



Genome-Wide Transcriptional Profile Analysis of *Prunus persica* in Response to Low Sink Demand after Fruit Removal

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Prunus persica fruits were removed from 1-year-old shoots to analysis photosynthesis, chlorophyll fluorescence and genes changes in leaves to low sink demand caused by fruit removal (–fruit) during the final stage of rapid fruit growth. A decline in net photosynthesis rate was observed, accompanied with a decrease in stomatal conductance. The intercellular CO₂ concentrations and leaf temperature increased as compared with a normal fruit load (+fruit). Moreover, low sink demand significantly inhibited the donor side and the reaction center of photosystem II. 382 genes in leaf with an absolute fold change ≥ 1 change in expression level, representing 116 up- and 266 down-regulated genes except for unknown transcripts. Among these, 25 genes for photosynthesis were down-regulated, 69 stress and 19 redox related genes up-regulated under the low sink demand. These studies revealed high leaf temperature may result in a decline of net photosynthesis rate through down-regulation in photosynthetic related genes and up-regulation in redox and stress related genes, especially heat shock proteins genes. The complex changes in genes at the transcriptional level under low sink demand provided useful starting points for in-depth analyses of source-sink relationship in *P. persica*.

Keywords: peach, low sink demand, photosynthesis, transcriptional profile, fruit removal

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INTRODUCTION

Photosynthesis is the basis of plant growth and development, and it plays a decisive role in crop yield and quality. The fruit is the most important sink organ for most horticultural plants. The presence or absence of the fruits has a significant effect on source leaf photosynthesis in many plant species including peach (Duan et al., 2008). Therefore, fruit removal has often been used to change the sink–source relationship in order to study photosynthetic responses under low sink demand.

Abbreviations: CAT, catalase; C_i, Intercellular CO₂ concentrations; DEGs, Differentially expressed genes; E, Transpiration rate; ESTs, Expressed sequence tags; FBPase, Fructose-1,6-bisphosphatase; GAPB, Glyceraldehyde-3-phosphate dehydrogenase B; GAPDH, Glyceraldehyde-3-phosphate dehydrogenase; g_s, Stomatal conductance; Hsp, Heat shock proteins; LHC, Light-harvesting complex; NDH, NAD(P)H dehydrogenase; OEC, oxygen-evolving complex; PAR, Photosynthetically active radiation; P_n, Net photosynthesis rate; PQ, Plastoquinone; PSI, photosystem I; PSII, Photosystem II; RCA, Rubisco activase; ROS, reactive oxygen species; SBPase, Seduheptulose bisphosphatase; sHsps, small Hsps; T_{leaf}, Leaf temperature; TPI, triose-phosphate isomerase; TPM, Tags per million clean tags.

Leaf net photosynthesis rate (P_n) was found to be reduced when the sink demand was lowered by removing fruits or flowers in herbaceous species such as potato (Basu et al., 1999), tomato (Walker and Ho, 1977), soybean (Setter et al., 1980), and Dahlia (Yan et al., 2011) as well as in woody plants such as grape (Downton et al., 1987), kiwifruit (Buwalda and Smith, 1990), apple (Gucci et al., 1995; Fan et al., 2010), citrus (Iglesias et al., 2002), coffee (DaMatta et al., 2008), peach (Li et al., 2005; Duan et al., 2008), and pine (López et al., 2015). A lot of studies support the hypothesis of end-product inhibition of photosynthesis to explain the response of the decline of P_n under the low sink demand (Paul and Foyer, 2001; Iglesias et al., 2002; Zhou and Quebedeaux, 2003; Wu et al., 2008). However, this conclusion is controversial (Li et al., 2007; DaMatta et al., 2008). In our previous work it was found that low sink demand increased leaf temperature (Li et al., 2001, 2005, 2007; Duan et al., 2008; Cheng et al., 2009; Fan et al., 2010). So we speculated high leaf temperature might cause irreversible damage to photosynthetic apparatus when it was above the optimum temperature of photosynthesis. To date, the specific mechanism for the effect of low sink demand on photosynthesis is unclear.

In order to understand the molecular basis of change in source-sink response, gene expression profiling using expressed sequence tags or microarray were carried out in some plant species. For example, leaf shading treatment in C_4 plants such as sugarcane resulted in the up-regulation of several genes associated with photosynthesis, mitochondrial metabolism, and sugar transport (McCormick et al., 2008). cDNA microarray analysis in sugarcane showed that elevated CO_2 levels modify the expression of genes related to photosynthesis and development (De Souza et al., 2008). Moreover, severely defoliated plants of perennial ryegrass showed increased abundance of photosynthesis-related gene transcripts (Lee et al., 2011). Changes in gene expression due to sink removal in soybean leaves were monitored using an oligonucleotide microarray in combination with targeted metabolite profiling (Turner et al., 2012). However, the genes related to metabolism and the selected signature genes showed diverse profiles in the above mentioned studies. Therefore, there is a lack of systematic analysis of changes in leaf gene expression under the source-sink regulation.

In this study, we studied the changes in photosynthesis and chlorophyll fluorescence parameters in *P. persica* leaves under normal sink demand and low sink demand by fruit removal. Moreover, we performed deep sequencing analysis using the Solexa digital gene expression system to compare the differentially expressed genes in response to –fruit and +fruit. These sequencing datasets allowed us to comprehensively characterize the molecular basis of the physiological processes under low sink demand and gain insight for further research.

MATERIALS AND METHODS

Plant Materials

In this study, we used 4-year-old peach “Zaojiubao” (mutant of “Okubo”) [*Prunus persica* (L.) Batch] trees, which have a mid-ripening peach with fruit maturity occurring in the middle of

July. The trees were planted 2 m apart within rows and 5 m apart between rows. They were trained to “Y” training systems and pruned using the long pruning method in winter (Li et al., 1994).

Treatments

During the final stage of rapid fruit growth (on 23 July 2010, about 85 days after full blossom), 1-year-old shoots located on the southwest and southeast sides of the tree in the outer part of the crown were used as the unit of sink-source manipulation. Those 1-year-old shoots with similar light exposure were selected according to their uniformity in length (40–50 cm) and growth status (at least one new shoot per 1-year-old shoot). Each selected 1-year-old shoot, which supported one fruit and one new shoot, was considered a plot. Eight mature leaves were retained on each new shoot by topping and removing the smaller basal leaves. Half of the shoots from the previous season had fruits while the fruit were removed from the other half after sunset on 23 July 2010. Moreover, the export of assimilates from the treated and untreated parts, including the base and top parts of the 1-year-old shoots, was strictly controlled by girdling of the 1-year-old shoots. Twenty one-year-old shoots per treatment were selected for measurements of gas exchange and chlorophyll fluorescence, and leaves were sampled from 12 one-year-old shoots per treatment for the gene analyses.

Measurement of Photosynthetic Gas Exchange Parameters

Photosynthetic gas exchange parameters including P_n , stomatal conductance (g_s), and intercellular CO_2 concentration (C_i) were measured using a Li-6400 portable photosynthesis system (Li-Cor Inc., Lincoln, NE, USA). The measurements were recorded between 0700 and 1800 h, on 25 July 2010, the 2nd day after initiating the source-sink manipulation on five leaves from each of five 1-year-old shoots per treatment. Photosynthetically active radiation (PAR), g_s , transpiration rate (E), C_i and leaf temperature (T_{leaf}) were obtained when P_n was measured.

