Plant Physiology and Biochemistry 135 (2019) 304–309

Contents lists available at ScienceDirect

Plant Physiology and Biochemistry

journal homepage: www.elsevier.com/locate/plaphy

Research article

Effect of post-silking drought stress on the expression profiles of genes involved in carbon and nitrogen metabolism during leaf senescence in maize (*Zea mays* L.)

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ARTICLE INFO

Keywords:
Drought
Senescence
RNA-Seq maize
Metabolism

ABSTRACT

Drought stress during reproductive growth stages greatly affects the growth and productivity of maize plants. To better understand the metabolic regulation during post-silking drought (PD) stress, an RNA sequencing (RNA-Seq) analysis was performed at the late stage of leaf senescence in maize. Physiological measurements showed that PD stress reduced both leaf carbon and nitrogen levels. A total of 4013 differentially expressed genes (DEGs) were found based on RNA-Seq analysis, 115 of which were identified to be involved in photosynthesis and in the metabolism of sucrose, starch, and amino acids. Among these DEGs, 14 genes involved in photosynthesis were down-regulated. The genes coding for sucrose and pectin synthesis were up-regulated under PD stress. The two genes of asparagine synthetase (*ZmAS3* and *ZmAS4*), which are responsible for nitrogen remobilization in leaves, were also significantly induced by the drought treatment. The expression profiles of these genes involved in carbon and nitrogen metabolism suggests their regulatory roles during drought-induced leaf senescence.

1. Introduction

Drought stress is one of the most important abiotic stresses that limit crop production. The adaptive response of plants to drought stress involves a series of morphological, metabolic, and molecular changes (Moore et al., 2009; Albacete et al., 2014). The imbalance in carbon and nitrogen metabolism is one of the major consequence of drought. Drought stress induces a decrease in photosynthesis, loss of canopy area, and reduction in carbon assimilation (Zheng et al., 2010; Savage et al., 2016; Yang et al., 2018). During reproductive growth stages, drought stress may cause premature senescence. The translocation of carbon and nitrogen molecules between the source and sink is also affected (Sadras and Richards, 2014; Chen et al., 2015a; Li et al., 2016).

Carbon and nitrogen metabolism are highly interconnected processes. Carbohydrates generated in photosynthesis provide basic carbon skeletons and energy source for various biological processes, including nitrogen absorption and inorganic nitrogen fixation (Huppe and Turpin, 1994). The levels of carbohydrates are considered as important regulators of nitrogen metabolism in plants (Chen et al., 2015a). However, photosynthetic carbon fixation is vulnerable to different environmental factors, including the availability of nitrogen (Masclaux-Daubresse et al., 2010; Albacete et al., 2014). The maintenance of normal growth requires an optimum coordination between carbon and nitrogen metabolism.

Natural leaf senescence is a genetically controlled process for nutrient recycling during reproductive growth stages. Under drought stress conditions, the senescence program may be accelerated. Carbohydrates were reported to be important metabolic regulators of drought-induced leaf senescence as they are involved in various responses for adaptation to drought (Bolouri-Moghaddam et al., 2010; Tang et al., 2015). Under drought stress, the inhibition of carbohydrate assimilation or its export from source organ may result in superfluous photosynthetic light energy, which may in turn lead to a burst of reactive oxygen species (ROS) production (Miller et al., 2010; Gururani et al., 2015; Checovich et al., 2016). These active oxygen-containing ROS can easily cause the peroxidation of membrane lipids and speed up the senescence program (Liu et al., 2015a). To cope with the oxidative damage, carbohydrates provide energy source and basic substance for the ROS scavenging systems (Zhang et al., 2017). In addition, some sugars or sugar alcohols, such as trehalose and mannitol, are the major

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Abbreviations: RNA-Seq, RNA sequencing; PD, post-silking drought; CK, well-watered control; DAS, days after silking stage; DEG, differentially expressed gene; SPS, sucrose phosphate synthase; SS, sucrose synthetase; AS, asparagine synthetase

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https://doi.org/10.1016/j.plaphy.2018.12.025
Received 28 September 2018; Received in revised form 2 December 2018; Accepted 24 December 2018

molecules involved in osmotic adjustments that prevent dehydration under drought stress (Yang et al., 2018).

