C47</i> <i>PSAT1</i>	Protein phosphatase 2C Phosphoserine aminotransferase
Chr.16	Plant height 6-g18; Pod shattering 1-g1.1; Seed height 1-g3.1	<i>Glyma.16g141100</i> <i>Glyma.16g141700</i>	<i>IDD1</i> <i>GGPS1</i>	C2H2-like zinc finger protein Geranylgeranyl pyrophosphate synthase 1

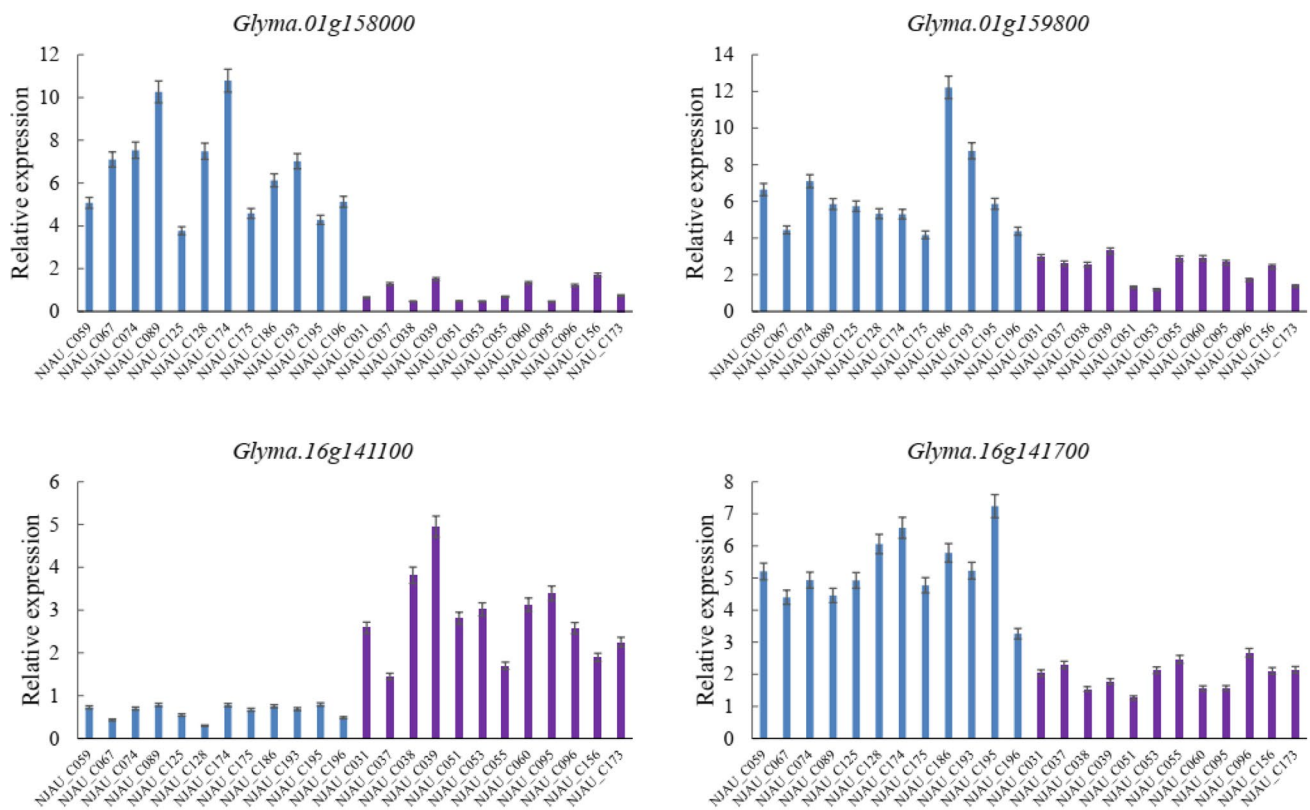


Fig. 3 Expression analysis of the four putative genes in the V4-stage leaves of 12 materials with high photosynthetic efficiency and 12 materials with low photosynthetic efficiency soybean accessions.

Error bar indicates the standard deviation. Results are representative of three biological replicates

functional mechanism of photosystem II (PS II) (Yin et al. 2010; Baker 2008; Liang et al. 2009).