Chlorophyll a Fluorescence Kinetics Transient Analysis (OJIP-Test)

The OJIP-test parameters were also measured on 25 July 2010, the same day as gas exchange measurement as Luo's methods (Luo et al., 2011). A Handy-Plant Efficiency Analyzer (Hansatech Instruments, King's Lynn, Norfolk, UK) was used for determine the fluorescence signals on the same leaves used for gas exchange measurements. The measurements were made after dark adaption for more than 15 min. The transients were induced by red light of about $3000 \mu\text{mol m}^{-2} \text{s}^{-1}$ provided by an array of six light emitting diodes (peak wavelength 650 nm). The fluorescence signals were recorded from 10 μs to 1 s with a data acquisition rate of 10 μs for the first 2 ms and every 1 ms thereafter. The following data from the original measurements were used: F_m : maximal fluorescence intensity; F_k : fluorescence intensity at 300 μs [required for calculation of the initial slope (M_0) of the relative variable fluorescence (V) kinetics and W_k]; F_j : the fluorescence intensity at 2 ms (the J-step), F_i : the fluorescence intensity at 30 ms (the I-step). The derived parameters were as follows: F_0 : fluorescence intensity at 50 μs . The parameter W_k

on donor side of photosystem II (PSII), represents the damage to oxygen evolving complex (OEC), $W_k = (F_k - F_o)/(F_j - F_o)$; the parameter RC_{QA} on reaction center of PSII, represents the density of Q_A -reducing reaction centers, $RC_{QA} = \varphi_{P_o} \times (V_j/M_o) \times (ABS/CS)$; the parameter φ_{P_o} on acceptor side of PSII, represents the maximum quantum yield of primary photochemistry at $t = 0$, $\varphi_{P_o} = TR_o/ABS = 1 - F_o/F_m$; the parameter φ_{E_o} on acceptor side of PSII, represents quantum yield for electron transport (at $t = 0$), $\varphi_{E_o} = ET_o/ABS = (F_m - F_j)/F_m$; the parameter ψ_{E_o} on acceptor side of PSII, represents the probability with which a trapped exciton moves an electron into the electron transport chain beyond Q_A^- , $\psi_{E_o} = ET_o/TR_o = (F_m - F_j)/(F_m - F_o)$. The calculation and derivation of a range of new parameters from O-J-I-P transients is shown in **Table S1**. Five independent replicates were used in both treatments and controls respectively.

Digital Expression Library Construction and Solexa Sequencing

Leaves were sampled at 1400 h on 25 July, the same day as gas exchange measurement. Total RNA was isolated from the pooled samples of three replicates with or without source-sink treatment, using plant total RNA isolation kit (Tiandz Inc.; Beijing, China). Gene Expression Sample Prep Kit (Illumina Inc.; San Diego, CA, USA) was used for sequence tag preparation according to the manufacturer's protocol. Six micrograms of total RNA were extracted and the mRNA was purified via Biotin-Oligo (dT) magnetic bead adsorption. First strand cDNA was synthesized with oligo (dT) on the bead. After second-strand cDNA synthesis, double strand cDNA was digested with NlaIII endonuclease producing a bead-bound cDNA fragment containing sequence from the 39-most CATG to the poly-A tail. These cDNA fragments were purified with magnetic bead precipitation and Illumina adapter 1 (GEX adapter 1) was added to newly formed 5' sticky end of cDNA fragments. The junction of GEX adapter 1 and CATG site was recognized by MmeI, which cuts 17 bp downstream of the CATG site, producing 17 bp cDNA sequence tags with GEX adapter 1. The 3' fragments were removed using magnetic bead precipitation; and the Illumina adapter 2 (GEX adapter 2) was ligated to the new 3' end of the cDNA fragment, which represented the tag library.

The cDNA fragments with GEX adapters 1 and 2 were subject to 15 cycles of linear PCR amplification by Phusion polymerase (Finnzymes, Espoo, Finland). The resulting 85 base fragments were purified by 6% TBE PAGE Gel electrophoresis. After double strand denaturation, the single chain molecules were fixed onto the Solexa Sequencing Chip (flow cell). Each molecule grew into a cluster sequencing template through *in situ* amplification, which represented a single tag derived from a single transcript. The sequencing was by the Beijing Genomics Institute (BGI, www.genomics.org.cn) using an Illumina HiSeq 2000 System. Four color-labeled nucleotides were added during sequencing; and the produced 49 bp sequences contained target tags and a 3' adaptor. Base-calling was performed using the Illumina Pipeline. After purity filtering and initial quality tests, the reads were sorted and counted for the following analysis. The clean reads data of -fruit and +fruit used in this manuscript have

been uploaded respectively to SRA database at NCBI (accession numbers: SAMN05178616 and SAMN05178617).

Sequence Annotation

"Clean Tags" were obtained by trimming adapter sequences and filtering adaptor-only tags and low-quality tags (containing ambiguous bases) using the Fastx-toolkit (http://hannonlab.cshl.edu/fastx_toolkit). Sequence alignment was done with Bowtie 0.12.8 using the Peach Genome database (http://www.rosaceae.org/species/prunus_persica/genome_v1.0). All clean tags were annotated based on transcript sequences of peach reference genes, masked peach genome sequences (excluding the repeating sequences), and NCBI. For conservative and precise annotation, only sequences with perfect homology or one nucleotide mismatch were considered for further annotation.

Identification of Differentially Expressed Genes

Numbers of annotated clean tags for each gene were calculated after alignment and then normalized to TPM (tags per million clean tags) (AC't Hoen et al., 2008; Morrissy et al., 2009). The genes that had <10 TPM in both +fruit and -fruit libraries were excluded first. The default value (tag number) of genes that not found in any of the libraries was one. Differentially expressed genes (DEGs) in -fruit as compared with +fruit were identified based on a rigorous algorithm (Audic and Claverie, 1997). *P*-value was used to test the authenticity of differential transcript accumulation (Audic and Claverie, 1997; Wu et al., 2010). In the *P*-value formula below, the total clean tag number of the +fruit library is noted as N_1 , and total clean tag number of -fruit library as N_2 ; gene A holds x tags in +fruit and y tags in -fruit library. The probability of gene A expressed equally between two samples can be calculated with:

$$P(y|x) = \left(\frac{N_2}{N_1}\right)^y \frac{(x+y)!}{x!y!(1 + \frac{N_2}{N_1})^{(x+y+1)}}$$

The Bonferroni corrected *P*-value was applied to control the false discovery rate (FDR) in the multiple comparison and analysis during the identification of DEGs (Benjamini et al., 2001). An $:FDR < 0.001$ and the absolute value of \log_2 ratio ≥ 1 was used as the threshold to determine the significance of gene expression differences. The differently expressed genes were categorized into functional groups and mapped using Mapman (version 3.5.1R2) according to the standard protocol (Usadel et al., 2009).