The status of nitrogen is closely related to leaf senescence. The senescence program is often associated with the degradation of chloroplasts and reutilization of nitrogen present in the chloroplast proteins. Rubisco, which is the central enzyme in the dark reaction of photosynthesis, is the largest source of leaf nitrogen (Distelfeld et al., 2014). With senescing of leaves, rubisco breaks down into amino acids, which are then reused as nitrogen supplement for grains (Masclaux-Daubresse et al., 2010). In crops, there is a close relationship between the level of leaf nitrogen and senescence (Moschen et al., 2016). Currently, a broadly accepted viewpoint is that higher leaf nitrogen levels are associated with delayed leaf senescence, which confers better drought tolerance (Sadras and Richards, 2014; Checovich et al., 2016; Sade et al., 2017).

RNA-sequencing (RNA-seq) analysis provides a practical way to screen genes that are associated with specific biological pathways during drought-induced leaf senescence. Previous studies revealed that the expression profiles of genes involved in carbon and nitrogen metabolism in maize leaves were significantly affected by drought stress (Zheng et al., 2010; Liu et al., 2015b; Li et al., 2017). However, most of these studies were performed at seedling stages, and few were done at very late growth stages. Maize could be extremely sensitive to drought stress at reproductive growth stages. The premature senescence of leaves may result in great loss to maize production. In the present study, we used RNA-seq analysis to investigate the involvement of specific genes associated with carbon and nitrogen metabolism under drought stress with the aim of understanding the transcriptional regulation of these processes in maize leaves in response to post-silking drought stress.

2. Materials and methods

2.1. Plant materials, growth conditions, and experimental design

This work was conducted at Northwest A&F University, Shaanxi province, China. ‘Zhengdan958’ (ZD958), one of the major cultivated maize hybrids of China, was used in this study. The experiments were carried out in large transparent plastic-covered rainproof sheds (32 m length × 15 m width × 3 m height), with four sides open. The maize plants were grown in pools (2 m length × 1.5 m width) with water-sealed walls beneath. The experiment was performed in 2016, and maize plants were cultivated from June to October.

The plants were exposed to well-watered and post-silking drought (PD) stress conditions. Firstly, all the plants were well-watered before the silking stage. After the silking stage, the well-watered control (CK) plants were normally irrigated until maturity, whereas the PD plants were watered in a controlled manner such that the relative water content (RWC) in the soil was maintained between 50% and 35%. Each treatment was done in triplicate.

2.2. Sampling

Ear-leaves (the leaves that were closest to the ear) were used for the measurement of biological parameters and for RNA sequencing. The chlorophyll concentration in the ear-leaves was measured in vivo by SPAD-502 (Japan) chlorophyll meter, and the results were recorded in terms of SPAD units (Zobiole et al., 2011).

Samples of ear-leaves were collected at 50 days after silking stage (DAS). Fresh samples were stored at $-80\,^{\circ}\mathrm{C}$ for total RNA extraction. The biomass was measured after drying the ear-leaves for 72 h in an oven at $80\,^{\circ}\mathrm{C}$. Thereafter, the dried samples were milled for determining the weight of dry matter, and the concentrations of carbohydrates (soluble sugar, sucrose, and starch) and nitrogen.

2.3. Physiological measurements

The concentration of amino-nitrogen was determined by the ninhydrin method (Xiong et al., 2006). The carbohydrate (soluble sugar, sucrose, and starch) concentrations were estimated by the anthrone method using glucose as the standard (Verma et al., 2011). The concentration of nitrogen was determined by the standard macro-Kjeldahl procedure (Horneck and Miller, 1998).

2.4. RNA preparation, library construction, and illumina HiSeq2500 sequencing

For RNA extraction and cDNA library preparation, total RNA was extracted using the TRIzol reagent, according to the manufacturer's instructions (Invitrogen, Canada). The extracted RNA was enriched by removing rRNA using Ribo-ZeroTM Magnetic Kit (Epicentre, Germany). The enriched mRNAs were then fragmented and reverse transcribed into cDNAs with random primers. The synthesized cDNA fragments were purified, phosphorylated, and poly (A) were added; they were then ligated to the adapters. The ligation products were subsequently size-selected and sequenced using Illumina HiSeqTM2500 by Gene Denovo Biotechnology Co. (Guangzhou, China). Three replicate samples were extracted for each treatment and a total of six cDNA libraries were constructed and sequenced. The raw reads were filtered and the sequences were aligned to the reference genome (*Zea mays* L.). The statistics of raw data assessment and sequence alignment is presented in Supplementary file S1.