In recent years, some photosynthesis-related QTLs identified using chlorophyll fluorescence parameters have been reported successively in different species (such as wheat, maize, soybean, and *Arabidopsis*) (Li et al. 2016; Czynyl-Mysza et al. 2013; Yin et al. 2015; Hao et al. 2012). In addition, photosynthetic physiological parameters closely related to the leaf photosynthetic rate measured by blade gas exchange and calculation are also basic indicators for evaluating photosynthetic capacity. In previous studies, we used photosynthetic phenotypic data measured in a recombinant inbred line (RIL) population at different phosphorus concentration levels, combined with linkage mapping and expression analysis, to reveal the genetic relationship between phosphorus utilization efficiency and photosynthesis in soybean and identified three major QTLs (Li et al. 2016). Moreover, a GWAS of photosynthetic phenotypic parameters (Pn, Tr, Co and Ci) in normal-phosphorus and low-phosphorus treatments under hydroponic conditions was carried out using different computational models, and five novel QTLs for the photosynthetic response to low-P stress were found (Lü et al. 2018). Nevertheless, it is not enough

to understand the basic metabolic mechanism of plant photosynthesis, and there are a few reports on photosynthesis-related QTLs in soybean in particular.

Therefore, this study performed a GWAS with four photosynthesis parameters measured in three different environments and identified some QTLs that may be related to photosynthesis. The results will be helpful for understanding plant photosynthesis.

Novel QTLs and potential candidate genes for photosynthesis

Natural variation in photosynthesis is widespread, and highly heritable variation in photosynthesis-related traits has been found for model and crop species such as wheat (Driever et al. 2014), *Arabidopsis*, soybean, sorghum, maize and rice (van Bezouw et al. 2019). Increasing crop yields by exploiting genetic variation in photosynthesis has been shown to be possible (Flood et al. 2011; Lawson et al. 2012). In recent years, positive genetic analysis using diverse panels has become a valuable method for identifying variations in functional alleles of important genes and traits related to photosynthesis, which not only provides important insights

into the regulation of photosynthesis but also provides important resources for promoting photosynthesis bioengineering (van Bezouw et al. 2019).

Common types of QTL mapping populations, such as RIL and near-isogenic line (NIL) populations, of multiple species have been used to identify genes related to changes in photosynthetic efficiency caused by genetic variation (Adachi et al. 2011; Gu et al. 2012; Yan et al. 2015; Oakley et al. 2018; de Oliveira Silva et al. 2018). In this study, 12 QTLs were identified by GWAS. Among these QTLs, *qP9* was detected for Ci in E2 and found to overlap with the reported *Shoot Fe 1-g12.1*, *Shoot Fe 1-g12.2* and *First flower 4-g29* QTLs (Dhanapal et al. 2018; Mao et al. 2017); *qP7*, *qP11*, *qP15* and *qP18*, which were associated with Cond in E1, Trmmol in E3, Ci in E1 and Pn in E3, respectively, overlapped with the *q7-2*, *q11-1*, *q12* and *q18-3* QTLs reported by Lü et al. (2018); *qP12* detected for Cond in E3 coincided with the partial region of the previously reported *Seed weight 3-g8*, *Seed yield 3-g2* and *Seed set 1-g19.2* QTLs (Contreras-Soto et al. 2017; Fang et al. 2017), which indicated that it may be a pleiotropic QTL associated with multiple traits. QTLs on chromosomes 1 and 16 were considered to be novel QTLs likely to be associated with photosynthesis. The amount of phenotypic variation explained by *qP1* and *qP16* was 16.89% and 16.3%, respectively. Furthermore, *qP1* was identified for Trmmol in E1 and Cond in E2, and *qP16* was associated with Pn in E1, E2 and E3, which showed that these two QTLs were more stable and representative than other targeted QTLs. Through further analysis, we found that this QTL was 461 kb long, overlapping with other reported QTLs, such as *Plant height 6-g18*, *Pod shattering 1-g1.1* and *Seed height 1-g3.1* (Fang et al. 2017; Zhang et al. 2015). Therefore, we considered these two genomic regions to be the key locations for finding genes of interest.