Real-Time PCR Analysis

Total RNA was isolated using the same method as used for DGE analysis. Real-time PCR was carried out using three independent biological replicates each containing three technical replicates. First-strand cDNA was synthesized using Oligo (dT)₁₅ (Sigma, Hamburg, Germany) and Superscript III Reverse Transcriptase (Invitrogen, Carlsbad, CA, USA). cDNAs were diluted 20 fold for use as template. Specific primer pairs of 10 transcripts were designed using Primer3 (v. 0.4.0; <http://frodo.wi.mit.edu/>) and shown in **Table S2**. Experiments were carried out using FastStart Universal SYBR Green Master

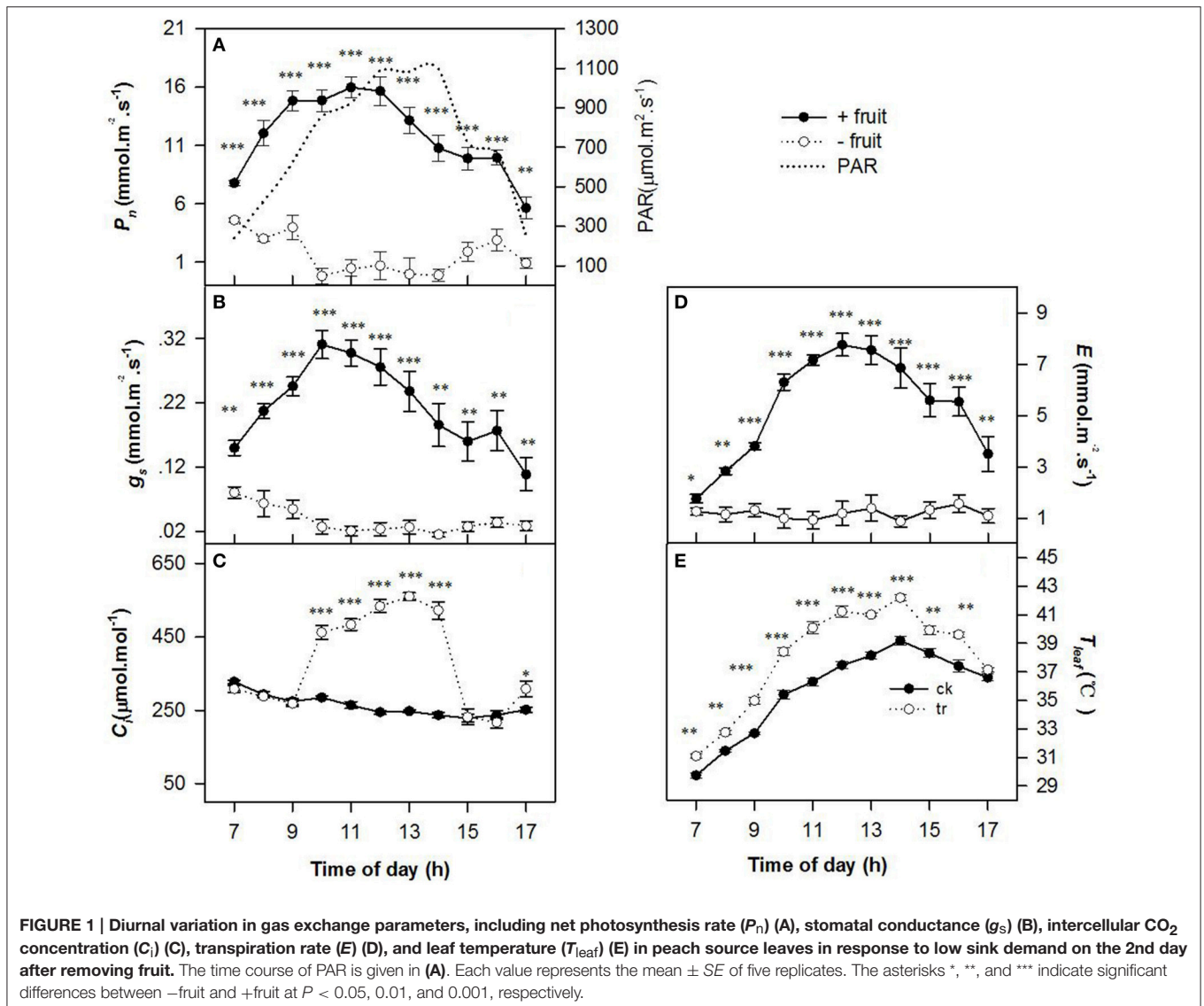
(Roche Diagnostics, Mannheim, Germany) with SteepOneplus™ Real-Time PCR system (Applied Biosystems). Data were analyzed using qbase^{PLUS} software (<http://www.biogazelle.com/products>). Transcript levels were normalized against the peach reference glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene (ppa006087m; Forward primer: 5-GAAATTCGATTGCA TGAGC-3, Reverse primer: 5-CAATGCCATTCAAGCTAAGG-3) according to Tong et al. (2009). The fold change in mRNA expression was estimated using threshold cycles, by the $\Delta\Delta CT$ method.

RESULTS

Diurnal Variations of Photosynthetic Parameters

Low sink demand had significant effects on the diurnal variations in P_n , g_s , C_i , E , and T_{leaf} (Figure 1) on the 2nd day after removing fruit. Values of P_n , g_s , and E were gradually increased until

1100 h reached maximum when PAR about $1000 \mu\text{mol m}^{-2} \text{s}^{-1}$, thereafter P_n , g_s , and E decreased slowly as PAR increased in +fruit shoots. The -fruit treatment significantly reduced P_n , g_s , and E throughout most of the day compared with the +fruit treatment (Figures 1A,B,D). At 1300 h, values of P_n , g_s , and E of -fruit were only 2.13, 11.05, and 1.38% of +fruit values respectively. P_n and g_s decreased to almost zero between 1000 h and 1400 h. Trends in T_{leaf} (Figure 1E) were similar to those in PAR. T_{leaf} reached the maximum (42.19°C) when PAR was about $1100 \mu\text{mol m}^{-2} \text{s}^{-1}$ at 1400 h. Throughout most of the day T_{leaf} values in leaves of -fruit shoots were significantly higher than in leaves of +fruit shoots (Figure 1E). The pattern of diurnal change in C_i , differed between the +fruit and -fruit treatments (Figure 1C). Maximal C_i occurred just after sunrise then decreased gradually in leaves in both treatments. Values of C_i decreased until the lowest value at 1100 h, and began to recover at 1600 h in the afternoon in +fruit, whereas it increased sharply at 0900 h, and high C_i was maintained between 1000 h to 1400 h



in –fruit. Moreover, significantly higher C_i values were obtained in –fruit than in +fruit from 1000 until 1400 h.

Diurnal Variations of Chl Fluorescence Parameters

We further investigated the relationship between P_n decline and electron transport chain of PSII under the low demand by chlorophyll *a* fluorescence kinetics transient (OJIP-test). W_k had similar diurnal variation patterns in both –fruit and +fruit (Figure 2A). In the morning W_k increased progressively up to about 1300 h, and then they decreased. Parameters RC_{QA} , φ_{P_0} , φ_{E_0} , ψ_{E_0} , and δ_{R_0} remained relatively stable throughout the day in +fruit plants, however they were at a maximum at 0700 h, then decreased progressively up to about mid-day, and remained at a low level in the afternoon (Figures 2B–E) except δ_{R_0} in –fruit plants. Low sink demand resulted in RC_{QA} , φ_{P_0} , φ_{E_0} , ψ_{E_0} about 24, 13, 16, 11% lower values, and W_k about 8% higher than

+fruit at 1300 h respectively. Parameter δ_{R_0} signifies the redox state of photosystem I (PSI). However there was not significant differences in δ_{R_0} between –fruit and +fruit although lower in the beginning and the end of day and higher values around noon were observed in –fruit than in +fruit (Figure 2F).

