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VARIATION IN LEAF PHOTOSYNTHETIC CHARACTERISTICS IN A NEW BUD MUTANT OF *POPULUS*

JUNQIANG. LI[1,2], LIHUA. LIN[2], FAN. ZHANG[1,*], XUEQIN WAN[1], JINGLONG ZHAO[1], JIFEI DONG[1], LINGXIA SUN AND QIBING CHEN[1]

[1]Sichuan Agricultural University, 611130, Chengdu, China;
[2]Vocational and Technical College, Sichuan, 644003, Yibin, China;
**Corresponding author's e-mail: nolady@163.com; Tel: 86-28- 86290880*

Abstract

Variations in leaf pigment contents, gas-exchange characteristics, and other important leaf traits were investigated in the mutant 'Quanhong poplar' (QHP) and the wild type *Populus Linn* 2025 (L 2025) poplar. The significant differences were observed in photosynthetic pigment contents and photosynthetic efficiency between the mutant type and the wild type. The mean chlorophyll (Chl), carotenoid (Car) and anthocyanin (Ant) contents in the leaves of QHP were higher than that of L2025. Remarkable differences were observed in the rate of photosynthesis (P_N), stomatal conductance (g_s), transpiration rate (R), and intercellular CO_2 concentration (C_i). The mean P_N, R, g_s and C_i in QHP were significantly lower than that in L2025. Transcriptome sequencing revealed that the reduced photosynthetic rate was probably due to the inhibition of the photosynthetic electron transport rather than the changes of photosynthetic pigment contents, because the plant photosynthetic capacity is closely associated with both the photosynthetic pigment content, especially Chl and photosynthetic electron transport rate.

Key words: Bud mutation, Leaf pigments, Photosynthesis, Gas-exchanges, Poplar plants.

Abbreviations

QHP: Quanhong Poplar; L2025: *Populus* sp. Linn. '2025'; Chl: Chlorophyll; Car: Carotenoid; Ant: Anthocyanin; P_N: Rate of photosynthesis; g_s :Stomatal conductance; R: Transpiration rate; C_i: Intercellular CO_2 concentration; F_m and F_m: Maximal fluorescence in dark- and light-adapted leaves, respectively; F_o and F_o': Minimal fluorescence in dark- and light-adapted leaves, respectively; F_v and F_m: Maximum variable fluorescence in dark- and light-adapted leaves, respectively; F_v/F_m: Maximum quantum efficiency of PS II; F_v'/F_m': Efficiency of excitation energy capture by open photosystem II reaction centers; *AQE*: Maximum quantum efficiency; PSI:Photosystem I; PSII: Photosystem II; Cyt b6/f: Cytochrome b6/f: Fd: Iron redox proteins; FNR: Iron redox proteins-NADP reductase; DEGs: Differentially expressed genes

Introduction

Leaf color mutant is an obvious character, which has been used in the study of plant photosynthesis, chlorophyll biosynthesis, genetic transformation, etc (Li *et al*., 2012; Córdoba *et al*., 2016). Many color-leafed garden plants originate from bud sports(Zhang *et al*., 2016). For example, the red maple originates from the bud sports of *Acer palmatum* Thunb f. (Van Gelderen & Van Gelderen, 1999) and a hybrid of 'Zhonghong' poplar derives from *Populus* sp. L. '2025' (Zhu & Cheng, 2008).

Color mutation (yellow, albino, red, and stay-green) is often caused by changes of both the content and concentration of anthocyanin (Ant) and chlorophyll (Chl) as well as by the changes of the ratios of Chl / Ant in flowers or fruits. It is generally accepted that color mutant leads to chlorophyll deficient, and hence leads to a decrease of light absorption and photosynthetic capacity. It is due to the inactivation of Ribulose-1,5-bisphosphate (RuBP) which restricts the application of the mutants in the practice (Walker *et al*., 2006). However, some studies found that the mutants showed higher photosynthetic efficiency compared to the wild type. Zhou *et al*. (2006) showed that the photosynthesis capacity of the *Xantha* mutant (yellow plant) was unexpectlly enhanced with less content of all photosynthesis pigments in comparison with the wide type. Therefore, it is in debate that the photosynthesis capacity is not always linear to the decrease of chlorophyll in the mutants (Gong *et al*., 2001; Xu *et al*., 2004).