A total of four potential candidate genes related to photosynthesis were found. *Glyma.01g158000* in *qP1* was annotated as a member of the PP2Cs. Several members of the PP2C group have been reported to play an important role in regulating protein kinase pathways activated by diverse environmental stresses or developmental signaling cascades, especially in response to ABA signals (Madrid et al. 2007; Sheen 1996; Merlot et al. 2001). *Glyma.01g158000* is homologous to *AtPP2C47* in *Arabidopsis*, which was reported to be expressed in the inflorescences and leaves and was also upregulated similarly to some photosynthetic genes under drought stress, suggesting that it might have potential links with photosynthesis (Xue et al. 2008; Gulyani and Khurana 2011). In addition, *Glyma.16g141100*, a member of the zinc finger protein family, was shown to be associated with soybean pods in previous studies (Lee et al. 2017). Furthermore, its homologous gene (*IDD1*) in *Arabidopsis* was shown to be involved in the regulation of seed development and maturation by regulating the GA and ABA signaling

pathways (Feurtado et al. 2011). ABA is a very important hormone in plant responses to various biological and abiotic stress signals (Jones 2016), so it is not surprising that some genes (such as *Glyma.16g141100*) involved in ABA signal regulation are associated with different traits. More research, if possible, will reveal exactly which role these genes play in photosynthesis and signaling.

Another gene (*Glyma.01g159800*) annotated as a phosphoserine aminotransferase has not been reported in soybeans, but its homologous gene (*PSAT1* in *Arabidopsis*) has been shown to play a role in the serine synthesis pathway. *PSAT1*-silenced lines exhibited strong inhibition of shoot and root growth and were hypersensitive to the inhibition of photorespiratory serine biosynthesis under elevated CO₂, which indicated that the gene plays an important role in light- and sugar-dependent growth promotion in plants (Wulfert and Krueger 2018).

Moreover, photorespiration and photosynthesis are closely related. Fifty percent of the organic compounds synthesized via photosynthesis are decomposed in the photorespiration process, but no ATP or NADPH is produced, which is obviously unfavorable to the accumulation of photosynthetic products. In *Arabidopsis*, *PSAT1* was shown to be an important intermediate in the photorespiration pathway and involved in the branch of the photorespiration pathway in the serine synthesis pathway (Wulfert and Krueger 2018). *Glyma.01g159800*, as a homologous gene of *PSAT1*, was presumed to be an important member that linked photosynthesis and photorespiration. On the other hand, some metabolites produced during plant metabolism, such as terpenoids and phenolic substances, are also important factors affecting the photosynthetic efficiency of plants. Among these metabolites, isoprene compounds are the most diverse in terms of function and structure reported so far, including molecules involved in respiration, photosynthesis and the regulation of growth and development (Ruiz-Sola et al. 2016; Pulido et al. 2012). The main groups of plastid isoprenoids, including photosynthesis-related metabolites such as carotenoids and the side chain of chlorophylls, tocopherols (vitamin E), phyloquinones (vitamin K), and plastoquinones, are derived from GGPP synthesized by geranylgeranyl pyrophosphate synthase (GGPPS) (Ruiz-Sola et al. 2016). Interestingly, the gene *Glyma.16g141700* found in *qP16* is a GGPPS, suggesting that it is very likely to be an important gene for photosynthesis in soybean. Because photosynthesis involves complex regulatory mechanisms and a large number of genes, QTL mapping alone may not be enough to fully understand the genetic structure. New and effective methods need to be developed to better analyze highly complex traits such as photosynthesis.

In general, 12 QTLs associated with Pn, Cond, Ci and Trmmol were identified via GWAS. Four candidate genes were proposed, including different types of molecules

involved in multiple pathways, such as PP2C, phosphoserine aminotransferase, C2H2-like zinc finger protein and GGPPS. The wide application of GWAS accelerates the identification of candidate genes in photosynthesis-related traits. It may be more beneficial to clarify functional genetic variation and characteristics by combining parental mapping populations. Nevertheless, the associated novel QTLs and candidate genes detected in this study could be used as targets of breeding or bioengineering to improve the photosynthetic efficiency of crops.

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Author contributions DY and YY developed the experimental design; LW, YY and ZC conducted and validated the phenotypic experiments; YY analyzed the data; SZ and WY extracted RNA; LW wrote the manuscript; and all authors contributed to final review and acceptance of the manuscript.