Digital Expression Libraries Construction and Tag Sequencing

Unique tags that perfectly matched reference genes in each library were normalized to tags per million clean tags (TPM) and used to evaluate the expression level of transcripts. The transcripts detected with at least two-fold differences in the two libraries are shown in Figure 3 (FDR < 0.001). The details of DEGs, including original TPM, fold-change, annotation, *P* value and FDR in both materials are shown in Table S3.

The distribution of unique tags with different copy numbers (clean tags) in +fruit and –fruit libraries were counted

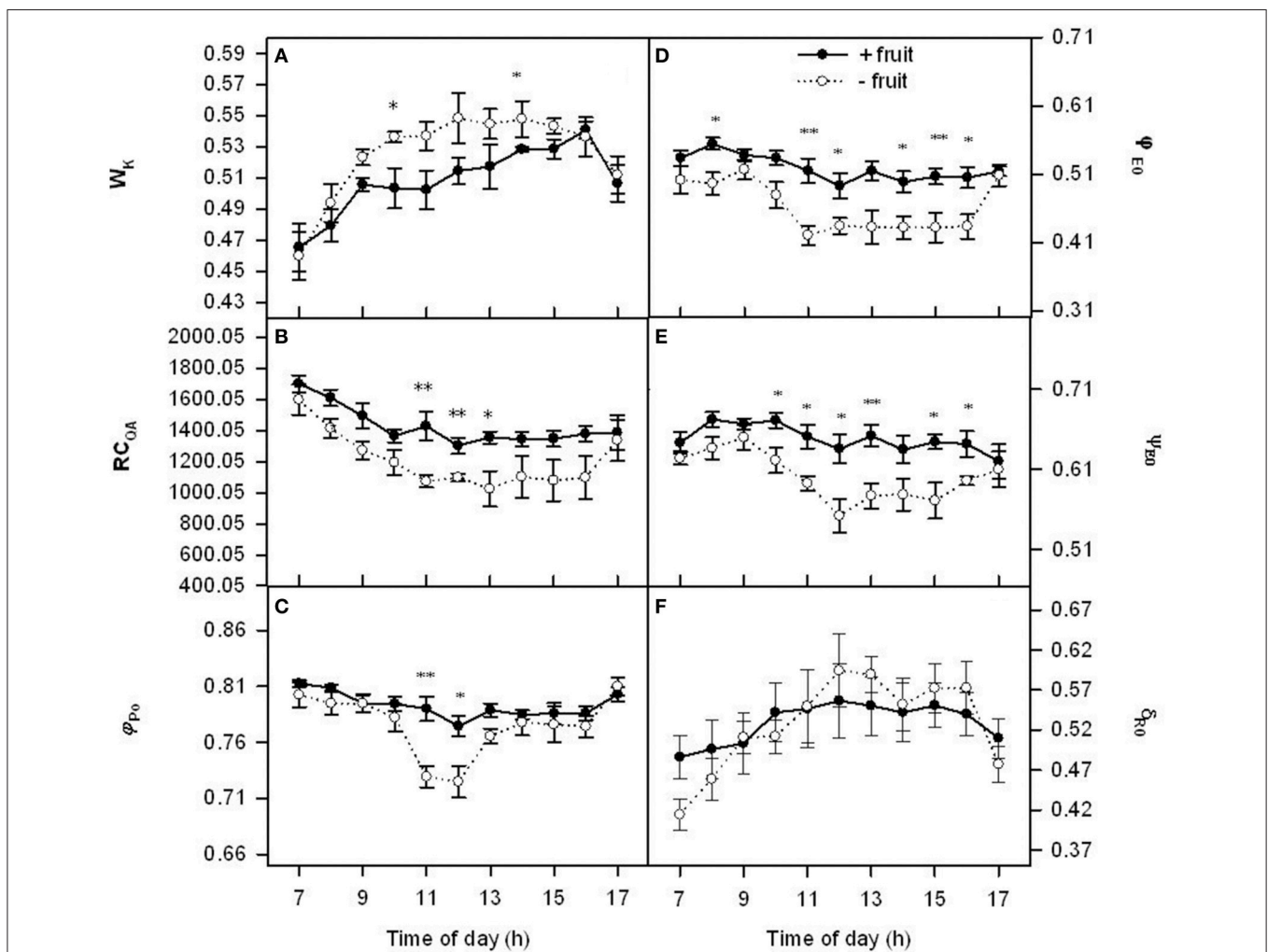


FIGURE 2 | Diurnal variations in donor side (W_k), reaction center (RC_{QA}), acceptor side (φ_{P_0} , φ_{E_0} , ψ_{E_0}) (A–E) parameters of PSII and δ_{R_0} (the efficiency with an electron can move from plastoquinone (PQ) through PSII to the PSI end electron acceptor) (F) in peach source leaves in response to low sink demand on the 2nd day after removing fruit. Each value represents the mean \pm S.E. of five replicates. The asterisks *, **, and *** indicate significant differences between –fruit and +fruit at $P < 0.05$, 0.01 , and 0.001 , respectively. The detailed meanings of W_k , RC_{QA} , φ_{P_0} , ψ_{E_0} , φ_{E_0} , and δ_{R_0} were shown in Table S1.

(Table 1). A total of 6,039,500 and 5,857,099 raw tags were sequenced in +fruit and –fruit libraries, including 247,102 and 243,331 distinct tags, respectively. Low quality tags and virus contaminations were filtered, and single-copy tags were excluded after which 118,192 and 104,826 distinct tags were obtained in each library. The majority of clean tags (about 82% from each library) were present in low copy numbers (<10 copies), and ~10% tags from each library were counted between 11 and 100 times. Approximately, 3.3% tags were detected more than a 100 times.

Analysis of Tag Mapping

The sequencing saturation was analyzed in the two libraries based on the number of identified genes to estimate whether the sequencing depth was sufficient for the transcriptome coverage. The number of tags reached saturation when no new genes were detected (Figure S1). All samples reached a plateau shortly after four million tags or higher were sequenced in both +fruit and –fruit libraries. No new genes were identified as the tag number approached six million in both libraries indicating that the capacity of the two libraries had approached saturation.

To identify the genes corresponding to 118,192 and 104,826 meaningful tags in each library, an essential dataset containing 286,689 reference genes expressed in the peach genome from

<http://www.rosaceae.org/node/355> was used. Altogether, 270,059 genes (94.32%) have the CATG sites, resulting in a total number of 147,813 unambiguous reference tags. By assigning the experimental Solexa tags to the virtual reference ones (Table S4), we observed that 44,173(37.4%) and 37,007 (35.3%) tags were perfectly matched to +fruit and –fruit libraries respectively for the reference genes. Moreover, ~18% tags in the two libraries were mapped to the antisense strands suggesting that those regions might be directionally transcribed.