Poplar (*Populus*) has been widely used for the public landscape gardening. In poplar breeding, bud sports are critical materials to improve plant morphological appearances. 'Quanhong' poplar (QHP) (*Populus deltoides* W.Bartram ex Humphry Marshall), a latest red mutation, has been used in this study. QHP originated from the bud sports of the 'Zhonghong' poplar (*Populus* × *euramericana*), which derived from *Populus* sp. L. '2025'. Recently, QHP has been widely cultivated throughout China. With its bright and attractive reddish-purple leaves which are rarely seen from the other parts of the world, this tree was successfully selected as a new landscape tree in the Shanghai World Expo in 2010. QHP, as a new red-leaf poplar variety. It has higher ornamental value but slower growth rate when compared with the wild type. The reason for this difference remains unclear. Therefore, the goal of the study was to compare the leaf pigment contents, gas-exchange characteristics, and other important leaf traits in the mutant 'Quanhong poplar' (QHP) and the wide type poplar (L2025), and to evaluate the physiological differences induced by bud sport.

Materials and Methods

Plant materials: The experiments were performed with the bud mutant QHP (red leaf) and the wild-type L2025 (green leaf). QHP and L2025 were one-year-old and cultivated at the farm of Sichuan Agricultural University in Chengdu Plain in July 2011. The climate in Chengdu Plain belongs to subtropical monsoon climate. The annual

average temperature is about 18ºC and the average annual rainfall is about 1000 mm. The physical and chemical characters of the soil are as following: pH 5.8, total organic matters 13. 87g·kg $^{-1}$, total nitrogen 0.65g·kg $^{-1}$, total phosphorus 0.31 g·kg $^{-1}$, total potassium 2.43 g·kg $^{-1}$. The two plant materials were provided by Zhonghong Nursery, Rucheng Town, Henan Province.

Growth parameters: The heights and stem diameters of all seedlings were measured at the end of the growth. The total dry mass of each seedling by destructive harvesting were also measured. Dry mass (DM) of all parts was determined by weighing after drying in an oven at 65ºC for 48 h.

Pigment determination: Chlorophyll (Chl) was extracted following the previous method described by Lichtenthaler (1987). Total anthocyanin (Ant) content was measured following the method of Ubi *et al*. (2006). For analyzing carotenoids (Caro) content, leaf samples were ground in liquid nitrogen. Caro pigments were then analyzed by the reversed phase high-performance liquid chromatography (RP-HPLC) according to the methods described by Liu *et al*. (2007).

Gas-exchange parameters and Chl fluorescence: In the mid-August, gas-exchange and Chl fluorescence parameters were monitored using a portable photosynthesis system (LI-6400, LI-COR, USA). The fully expanded third, fourth and fifth leaves (from the apex) of each accession was used to quantify the gas-exchange and Chl fluorescence parameters. Measurements of each leaf were conducted with five replicates. Gas-exchange and Chl fluorescence parameters were determined according to the methods described by Zhang *et al.* (2011). It should be pointed out that gas-exchange measurements were not made simultaneously with fluorescence measurements. However, the gas-exchange and fluorescence measurements were conducted on the same part of the leaves.

RNA extraction and transcriptome sequencing: Two separate libraries were generated from the leaves of the bud mutant and the wild-type plants, respectively. For Illumina sequencing, the total RNA of each sample was isolated using an RNAiso^{TM} Plus (TaKaRa) protocol and then further purified with RNase-free DNase I (TaKaRa). After RNA extraction, Magnetic Oligo (dT) beads (Illumina) were used to isolate poly (A) mRNA. For each library, RNA samples of leaves from QHP or L2025 were equally pooled. Fragmentation buffer was added for interrupting mRNA to short fragments of length about 200 nt. Then these short fragments as templates, random hexamer (N6) primers (Illumina) was used to synthesize the first-strand cDNA. The second-strand cDNA was synthesized using 5X buffer, 10 Mm each dNTPs, 2U RNase H and 40U DNA polymerase I. Paired-end library was constructed by the Genomic Sample Prep kit (Illumina, San Diego, CA) according to manufacturer's instructions. Short fragments were purified with QiaQuick PCR extraction kit and resolved with EB buffer for end repair and adding poly (A). After that, the short fragments were connected with sequencing adapters. Transcriptome sequencing was undertaken using the Illumina Genome Analysis II platform. Transcriptome sequencing was finished by Shenzhen Huada Gene Company.