Compliance with ethical standards

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

References

- Adachi S, Tsuru Y, Nito N, Murata K, Yamamoto T, Ebitani T, Ookawa T, Hirasawa T (2011) Identification and characterization of genomic regions on chromosomes 4 and 8 that control the rate of photosynthesis in rice leaves. *J Exp Bot* 62:1927–1938
- Alexandratos N, Bruinsma J (2012) World agriculture towards 2030/2050: the 2012 revision. ESA Working paper No. 12-03. Rome, FAO
- Asseng S, Ewert F, Martre P, Ewert F, Martre P, Rötter RP, Lobell DB, Cammarano D, Kimball BA, Ottman MJ, Wall GW, White JW, Reynolds MP, Alderman PD, Prasad PVV, Aggarwal PK, Anothai J, Basso B, Biernath C, Challinor AJ, De Sanctis G, Doltra J, Fereres E, Garcia-Vila M, Gayler S, Hoogenboom G, Hunt LA, Izaurralde RC, Jabloun M, Jones CD, Kersebaum KC, Koehler AK, Müller C, Naresh Kumar S, Nendel C, O'Leary G, Olesen JE, Palosuo T, Priesack E, Eyshi Rezaei E, Ruane AC, Semenov MA, Shcherbak I, Stöckle C, Stratonovitch P, Streck T, Supit I, Tao F, Thorburn PJ, Waha K, Wang E, Wallach D, Wolf J, Zhao Z, Zhu Y (2015) Rising temperatures reduce global wheat production. *Nature Clim Change* 5:143–147
- Baker NR (2008) Chlorophyll fluorescence: a probe of photosynthesis in vivo. *Annu Rev Plant Biol* 59:89–113
- Bhusal N, Sharma P, Sareen S, Sorial AK (2018) Mapping QTLs for chlorophyll content and chlorophyll fluorescence in wheat under heat stress. *Biol Plant* 62:721–731
- Contreras-Soto RI, Mora F, de Oliveira MA, Higashi W, Scapim CA, Schuster I (2017) A genome-wide association study for agronomic traits in soybean using SNP markers and SNP-based haplotype analysis. *PLoS ONE* 12:e0171105
- Czyczylo-Mysza I, Tyrka M, Marcinska I, Skrzypek E, Karbarz M, Dziurka M, Hura T, Dziurka K, Quarrie SA (2013) Quantitative trait loci for leaf chlorophyll fluorescence parameters, chlorophyll and carotenoid contents in relation to biomass and yield in bread wheat and their chromosome deletion bin assignments. *Mol Breed* 32:189–210
- de Oliveira Silva FM, Lichtenstein G, Alseekh S, Rosado-Souza L, Conte M, Suguiyama VF, Lira BS, Fanourakis D, Usadel B, Bhering LL, DaMatta FM, Sulpice R, Araújo WL, Rossi M, de Setta N, Fernie AR, Carrari F, Nunes-Nesi A (2018) The genetic architecture of photosynthesis and plant growth-related traits in tomato. *Plant Cell Environ* 41:327–341
- Dhanapal AP, Ray JD, Singh SK, Hoyos-Villegas V, Smith JR, Purcell LC, Andy King C, Cregan PB, Song Q, Frittschi FB (2015) Genome-wide association study (GWAS) of carbon isotope ratio ($\delta^{13}C$) in diverse soybean [*Glycine max* (L.) Merr.] genotypes. *Theor Appl Genet* 128:73–91
- Dhanapal AP, Ray JD, Smith JR, Purcell LC, Frittschi FB (2018) Identification of novel genomic loci associated with soybean shoot tissue macro- and micronutrient concentrations. *Plant Genome* 11:170066
- Dogara AM, Jumare AI (2014) Origin, distribution and heading date in cultivated rice. *Int J Plant Biol Res* 2:1008
- Driever SM, Lawson T, Andralojc PJ, Raines CA, Parry MA (2014) Natural variation in photosynthetic capacity, growth, and yield in 64 field-grown wheat genotypes. *J Exp Bot* 65:4959–4973
- Fang C, Ma Y, Wu S, Liu Z, Wang Z, Yang R, Hu G, Zhou Z, Yu H, Zhang M, Pan Y, Zhou G, Ren H, Du W, Yan H, Wang Y, Han D, Shen Y, Liu S, Liu T, Zhang J, Qin H, Yuan J, Yuan X, Kong F, Liu B, Li J, Zhang Z, Wang G, Zhu B, Tian Z (2017) Genome-wide association studies dissect the genetic networks underlying agronomical traits in soybean. *Genome Biol* 18:161
- Feurtado JA, Huang D, Wicki-Stordeur L, Hemstock LE, Potentier MS, Tsang EW, Cutler AJ (2011) The *Arabidopsis* C2H2 zinc finger INDETERMINATE DOMAIN1/ENHYDROUS promotes the transition to germination by regulating light and hormonal signaling during seed maturation. *Plant Cell* 23:1772–1794
- Flood PJ, Harbinson J, Aarts MG (2011) Natural genetic variation in plant photosynthesis. *Trends Plant Sci* 16:327–335
- Grundy MJ, Bryan BA, Nolan M, Battaglia M, Hatfield-Dodds S, Connor JD, Keating BA (2016) Scenarios for Australian agricultural production and land use to 2050. *Agric Syst* 142:70–83
- Gu J, Yin X, Struik PC, Stomph TJ, Wang H (2012) Using chromosome introgression lines to map quantitative trait loci for photosynthesis parameters in rice (*Oryza sativa* L.) leaves under drought and well-watered field conditions. *J Exp Bot* 63:455–469
- Gulyani V, Khurana P (2011) Identification and expression profiling of drought-regulated genes in mulberry (*Morus* sp.) by suppression subtractive hybridization of susceptible and tolerant cultivars. *Tree Genet Genomes* 7:725–738
- Hao D, Chao M, Yin Z, Yu D (2012) Genome-wide association analysis detecting significant single nucleotide polymorphisms for chlorophyll and chlorophyll fluorescence parameters in soybean (*Glycine max*) landraces. *Euphytica* 186:919–931
- Hernandez-Ochoa IM, Asseng S, Kassie BT, Xiong W, Robertson R, Luz Pequeno DN, Sonder K, Reynolds M, Babar MA, Molero Milan A, Hoogenboom G (2018) Climate change impact on Mexico wheat production. *Agric Forest Meteorol* 263:373–387
- Hervé D, Fabre F, Berrios EF, Leroux N, Chaarani GA, Planchon C, Sarrafi A, Gentzbittel L (2001) QTL analysis of photosynthesis and water status traits in sunflower (*Helianthus annuus* L.) under greenhouse conditions. *J Exp Bot* 52:1857–1864
- Huang JK, Wei W, Cui Q, Xie W (2017) The prospects for China's food security and imports: will China starve the world via imports? *J Integr Agr* 16:2933–2944
- Huang X, Wei X, Sang T, Zhao Q, Feng Q, Zhao Y, Li C, Zhu C, Lu T, Zhang Z, Li M, Fan D, Guo Y, Wang A, Wang L, Deng L, Li W, Lu Y, Weng Q, Liu K, Huang T, Zhou T, Jing Y, Li W, Lin Z, Buckler ES, Qian Q, Zhang Q, Li J, Han B (2010) Genome-wide