Altogether, there were 52,347 (44.3%) tags in the +fruit library and 43,863 (41.8%) tags in the –fruit library were found to match the annotated reference genes. The unmatched tags were then blasted against the peach genome, and ~40% tags were matched to the genomic sequences in the two libraries. As a result of the significant sequencing depth of Solexa technology and incomplete annotation of the peach genome. However, there were 19.3 and 17.8% unmatched tags in each library as result of the significant sequencing depth of Solexa technology and incomplete annotation of the peach genome.

Function Categories of Differentially Expressed Genes

The functional classification of DEGs was further examined in peach to investigate the pattern of transcriptome regulation that

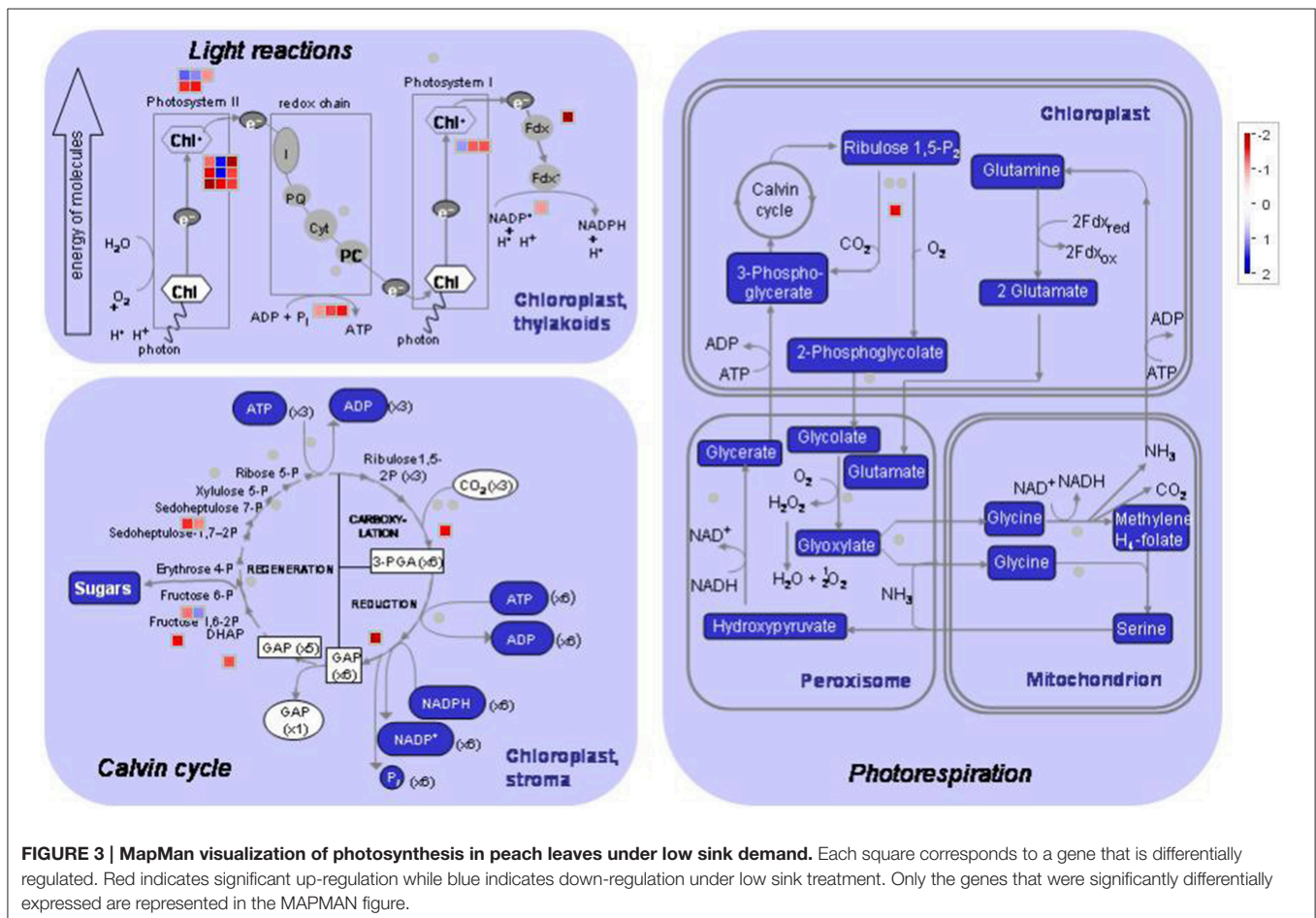


TABLE 1 | Distribution of the sequenced tags from libraries of peach leaves under normal sink demand (+fruit) or low sink demand (–fruit).

	+fruit	–fruit
Total tags	6,039,500	5,857,099
Clean tags	5,902,114	5,709,974
Total number of distinct tags	247,102	243,331
Unique tag	118,192	104,826
Tag copy number <2 (clean tag)	121,198(49.05%)	130,727(53.72%)
2–5	67,343 (27.25%)	57,105 (23.47%)
6–10	15,889 (6.43%)	14,452 (5.93%)
11–20	11,000 (4.45%)	10,247 (4.21%)
21–50	10,093 (4.08%)	9697 (3.99%)
51–100	5514 (2.23%)	5315 (2.18%)
>100	8353 (3.38%)	8010 (3.29%)

occurred under the low sink demand. These genes were found to cover a lot of functions by using MapMan functional categories. Thereafter, the 1765 differently expressed proteins were classified into functional categories with the exception of 554 genes that were not assigned to any groups (Figure 4). The main categories included protein (16.4%), RNA (10.5%), and transport (5.4%). Miscellaneous enzyme families, signaling, stress, cell, hormone metabolism, development, and photosynthesis categories each accounted for 2.0–5.0% of the DEGs. Each of the other categories accounted for <2% of DEGs. Full datasets are available online in Table S3. The photosynthesis related genes were regulated by source-sink relationship treatment.

As regards genes related to photosynthesis, a total of 25 genes were down-regulated under low sink demand (Table 2, Figure 3). 17 genes were involved in the light reaction. Among these groups, one oxygen-evolving complex-related gene was severely inhibited. The expression levels of LHCB3 and LHCA2 were inhibited under low sink demand. However, the expression of LHCB6 and LHC2.1 increased in the expression of genes. The PsaO subunit of PSI is declined under low sink demand. PPL2 (PsbP-like protein 2), PsbP, PsbY, and thylakoid lumenal 19 kDa protein were repressed while PsbR increased under low sink demand. In the Calvin cycle, seven genes included seduheptulose biphosphatase (SBPase), fructose-1,6-biphosphatase (FBPase), aldolase, triose-phosphate isomerase (TPI), glyceraldehyde-3-phosphate dehydrogenase B subunit (GAPB), and Rubisco activase (RCA) were severely repressed. Only RCA was down-regulated in photorespiration.