2.5. Differential expression analysis and functional enrichment

The expression levels of genes were determined by the fragments per kilobase of exon per million mapped reads (FPKM) method. To identify the differentially expressed genes (DEGs) between the two conditions, the edgeR package (http://www.r-project.org/) was used. The criteria for identifying the significant DEGs between PD and CK plants was set at $|\log_2 \text{fold-change (FC)}| > 1$ and a false discovery rate $(\text{FDR}) < 0.05$. The identified DEGs were then subjected to further analysis. The gene ontology (GO) classification of DEGs was performed using the Gene Ontology database (http://www.geneontology.org/). The metabolic pathway enrichment for the DEGs was performed by MapMan (Usadel et al., 2009).

2.6. Real-time qPCR

The results of RNA-Seq were validated by quantitative real-time polymerase chain reaction (RT-qPCR) (Tombuloglu et al., 2015). Six genes involved in nitrogen metabolism (*ZmNR*, *ZmNir*, *ZmGS1-3*, *ZmGS2*, *ZmAS1*, and *ZmAS3*) were selected as the targets. RT-qPCR was performed on CFX96 real-time PCR platform (Bio-rad, USA) using SYBR Green reagent (Takara, Japan), and the relative levels of gene expression were calculated by the $2^{-\Delta\Delta CT}$ method, using maize actin gene as an internal control. The pairs of primers used for RT-qPCR in this study are given in Supplementary file S2. The validation of the results of RNA-Seq and RT-qPCR was performed by linear regression analysis. The two variables for the regression analysis for RNA-Seq and RT-qPCR were $\log_2$FC and $\log_2$ (relative expression level), respectively.

2.7. Statistical analysis

The data presented in Table 1 were analysed by One-way analysis of variance (ANOVA) and were compared using Least Significant Difference (LSD) method $(P < 0.05)$.

Table 1
Effect of post-silking drought stress on biological parameters of maize ear-leaves.

Parameters	CK	PD
Biomass (g)	8.83 ± 0.06 a	8.20 ± 0.26 b
Chlorophyll (SPAD units)	43.7 ± 1.78 a	39.1 ± 1.42 b
Total N (g/kg DM)	13.83 ± 1.08 a	11.66 ± 0.57 b
Amino N (mg/g DM)	0.63 ± 0.12 a	0.32 ± 0.07 b
Soluble sugar (% DM)	17.87 ± 0.73 a	12.25 ± 0.55 b
Sucrose (% DM)	4.20 ± 0.06 a	3.46 ± 0.18 b
Starch (% DM)	6.43 ± 0.22 a	4.84 ± 0.31 b

N: nitrogen; DM: dry matter. Data are presented as means ± SD of three biological replicates. The letters indicate significant differences at $P \leq 0.05$.

3. Results

3.1. Effect of PD stress on carbon and nitrogen concentrations in maize ear-leaves

As shown in Table 1, the biomass and chlorophyll content (SPAD) of maize ear-leaves were significantly reduced under PD stress at 50 DAS. Also, PD stress caused substantial reduction in the concentrations of carbohydrates and nitrogen, including soluble sugar, sucrose, starch, total nitrogen, and amino-nitrogen.

3.2. Gene expression profiles in maize ear-leaves in response to PD stress

To identify the DEGs between the ear-leaves of plants exposed to PD and CK conditions, we performed transcriptome analysis of the leaf tissues by RNA-Seq. Over 7 million high quality clean reads were generated from each RNA-Seq library. Most of the reads could be aligned to the reference genome. Quality assessment of the sequencing reactions is shown in Supplementary file S1. Raw sequencing data is uploaded and released in the NCBI SRA database (accession number: PRJNA506465). A total of 28,476 genes, covering 72.41% of the reference genome, were detected from the RNA-Seq data. Overall, 4013 DEGs were identified for the CK vs. PD comparison at 50 DAS. Among these, 2367 genes were up-regulated and 1646 genes were down-regulated under PD stress. The identification, expression level of the 4013 DEGs were listed in Supplementary file S3.