Statistical analysis: Analyses of variance (ANOVA) with orthogonal contrasts and mean comparison procedures were used to detect differences among the bud mutant and the wild-type. Mean separation procedures were carried out using the multiple range test with Fisher's least significant difference (LSD) ($p<0.01$).

Results

Morphological and growth responses: The poplar mutants QHP were propagated by grafting onto different rootstocks or by rooting cuttings; these mutants have remained stable and no reversion to the parental phenotype has been found. Tree habit, leaf morphology, and agronomic traits of the mutant trees were normal and indistinguishable from those of the wild type. However, the color of their leaves and shoots and the growth were remarkably distinguishable from that of L2025 (Fig. 1). The leaves and new shoots of QHP were dark purple from bud flashy in spring to late June. The leaves then showed a medium shade of purple from July to September. After October, they turned into bright red. The leaves of one-year-old seedlings turned into dark purple at the end of July in the first year while the petioles and veins of leaves were still bright red. In comparison, the leaves and shoots of L2025 always stay green throughout the whole growth stages. In addition, since bud sport inhibited growth, a significant decrease in plant height, plant basal diameter and biomass of one-year old QHP plants was observed in comparison with L2025. On average the plant height, basal diameter and dry weight of QHP decreased 37.9%, 41.8% and 22.7% respectively compare to L2025 (Table 1).

The dynamic changes of pigment contents: The seasonal changes of each pigment contents in the leaf of *Populus deltoids* 'QHP' showed regularly. Chlorophyll in the leaves of QHP was increased from April to September but decreased after October. Carotenoids content was maintained low in spring and autumn and increased in summer. In the contrast, anthocyanin content was lower in summer and higher in spring and autumn. In addition, the anthocyanin contents in the leaves of QHP were about 9 to 37 times than that of *Populus Linn* 2025, and the photosynthetic pigment contents were 1.3 to 2.2 times than *Populus Linn* 2025 during the study periods (Fig. 2). Therefore , the results suggested that high foliar levels of Ant as well as the Ant / Chl balance play an important role in coloration in the leaf of QHP poplar plants.

Table 1. The height, plant basal diameter and dry weight (mg plant^{-1}) of one-year old plants.

	Height / cm	Basal diameter/ mm	Dry weight (mg plant^{-1})
QHP	72.3 ± 3.4A	8.5 ± 1.3A	146.7 ± 3.3A
L2025	116.53 ± 4.6B	4.6 ± 1.2B	189.8 ± 4.6B

Each value is the mean ± SE of six replicates, and the terms A and B within each column indicate significant differences between means ($p<0.01$)