- association studies of 14 agronomic traits in rice landraces. *Nat Genet* 42:961–967
- Huang X, Zhao Y, Wei X, Li C, Wang A, Zhao Q, Li W, Guo Y, Deng L, Zhu C, Fan D, Lu Y, Weng Q, Liu K, Zhou T, Jing Y, Si L, Dong G, Huang T, Lu T, Feng Q, Qian Q, Li J, Han B (2012) Genome-wide association study of flowering time and grain yield traits in a worldwide collection of rice germplasm. *Nat Genet* 44:32–39
- Jee G (1995) Sixty-three years since Kautsky: chlorophylla fluorescence. *Aust J Plant Physiol* 22:131–160
- Jones AM (2016) A new look at stress: abscisic acid patterns and dynamics at high-resolution. *New Phytol* 210:38–44
- Knapp SJ, Stroup WW, Ross WM (1985) Exact confidence intervals for heritability on a progeny mean basis 1. *Crop Sci* 25:192–194
- Lawson T, Kramer DM, Raines CA (2012) Improving yield by exploiting mechanisms underlying natural variation of photosynthesis. *Curr Opin Biotechnol* 23:215–220
- Lee JS, Kim KR, Ha BK, Kang S (2017) Identification of SNPs tightly linked to the QTL for pod shattering in soybean. *Mol Breed* 37:54
- 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
- Liang Y, Zhang K, Zhao L, Liu B, Meng Q, Tian J, Zhao S (2009) Identification of chromosome regions conferring dry matter accumulation and photosynthesis in wheat (*Triticum aestivum* L.). *Euphytica* 171:145–156
- 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* 28:2397–2399
- Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2^(−ΔΔC_T) Method. *Methods* 25:402–408
- Long SP, Marshall-Colon A, Zhu XG (2015) Meeting the global food demand of the future by engineering crop photosynthesis and yield potential. *Cell* 161:56–66
- Long SP, Ort DR (2010) More than taking the heat: crops and global change. *Curr Opin Plant Biol* 13:241–248
- Long SP, Zhu XG, Naidu SL, Ort DR (2006) Can improvement in photosynthesis increase crop yields? *Plant Cell Environ* 29:315–330
- 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
- Madrid M, Nunez A, Soto T, Vicente-Soler J, Gacto M, Cansado J (2007) Stress-activated protein kinase-mediated down-regulation of the cell integrity pathway mitogen-activated protein kinase Pmk1p by protein phosphatases. *Mol Biol Cell* 18:4405–4419
- Mao T, Li J, Wen Z, Wu T, Wu C, Sun S, Jiang B, Hou W, Li W, Song Q, Wang D, Han T (2017) Association mapping of loci controlling genetic and environmental interaction of soybean flowering time under various photo-thermal conditions. *BMC Genom* 18:415
- Merlot S, Gosti F, Guerrier D, Vavasseur A, Giraudat J (2001) The ABI1 and ABI2 protein phosphatases 2C act in a negative feedback regulatory loop of the abscisic acid signalling pathway. *Plant J* 25:295–303
- Oakley CG, Savage L, Lotz S, Larson GR, Thomashow MF, Kramer DM, Schemske DW (2018) Genetic basis of photosynthetic responses to cold in two locally adapted populations of *Arabidopsis thaliana*. *J Exp Bot* 69:699–709
- Ort DR, Merchant SS, Alric J, Barkan A, Blankenship RE, Bock R, Croce R, Hanson MR, Hibberd JM, Long SP, Moore TA, Moroney J, Niyogi KK, Parry MA, Peralta-Yahya PP, Prince RC, Redding KE, Spalding MH, van Wijk KJ, Vermaas WF, von Caemmerer S, Weber AP, Yeates TO, Yuan JS, Zhu XG (2015) Redesigning photosynthesis to sustainably meet global food and bioenergy demand. *Proc Natl Acad Sci USA* 112:8529–8536
- Pulido P, Perello C, Rodriguez-Concepcion M (2012) New insights into plant isoprenoid metabolism. *Mol Plant* 5:964–967