The GO terms for the DEGs were annotated in three categories, namely biological processes, molecular functions, and cellular components (Fig. 1). For GO terms associated with biological processes, most of the DEGs were categorized in the metabolic processes. Some of the GO terms for the DEGs were related to abiotic stresses, such as cell killing, developmental process, reproduction, response to stimulus, and signalling. The distribution of DEGs in the cellular components category was mainly in cell part, organelle, and membrane. The distribution of DEGs in the molecular function category was mainly in binding, catalytic activity and transporter activity.

3.3. Analysis of DEGs in relation to carbon and nitrogen metabolism under PD stress

To determine their involvement in metabolic pathways, all the DEGs were mapped using the MapMan software (Usadel et al., 2009). Most of the DEGs involved in photosynthetic light reactions and tetrapyrrole metabolism were down-regulated under PD stress. Nine of the 11 DEGs involved in starch breakdown and 15 of the 19 genes coding for the enzymes of amino acid degradation pathways were up-regulated under PD stress. Other major metabolic pathways, such as those of cell wall, lipid, and secondary metabolism, were also affected by the PD stress.

For further investigating the effects of PD stress on the expression patterns of genes involved in carbon and nitrogen metabolism, three major processes, viz. photosynthesis, carbohydrate (sucrose and starch) metabolism, and amino acid metabolism, were considered. The most remarkable DEGs ($|\text{Log2 (FC)}| > 2$) belonging to these metabolic pathways were listed in Table 2, and the others were listed in Supplementary file S4.

Photosynthesis was significantly affected by the PD stress. Seventeen DEGs related to photosynthesis were revealed upon analysis of RNA-Seq results; of these, 14 were down-regulated and three were up-regulated under PD stress. Six of the seven genes encoding reaction centre subunits of the two photosystems, PSI and PSII, were down-regulated under PD stress; these genes coded for PSII W, PSII V, PSII 11 kD, PSI IV A, and PSI V proteins. The expression of the gene encoding the ATP synthase subunit (Zm00001d006403) was most strongly down-regulated ($\log_2 FC = -8.20$) under PD stress. Only three genes belonging to the photosynthesis pathway were up-regulated in response to PD stress; two of these encoded ferredoxin (Zm00001d012293 and Zm00001d034760) and one coded for the PS II reaction centre 28 protein (Zm00001d042178).

Forty-four genes related to sucrose and starch metabolism were differentially expressed under PD stress. With the exception of seven (out of eight) genes encoding amylases that were up-regulated under PD stress, the other up-regulated genes mainly coded for polygalacturonase (Zm00001d034552 and Zm00001d042556), sucrose synthase (Zm00001d045042, *ZmSH1*), sucrose-phosphate synthase (Zm00001d048979), and ADP-glucose pyrophosphorylase (Zm00001d033910). All the three genes coding for invertases

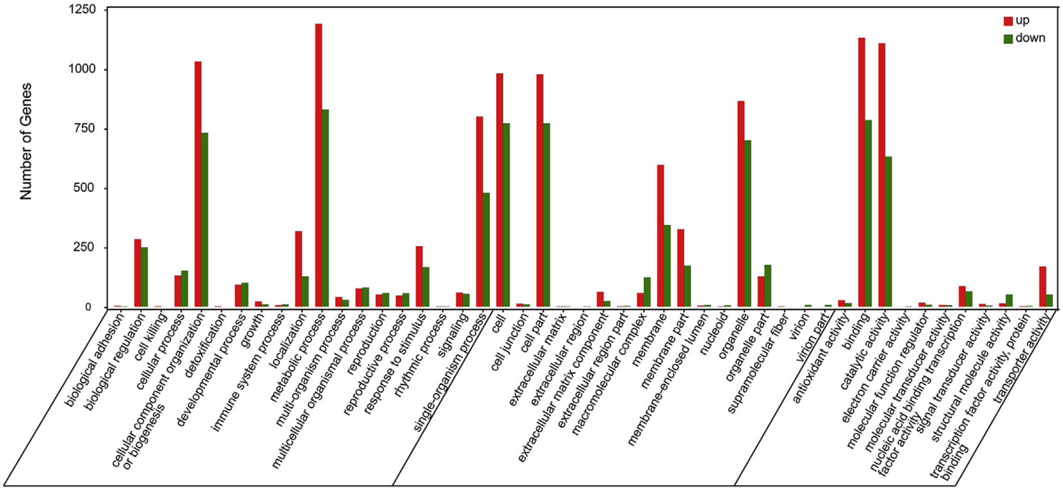

Fig. 1. Gene ontology enrichment of differentially expressed genes in maize ear-leaves under post-silking drought stress.