Confirmation of DEGs by Real-Time PCR Analysis

Ten candidate genes that showed change in the pattern of expression in response to low sink demand were randomly selected from the peach DEGs for Real-time PCR analysis. Among them, six genes were up-regulated and four genes were down-regulated. The list of the genes and the comparison of fold changes between deep sequencing and Real-time PCR in +fruit and –fruit were shown in Table S5. The primers used for Real-time PCR of the selected genes are listed in Table S2. The

Real-time PCR based expression patterns of all 10 selected genes showed a trend similar to that detected by the Solexa-sequencing method, which confirmed the reliability of our transcriptome analysis (Table S5).

DISCUSSION

Leaf transpiration and temperature play an important role on the source-sink relationship. Low sink demand by removing fruit or tuberous root sink resulted in significantly decreased g_s but increased T_{leaf} in higher plants (DaMatta et al., 2008; Duan et al., 2008; Wu et al., 2008; Fan et al., 2010; Yan et al., 2011). So Li et al. (2001) suggested that the decreased g_s may be considered as the trigger or promoter and increased T_{leaf} as the actor for regulating photosynthesis under a lower sink-source ratio. Low sink by fruit removal resulted in a decreased P_n with lower g_s and higher T_{leaf} in this study (Figure 1), which corroborates the results of previous studies in peaches or in other higher plants (Li et al., 2005; Fan et al., 2010; Yan et al., 2013). Moreover, significantly higher C_i was observed in –fruit than in +fruit (Figure 1C). In general, leaf C_i increases with a decrease in g_s and P_n when there is non-stomatal limitation in higher plant (Farquhar and Sharkey, 1982). Thus, the lower P_n under low sink demand in –fruit in this study was primarily due to non-stomatal limitation.

In OJIP-test, RC_{QA} shows the density of the of Q_A -reducing PSII reaction centers. W_k is used as a specific indicator of damage to PSII donor side (Strasser, 1997), while ϕPo , ϕEo , and ψEo represent the acceptor side parameters of PSII. Low sink demand mainly resulted in a decrease in the acceptor side parameters ϕPo and ψEo of PSII and PSII reaction centers parameters RC_{QA} (Figure 2). These results in the present study were similar to the results obtained on bean at late stages after the removal of the sink of roots and pods plants (Yan et al., 2013). The P_n reduction could be attributed to essentially the probability that a trapped exciton moves an electron into the electron transport chain beyond Q_A^- and ϕPo (Xiang et al., 2013).

Photosynthesis is one of the most heat sensitive processes and it can be completely inhibited by high temperature before other symptoms of the stress are detected (Berry and Björkman, 1980). In this study 31 genes involved in the light reaction, Calvin cycle and photorespiration were down-regulated under low sink demand (Table 2, Figure 3). These changes in photosynthesis-related genes were similar to those observed in the application of a cold-girdle to C_4 sugarcane (McCormick et al., 2008). Linear electron flow involves light-stimulated electron transfer between PSII and PSI, which stores the majority of photosynthetic energy. A total of 17 genes related to electron transfer were significantly down-regulated (Table 2), suggesting that the light reaction might be repressed by low sink demand. The repression electron transport causes the production of significant reactive oxygen species (ROS) early in the low sink response resulting in the inhibition of plant photosynthesis (Duan et al., 2008).

The light-harvesting complex (LHC) functions as a light receptor, and captures and delivers excitation energy to photosystems. LHCB3 serves as an intermediary in light energy transfer from the main LHCB1/LHCB2 antenna to the core

of PSII (Standfuss and Kühlbrandt, 2004). In this study, the expression levels of LHCB7, LHCB3, and LHCA2 were inhibited under low sink demand. However, the expression of LHCB6 and LHC2.1 increased, indicating that they may be stable under low sink demand. The PsaO subunit of PSI is involved in balancing the excitation pressure between the two photosystems. Consistent with this observation, the levels of PsaE-2 and PsaO declined under low sink demand (Table 2).

PsbP (23 kD) is one of three extrinsic nuclear-encoded subunits of eukaryotic PSII oxygen-evolving complex (OEC). PsbR (10 kD) protein found in plant PSII plays a role in water oxidation (Roose et al., 2007). PsbY is one of the low molecular mass subunits of oxygen-evolving PSII (Kawakami et al., 2007). A PsbP-like protein 2 was previously shown to be essential for the accumulation of the chloroplast NAD(P)H dehydrogenase (NDH) complex (Ishihara et al., 2007). In the present study, PPL2 (PsbP-like protein 2), PsbP, PsbY, and thylakoid luminal 19 kDa protein were repressed while PsbR increased under low sink demand. Moreover, the chlorophyll fluorescence parameter W_k also showed that the OEC of PSII was damaged under low sink demand.

In the Calvin cycle, seven genes (SBPase, FBPase, TPI, GAPB, RCA) were severely repressed involved in the reduction,

regeneration, and carboxylation (Table 2). The repression of these genes suggested that these processes were negatively regulated by low sink demand. Only one gene was down-regulated in photorespiration indicating that most genes involved in photorespiration are not responsive to low sink demand. Arabidopsis plants growing for long periods under high CO_2 resulted in a significant decrease in *rbcL* and *rbcS* transcripts, which encode the large and small subunits of Rubisco, respectively (Cheng et al., 1998).

In this study, nine Hsps were up-regulated under the low sink demand (Table 3). Most of them were belonged to one of the three major classes of molecular chaperones, HSP90, HSP70, and sHSPs. Four sHSPs were up-regulated in low sink demand compared to control. In plants, sHSPs have been reported to be involved in protecting macromolecules like enzymes, lipids, nucleic acid, and mRNAs from dehydration (Yamaguchi-Shinozaki et al., 2002). sHSPs are the most abundant and diverse HSPs produced at high temperatures (Palmlblad et al., 2008). Furthermore, some sHSPs are also known to be induced by various abiotic stresses such as cold, salinity, drought, and chemical pollution (Palmlblad et al., 2008). Proteins from the HSP70 family are essential for preventing aggregation and assisting re-folding of non-native proteins under stressing