- Ray DK, Ramankutty N, Mueller ND, West PC, Foley JA (2012) Recent patterns of crop yield growth and stagnation. *Nat Commun* 3:1293
- Reeves T, Thomas G, Ramsay G (2016) Save and grow in practice: maize, rice, wheat. A guide to sustainable cereal production (FAO UN, 2016)
- RStudio Team (2015) RStudio: integrated development for R, 42nd edn. RStudio Inc, Boston, p 14
- Ruiz-Sola MA, Coman D, Beck G, Barja MV, Colinas M, Graf A, Welsch R, Rutimann P, Buhlmann P, Bigler L, Gruissem W, Rodriguez-Concepcion M, Vranova E (2016) *Arabidopsis* GERANYLGERANYL DIPHOSPHATE SYNTHASE 11 is a hub isozyme required for the production of most photosynthesis-related isoprenoids. *New Phytol* 209:252–264
- Sheen J (1996) Ca²⁺-dependent protein kinases and stress signal transduction in plants. *Science* 274:1900–1902
- Takai T, Kondo M, Yano M, Yamamoto T (2010) A quantitative trait locus for chlorophyll content and its association with leaf photosynthesis in rice. *Rice* 3:172–180
- Teng S, Qian Q, Zeng D, Kunihiro Y, Fujimoto K, Huang D, Zhu L (2004) QTL analysis of leaf photosynthetic rate and related physiological traits in rice (*Oryza sativa* L.). *Euphytica* 135:1–7
- Tigchelaar M, Battisti DS, Naylor RL, Ray DK (2018) Future warming increases probability of globally synchronized maize production shocks. *Proc Natl Acad Sci USA* 115:6644–6649
- Tilman D, Balzer C, Hill J, Befort BL (2011) Global food demand and the sustainable intensification of agriculture. *Proc Natl Acad Sci USA* 108:20260–20264
- Turner SD (2014) qqman: an R package for visualizing GWAS results using QQ and manhattan plots. *Biorxiv*. <https://doi.org/10.1101/005165>
- van Bezouw R, Keurentjes JJB, Harbinson J, Aarts MGM (2019) Converging phenomics and genomics to study natural variation in plant photosynthetic efficiency. *Plant J* 97:112–133
- VanRaden PM (2008) Efficient methods to compute genomic predictions. *J Dairy Sci* 91:4414–4423
- Wang B, Liu L, O’Leary GJ, Asseng S, Macadam I, Lines-Kelly R, Yang X, Clark A, Crean J, Sides T, Xing H, Mi C, Yu Q (2018) Australian wheat production expected to decrease by the late 21st century. *Glob Change Biol* 24:2403–2415
- 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* 6:20728
- Wang Y, Gai J (2002) Study on the ecological regions of soybean in China. II. Ecological environment and representative varieties. *Chin J Appl Ecol* 13:71–75
- Wulfert S, Krueger S (2018) Phosphoserine aminotransferase 1 is part of the phosphorylated pathways for serine biosynthesis and essential for light and sugar-dependent growth promotion. *Front Plant Sci* 9:1712
- Xu DQ, Shen YK (2002) Photosynthetic efficiency and crop yield. In: Pessaraki M (ed) *Handbook of Plant and Crop Physiology*, 2nd edn. Marcel Dekker Inc, New York, pp 821–834
- Xue T, Wang D, Zhang S, Ehling J, Ni F, Jakab S, Zheng C, Zhong Y (2008) Genome-wide and expression analysis of protein phosphatase 2C in rice and *Arabidopsis*. *BMC Genomics* 9:550
- Yan X, Qu C, Li J, Chen L, Liu L (2015) QTL analysis of leaf photosynthesis rate and related physiological traits in *Brassica napus*. *J Integr Agric* 14:1261–1268
- Yang F, Xu X, Wang W, Ma J, Wei D, He P, Pampolino MF, Johnston AM (2017) Estimating nutrient uptake requirements for soybean using QUEFTS model in China. *PLoS ONE* 12:e0177509