Table 2
Remarkable DEGs for photosynthesis, carbohydrates and amino acids metabolism pathways in maize ear-leaves under PD stress.

Gene ID	Description	Log_2 (FC)
Photosynthesis		
Zm00001d012293	ferredoxin-6 [Zea mays]	2.23
Zm00001d006403	ATP synthase subunit beta, chloroplastic	−8.20
Carbohydrates		
Zm00001d023994	4-hydroxy-7-methoxy-3-oxo-3,4-dihydro-2H-1,4-benzoxazin-2-yl glucoside beta-D-glucosidase 1, chloroplastic	9.24
Zm00001d034552	polygalacturonase ADPG1-like isoform X1 [Zea mays]	6.95
Zm00001d018159	alpha-amylase precursor [Zea mays]	6.79
Zm00001d042556	polygalacturonase-like [Setaria italica]	6.65
Zm00001d022104	pectinesterase QRT1 isoform X1 [Zea mays]	5.34
Zm00001d020350	alpha-amylase type A isozyme-like [Zea mays]	3.20
Zm00001d020351	alpha-amylase type A isozyme [Setaria italica]	3.07
Zm00001d037918	non-cyanogenic beta-glucosidase [Zea mays]	2.60
Zm00001d005687	trehalose 6-phosphate synthase [Zea mays]	2.48
Zm00001d005546	ADP-glucose pyrophosphorylase small subunit [Zea mays]	2.32
Zm00001d011889	hexokinase-3-like [Zea mays]	2.12
Zm00001d019756	beta-amylase 7 isoform X3 [Malus domestica]	2.04
Zm00001d050082	pectinesterase PPE8B precursor [Zea mays]	−2.81
Zm00001d022460	pectinesterase-like [Zea mays]	−6.74
Amino acids		
Zm00001d003058	L-allo-threonine aldolase [Zea mays]	3.59
Zm00001d047736	asparagine synthetase [Zea mays]	3.41
Zm00001d043845	glutamate synthase 1 [NADH], chloroplastic-like isoform X1 [Zea mays]	3.01
Zm00001d004171	transketolase, chloroplastic-like [Zea mays]	2.93
Zm00001d046676	tryptophan synthase beta chain 2 isoform X1 [Setaria italica]	2.62
Zm00001d029025	2,3-bisphosphoglycerate-independent phosphoglycerate mutase-like isoform X1 [Zea mays]	2.54
Zm00001d040850	bifunctional 3-dehydroquinate dehydratase/shikimate dehydrogenase, chloroplastic-like [Setaria italica]	2.37
Zm00001d032114	LOC100280688 isoform X1 [Zea mays]	2.33
Zm00001d024908	arginase 1, mitochondrial [Setaria italica]	2.28
Zm00001d028303	branched-chain-amino-acid aminotransferase isoform X1 [Zea mays]	2.14
Zm00001d026619	pyruvate kinase [Zea mays]	2.06
Zm00001d049409	aconitate hydratase, cytoplasmic [Jatropha curcas]	2.04
Zm00001d045350	cysteine synthase-like [Zea mays]	−2.06
Zm00001d017168	acetolactate synthase/amino acid binding protein [Zea mays]	−2.10
Zm00001d030557	alanine aminotransferase [Zea mays]	−2.49
Zm00001d043628	ketol-acid reductoisomerase, chloroplastic [Setaria italica]	−2.66
Zm00001d052012	oxysterol-binding protein-related protein 3C-like [Zea mays]	−3.43
Zm00001d003717	cysteine synthase-like [Zea mays]	−4.58

(Zm00001d025943, Zm00001d001941, and Zm00001d025943) were down-regulated under PD stress. The genes categorized as pectinesterases (Zm00001d050082 and Zm00001d022460) and trehalose-6-phosphate synthase isoforms (Zm00001d038728 and Zm00001d047077) were also down-regulated under PD stress.