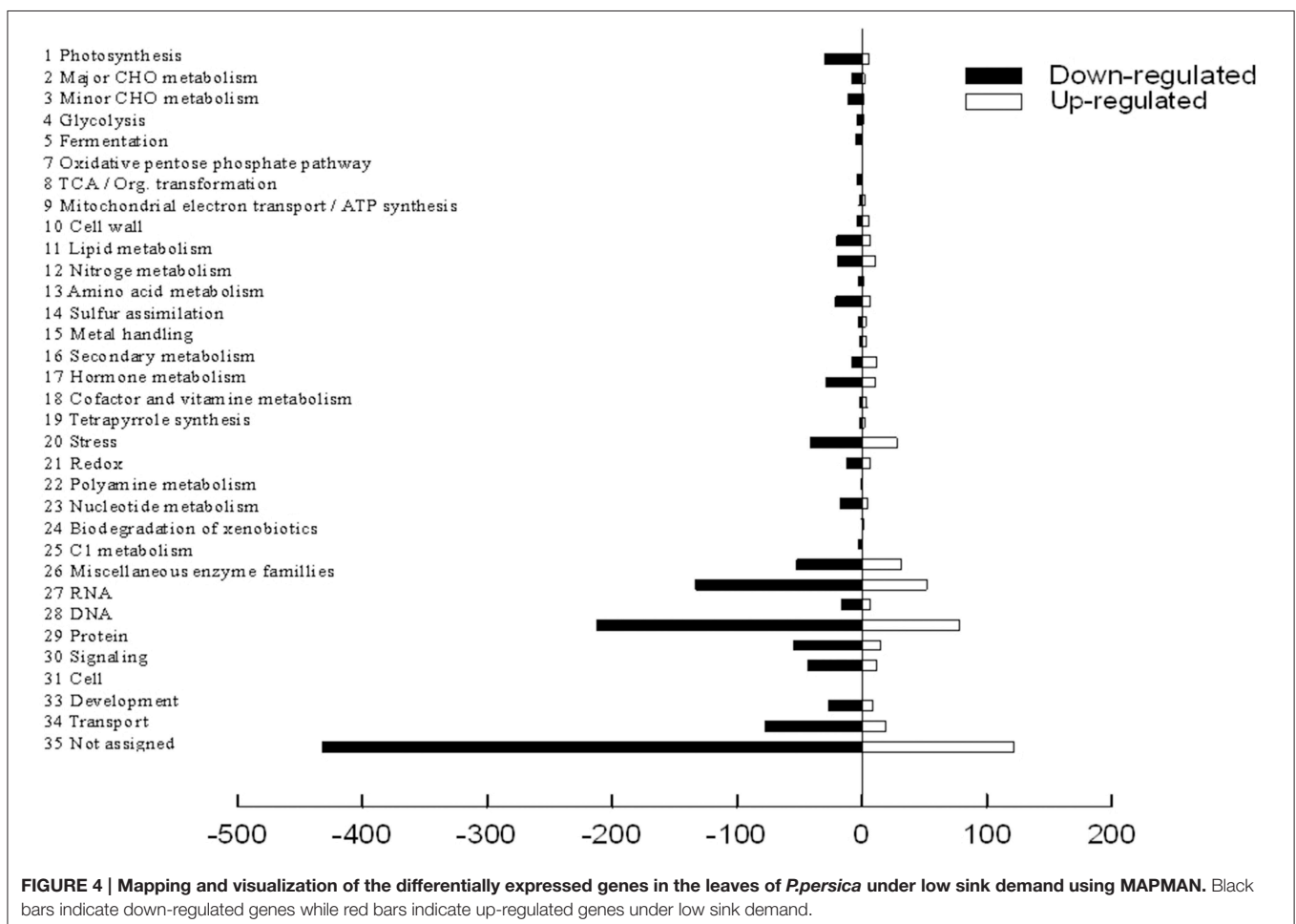


TABLE 2 | The list of genes photosynthesis regulated under low sink demand, based on MapMan functional categories.

Biological process	Accession number	Fold change	Bin	Species	Annotation	
Light reaction	PSII					
	XP_002298178.1	1.27	1.1.1.1	<i>Populus trichocarpa</i>	Light-harvesting complex II protein Lhcb6	
	AAC34983.1	1.09	1.1.1.1	<i>Prunus persica</i>	Light harvesting chlorophyll a/b binding protein	
	XP_002510744.1	-1.05	1.1.1.1	<i>Ricinus communis</i>	Chlorophyll a/b binding protein, Lhcb7	
	XP_002525758.1	-1.48	1.1.1.1	<i>Ricinus communis</i>	Chlorophyll a/b binding protein, Lhcb3	
	XP_002299309.1	-1.56	1.1.1.1	<i>Populus trichocarpa</i>	Light-harvesting complex I protein Lhca2	
	NP_565906.1	-1.23	1.1.1.2	<i>Arabidopsis thaliana</i>	PPL2 (psbp-like protein 2); calcium ion binding	
	ADB93062.1	1.57	1.1.1.2	<i>Jatropha curcas</i>	Chloroplast photosystem II 10 kDa polypeptide	
	XP_002526766.1	-1.97	1.1.1.2	<i>Ricinus communis</i>	Thylakoid lumenal 19kDa protein, chloroplast precursor, putative	
	XP_002521576.1	-1.71	1.1.1.2	<i>Ricinus communis</i>	Oxygen-evolving enhancer protein 2, chloroplast precursor, putative	
	XP_002512708.1	1.66	1.1.1.2	<i>Ricinus communis</i>	Photosystem II 11 kDa protein precursor, putative	
	XP_002515034.1	-1.39	1.1.1.2	<i>Ricinus communis</i>	Photosystem II core complex proteins psbY, chloroplast precursor	
	NP_196706.2	-2.49	1.1.1.2	<i>Arabidopsis thaliana</i>	PsbP domain-containing protein 5	
	AAM61552.1	-1.69	1.1.1.2	<i>Arabidopsis thaliana</i>	Thylakoid lumen protein, chloroplast precursor	
	NP_563737.1	-1.42	1.1.1.2	<i>Arabidopsis thaliana</i>	Photosystem II D1 precursor processing protein PSB27-H2	
	PSI	AAO85557.1	1.00	1.1.2.2	<i>Nicotiana attenuata</i>	Photosystem I subunit XI
		BAA07667.1	-1.27	1.1.2.2	<i>Nicotiana sylvestris</i>	PSI-E subunit of photosystem I
		CAB75430.1	-1.32	1.1.2.2	<i>Nicotiana tabacum</i>	Putative 16kDa membraneprotein
Redox chain	NP_565711.1	-1.01	1.1.4	<i>Arabidopsis thaliana</i>	ATP synthase protein I -related	
	XP_002518477.1	-1.39	1.1.4.4	<i>Ricinus communis</i>	ATP synthase gamma chain 2, chloroplast, putative	
	NP_194953.1	-1.56	1.1.4.9	<i>Arabidopsis thaliana</i>	ATP synthase family	
	XP_002516617.1	-2.63	1.1.5.2	<i>Ricinus communis</i>	Electron carrier, putative	
	XP_002533800.1	-1.03	1.1.5.3	<i>Ricinus communis</i>	Ferredoxin-NADP reductase, putative	
Calvin cycle	ABK76304.1	-1.47	1.3.9	<i>Morus alba var. multicaulis</i>	Chloroplast sedoheptulose-1,7-bisphosphatase	
	XP_002530415.1	-1.09	1.3.9	<i>Ricinus communis</i>	Sedoheptulose-1,7-bisphosphatase, chloroplast, putative	
	ABW38330.1	-1.18	1.3.7	<i>Fragaria X ananassa</i>	Chloroplast fructose-1,6-bisphosphatase I	
	ABW38331.1	1.08	1.3.7	<i>Fragaria X ananassa</i>	Chloroplast fructose-1,6-bisphosphatase II	
	AAR86689.1	-1.66	1.3.6	<i>Glycine max</i>	Fructose-bisphosphate aldolase	
	XP_002529248.1	-1.34	1.3.5	<i>Ricinus communis</i>	Triosephosphate isomerase, putative	
	ABA86964.1	-1.89	1.3.4	<i>Glycine max</i>	Glyceraldehyde-3-phosphate dehydrogenase B subunit	
	ADD60242.1	-1.67	1.3.13	<i>Glycine max</i>	Alpha-form rubisco activase	
Photorespiration	ADD60242.1	-1.67	1.3.13	<i>Glycine max</i>	Alpha-form rubisco activase	

environmental conditions (Boston et al., 1996). HSP70 were accumulated under heat stress (Kosova et al., 2011; Liu et al., 2014). In this study, two members of the HSP70 family were up-regulated (Table 3). Hsp90 is one of the most common of the heat-related proteins. The majority of HSP90 known substrates are signal transduction proteins (Richter and Buchner, 2001), and it also uses a novel protein-folding strategy (Young et al., 2001). A putative HSP90 was up-regulated in *P. euphratica* at the early stage of heat stress. The -fruit treatment resulted in up-regulating two members of the HSP90 family (Table 3), which should play a role for preventing aggregation and assisting re-folding of

non-native proteins. Therefore, we should say the Hsps may have important functions when the sink demand is low in *P. persica*.