- Yang J, Zaitlen NA, Goddard ME, Visscher PM, Price AL (2014) Advantages and pitfalls in the application of mixed-model association methods. *Nat Genet* 46:100–106
- Yin Z, Meng F, Song H, He X, Xu X, Yu D (2010) Mapping quantitative trait loci associated with chlorophyll a fluorescence parameters in soybean (*Glycine max* (L.) Merr.). *Planta* 231:875–885
- Yin Z, Qin Q, Wu F, Zhang J, Chen T, Sun Q, Zhang Y, Wang H, Deng D (2015) Quantitative trait locus mapping of chlorophyll a fluorescence parameters using a recombinant inbred line population in maize. *Euphytica* 205:25–35
- Yu J, Buckler ES (2006) Genetic association mapping and genome organization of maize. *Curr Opin Biotechnol* 17:155–160
- Zhang J, Song Q, Cregan PB, Nelson RL, Wang X, Wu J, Jiang GL (2015) Genome-wide association study for flowering time, maturity dates and plant height in early maturing soybean (*Glycine max*) germplasm. *BMC Genomics* 16:217
- Zhang Z, Ersoz E, Lai CQ, Todhunter RJ, Tiwari HK, Gore MA, Bradbury PJ, Yu J, Arnett DK, Ordoñas JM, Buckler ES (2010) Mixed linear model approach adapted for genome-wide association studies. *Nat Genet* 42:355–360
- Zhang D, Song H, Cheng H, Hao D, Wang H, Kan G, Jin H, Yu D (2014) The acid phosphatase-encoding gene GmACP1 contributes to soybean tolerance to low-phosphorus stress. *PLoS Genet* 10:e1004061
- Zhu XG, Long SP, Ort DR (2008) What is the maximum efficiency with which photosynthesis can convert solar energy into biomass? *Curr Opin Biotechnol* 19:153–159

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