Fifty-four DEGs were determined to be involved in amino acid metabolism: 39 were up-regulated and 15 were down-regulated under PD stress. The most significantly up-regulated gene was L-allo-threonine aldolase (Zm00001d003058, $\log_2 FC = 3.59$). The two genes encoding asparagine synthetase (Zm00001d047736 and Zm00001d028750) were also conspicuously up-regulated under PD stress. Other DEGs that were highly up-regulated were glutamate synthase 1 (Zm00001d043845), transketolase (Zm00001d004171), and tryptophan synthase (Zm00001d046676). Three DEGs coding for cysteine synthase (Zm00001d043101, Zm00001d045350, and Zm00001d003717) were most obviously down-regulated under PD stress. Two alanine aminotransferase genes (Zm00001d007937 and Zm00001d030557) were also down-regulated.

3.4. Validation of RNA-Seq results by RT-qPCR

Six genes involved in the major nitrogen metabolic pathway were used for validation of the sequencing results. The relative gene expression levels (RT-qPCR) and FPKM (RNA-Seq) of the six genes showed similar regulatory patterns by PD stress (Fig. 2a). As shown in Fig. 2b, a linear regression coefficient (R^2) of 0.9969 was obtained between the results of RT-qPCR and RNA-Seq. These consistencies validated the results of RNA-Seq analysis (Tombuloglu et al., 2013).

4. Discussion

Yellowing of leaves is the most notable manifestation of leaf senescence (Distelfeld et al., 2014). The reduction in the chlorophyll content (in SPAD units) suggested that leaf senescence was accelerated under PD stress (Table 1). We observed that the concentration of leaf carbon and nitrogen assimilates declined substantially under PD stress. This was in accordance with the results of previous studies in maize and other crops (Li et al., 2016). The stress-induced leaf senescence could be a reflection of increased degradation and recycling of cellular macromolecules, such as leaf carbohydrates and proteins (Chen et al., 2015b), and is considered as a survival tactic for ensuring the growth of the developing grains (Sade et al., 2018).

In plants, photosynthesis plays a decisive role in carbon fixation and biomass accumulation. In higher plants, the light reaction of photosynthesis is accomplished by the two photosystems—PSI and PSII. These two photosystems work in series through the photosynthetic energy transport chain and are involved in the reactions of light-dependent carbon fixation (Gururani et al., 2015). In the present study, six of the seven genes encoding the PSI and PSII reaction centre subunits were observed to be down-regulated under PD stress, suggesting that PD stress might have impaired the activities of the two photosystems. Overall, 14 of the 17 genes involved in the photosynthesis pathway were down-regulated in response to PD stress, indicating that photosynthetic light reaction might be inhibited. Two genes of ferredoxins were up-regulated under PD stress, which might be attributed to the stimulation of cyclic electron transport under drought stress conditions (Lehtimäki et al., 2010).

Carbohydrates serve basic roles as structural components, energy

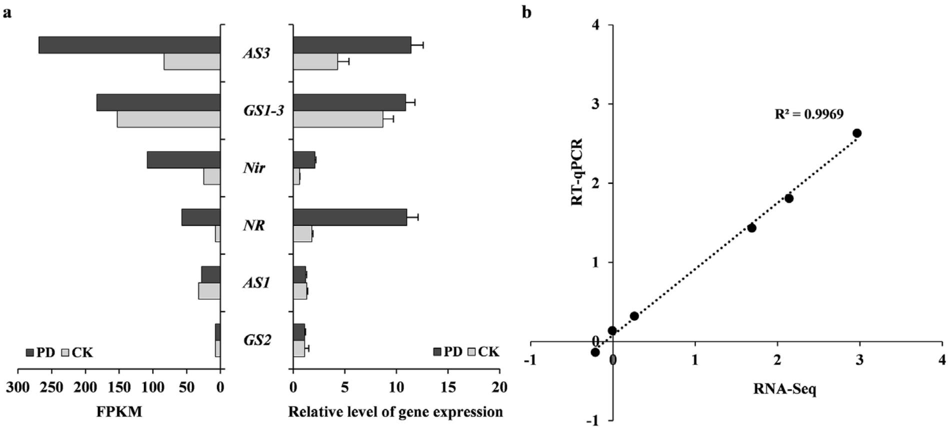

Fig. 2. Validation of RNA-Seq results by RT-qPCR. (a) Comparison between FPKM and relative gene expression level (the values were the means ± SD (n = 3)) of six genes. (b) Regression analysis between the results of RNA-Seq and RT-qPCR experiments.