Antioxidant enzymes play important roles in scavenging or reducing excessive ROS produced under stress conditions (Lee et al., 2007). Fruit removal remarkably increased the activities of antioxidant enzymes (Duan et al., 2008). However, only the antioxidant enzyme catalase (CAT) was up-regulated in our study. Thioredoxins are proteins that act as antioxidants by catalyzing thiol-disulfide interchange involved in the regulation of the redox environment in cells (Serrato et al., 2002; Gelhaye et al., 2005). Four

TABLE 3 | The list of genes up-regulated of stress and redox under low sink demand, based on MapMan functional categories.

Accession number	Fold change	BIN	Species	Annotation
BIOTIC STRESS				
ABA26457.1	3.70	20.10	<i>Citrullus lanatus</i>	Acidic class III chitinase
ACE80957.1	3.03	20.10	<i>Prunus dulcis X Prunus persica</i>	Allergen prup 2.01a, putative
ADM22305.1	2.93	20.10	<i>Prunus domestica</i>	Pathogenesis related protein 5
ACZ52964.1	2.21	20.10	<i>Dimocarpus longan</i>	Chitinase
ACM45716.1	1.31	20.10	<i>Pyrus pyrifolia</i>	Class IV chitinase
AAK82460.1	1.30	20.10	<i>Cinnamomum camphora</i>	Type 2 ribosome-inactivating protein cinnamomin III precursor
AAR28754.1	1.25	20.10	<i>Solanum lycopersicum</i>	Bax inhibitor
ACM45716.1	1.12	20.10	<i>Pyrus pyrifolia</i>	Class IV chitinase
ABC47922.1	1.07	20.10	<i>Malus X Domestica</i>	Pathogenesis-related protein 1a
XP_002519358.1	1.56	20.1.7	<i>Ricinus communis</i>	Leucine-rich repeat-containing protein, putative
ABIOTIC STRESS				
XP_002285199.1	2.10	20.20	<i>Vitis vinifera</i>	Spx domain-containing protein 2 isoform 1
XP_002318460.1	3.23	20.2.1	<i>Populus trichocarpa</i>	Heat shock 22k family protein
XP_006486450.1	3.18	20.2.1	<i>Citrus sinensis</i>	18.2 kDa class I heat shock protein-like
P30236.1	2.49	20.2.1	<i>Glycine max</i>	22.0 kDa class iv heat shock protein
NP_200076.1	1.94	20.2.1	<i>Arabidopsis thaliana</i>	Heat shock protein 90.1
EOX91407.1	1.82	20.2.1	<i>Theobroma cacao</i>	Heat shock factor 4
XP_002332067.1	1.72	20.2.1	<i>Populus trichocarpa</i>	Heat shock protein 70 cognate
CAA52149.1	1.28	20.2.1	<i>Cucumis sativus</i>	Heat shock protein 70
XP_004306709.1	1.26	20.2.1	<i>Fragaria vesca subsp. vesca</i>	Bag family molecular chaperone regulator 6-like
XP_002515568.1	1.19	20.2.1	<i>Ricinus communis</i>	Heat shock protein binding protein, putative
XP_002879575.1	1.18	20.2.1	<i>Arabidopsis lyrata subsp. lyrata</i>	DNAJ/Hsp40 heat shock N-terminal domain-containing protein
NP_178487.1	1.01	20.2.1	<i>Arabidopsis thaliana</i>	Heat shock protein 90
ADN33944.1	1.15	20.2.2	<i>Cucumis melo subsp. melo</i>	Cold-shock DNA-binding family protein
ADP30960.1	1.60	20.2.3	<i>Gossypium hirsutum</i>	Dehydration-induced 19-like protein
XP_002535200.1	1.93	20.2.99	<i>Ricinus communis</i>	Major latex protein, putative
XP_002864359.1	1.26	20.2.99	<i>Arabidopsis lyrata subsp. lyrata</i>	Pollen ole e 1 allergen and extensin family protein
ABD33344.1	1.18	20.2.99	<i>Medicago truncatula</i>	Pollen ole e 1 allergen and extensin
NP_850016.1	1.03	20.2.99	<i>Arabidopsis thaliana</i>	Rd2
REDOX				
AAD33596.1	1.77	21.10	<i>Hevea brasiliensis</i>	Thioredoxin H
XP_003517423.1	1.45	21.10	<i>Glycine max</i>	Thioredoxin-like 2, Chloroplastic-Like
CAH59452.1	1.44	21.10	<i>Plantago major</i>	Thioredoxin 3
NP_196046.2	1.06	21.10	<i>Arabidopsis thaliana</i>	WCRKC2 (WCRKC thioredoxin 2)
XP_002878810.1	1.57	21.20	<i>Arabidopsis lyrata subsp. lyrata</i>	Membrane-associated progesterone binding protein 2
XP_002869447.1	1.05	21.2.2	<i>Arabidopsis lyrata subsp. lyrata</i>	Gamma-glutamyl transpeptidase 3
CAD42908.1	2.41	21.60	<i>Prunus persica</i>	Catalase

thioredoxins (thioredoxin h, thioredoxin 3, thioredoxin 2, and thioredoxin 3-2) were up-regulated in our study (Table 3) suggesting that CAT and thioredoxin play an important role in maintaining redox homeostasis in *P. persica* cells under low sink demand.

CONCLUSION

This study provided a global picture of gene changes in peach leaves under low sink demand using the Solexa digital gene expression system. Under low sink demand condition, net photosynthesis rate may be reduced due to increased leaf

temperature, during which some genes related to the electron transport chain of photosynthesis and HSPs were differentially regulated. It helped to gain insight into how peach leave photosynthesis adapted to low demand.

AUTHOR CONTRIBUTIONS

WD performed the experiments and wrote the manuscript. HX and GL helped perform the experiments and data analysis. ZL and PF helped design the experiment. SL designed the experiment and reviewed the manuscript. All authors have read and approved the final manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <http://journal.frontiersin.org/article/10.3389/fpls.2016.00883>

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Figure S1 | Sequencing saturation analysis of peach leaf libraries under normal sink demand (+fruit) or low sink demand (–fruit).

Table S1 | Summary of parameters, formulae and their description using data extracted from chlorophyll a fluorescence transient (O–J–I–P–test).

Table S2 | List of primers used for the Real-time PCR.

Table S3 | Detail information of peach DEGs under low sink demand.

Table S4 | Alignment of the unique tags to the reference genome and annotated genes from peach leaves under normal sink demand (+fruit) or low sink demand (–fruit) peach trees.

Table S5 | List of genes selected for Real-time PCR.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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