sources, and osmotic regulators in the growth of plants. Sucrose and starch are the major forms of non-structural carbohydrates in plant tissues (Savage et al., 2016; Yang et al., 2018). Sucrose is the major intermediate in carbon metabolism. The synthesis of sucrose provides the sink strength required for photosynthetic carbon fixation, and its conversion provides raw material for starch accumulation (Keeling and Myers, 2010). Sucrose phosphate synthase (SPS) and sucrose synthetase (SS) are key enzymes of sucrose metabolism in plants. Sucrose phosphate synthase is required for the generation of sucrose-6-phosphate and is considered as the central enzyme in sucrose synthesis. Sucrose synthetase catalyses the reversible reaction between sucrose and fructose, which is generally thought to be critical for the processing of sucrose in several downstream metabolic reactions, including starch synthesis (Whittaker et al., 2007). In the present study, PD stress was found to reduce the sucrose content in leaves. The expression of both SPS and SS genes was up-regulated. The reduction in the sucrose content could be a reflection of the decline in photosynthetic activity, whereas the induction of SPS and SS might be an active mechanism of stimulation for ensuring the conversion of sucrose when photosynthetic carbon fixation is inhibited (Whittaker et al., 2007; Buchner et al., 2015; Savage et al., 2016). Most of the genes coding for amylases were up-regulated under drought stress, implying that PD stress might stimulate the hydrolysis of starch in leaves (Buchner et al., 2015).

Pectin is one of the most abundant carbohydrates in cell wall. Previous studies found that drought stress reduced the content of pectin in leaves and decreased the rigidity of the cell walls (Moore et al., 2009; Yang et al., 2010). Polygalacturonase is responsible for the degradation of pectin in cells; the overexpression of polygalacturonase gene in rice increased its sensitivity to drought stress (Liu et al., 2014). In the present study, the two genes coding for polygalacturonase were found to be up-regulated under PD stress, in consistency with previous findings.

Post-silking senescence is associated with the degradation and remobilization of leaf nitrogen (Masclaux-Daubresse et al., 2010). Asparagine synthetase (AS), which catalyses the generation of asparagine, is recognized as an important enzyme in the transportation of nitrogen in plants (Cañas et al., 2010). In maize, *ZmAS3* and *ZmAS4* were reported to be highly up-regulated at very late stages of leaf senescence and were proposed to function in nitrogen remobilization (Cañas et al., 2010; Li et al., 2016). Based on the results of RNA Seq in this study, *ZmAS3* (Zm00001d028750) and *ZmAS4* (Zm00001d047736) were determined to be up-regulated under PD stress. This was consistent with our previous result regarding PD stress-induced nitrogen partitioning and determination of relative expression levels of maize AS genes using RT-qPCR (Li et al., 2016). Cysteine is a major precursor for various sulphur-containing molecules, such as glutathione. Its synthesis is catalysed by cysteine synthase and is considered as the primary route for sulphur assimilation in plants (Koprivova and Kopriva, 2016). In this study, the expression levels of three cysteine synthase genes were suppressed under PD stress, which reflected the inhibitory effect of drought stress on the metabolism of sulphur-containing amino acids.

In summary, PD stress was found to accelerate leaf senescence and cause prominent changes in the expression profile of several genes. A number of genes were found to be differentially regulated under PD stress, and some of these are crucial players in carbon and nitrogen metabolism. Drought stress at reproductive growth stages is one of the major abiotic stresses that limit the production of maize. The results of the present study may provide a comprehensive picture of the metabolic regulation under drought conditions.

Conflicts of interest

None.

CRediT authorship contribution statement

Miao Yang: Investigation, Methodology, Data curation. **Mengyao Geng:** Investigation, Methodology, Data curation. **Pengfei Shen:** Investigation, Methodology, Data curation. **Xiaohong Chen:** Resources. **Yajun Li:** Project administration, Writing – original draft. **Xiaoxia Wen:** Project administration, Writing – original draft.

Acknowledgements

This study was supported by the National Natural Science Foundation of China (Grant No. 31500439 and 31401303) and Fundamental Research Funds for the Central Universities of China (Grant No. Z109021804).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.plaphy.2018.12.025.

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