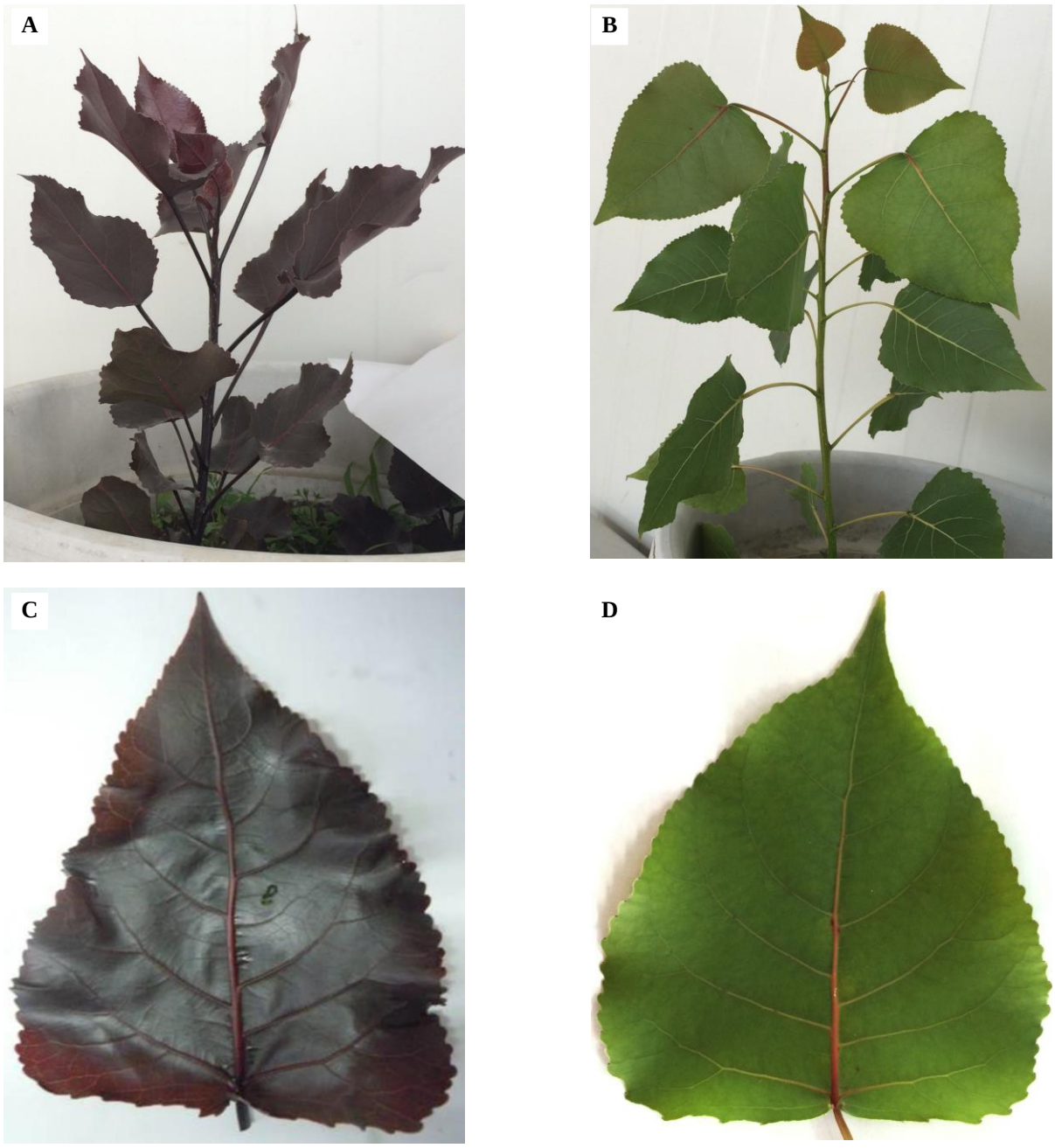

Fig. 1. QHP and L2025. QHP plants (a); L2025 plants (b); Leaf samples of QHP(c) and L2025 (d).

Photosynthetic gas exchange and chl fluorescence: Photosynthetic gas-exchange parameters were estimated in the leaves of the mutant and the wild type. Seedlings of QHP showed 37.4% lower net photosynthetic rate (P_{nmax}) and highly reduced stomatal conductance (g_s) and transpiration rate (R) than those of the L2025, QHP seedlings showed a tendency for reduction of the intercellular CO_2 concentration (C_i) (Table 2). This pattern also occurred in most of the Chl fluorescence parameters. F_v / F_m, F_v' / F_m', Φ_{PSII} and qP were significantly reduced in QHP when compared to L2025. In contrast, QHP seedlings showed that the minimum fluorescence (F_o) is about 43.3% higher than that of the L2025 and there was no significant difference in the maximum fluorescence (F_m) between QHP and L2025 plants (Table 3). It can be deduced that the normal level of F_m in QHP was consistent with the normal level of Chl content in comparison with L2025 since F_m is proportional to the total amount of Chl (Kauser *et al*., 2006). At the same time, the increase of F_o indicated that the capacity of the plant to convert light energy into electrical energy was inhibited by the bud mutation.

The differentially expressed genes (DEGs) related to the photosynthesis capacity of QHP plants: Based on the data of transcriptome sequencing, the DEGs related to the photosynthesis capacity of QHP were screened (Table 4). There were no differences in Photosynthetic system I and II, but PetC, Pet F, Pet H and delta were down-regulated in QHP (Table 4). As a result, the photosynthetic electron transfer had been slowed down, so as to the production of ADP and NADPH was dropped.

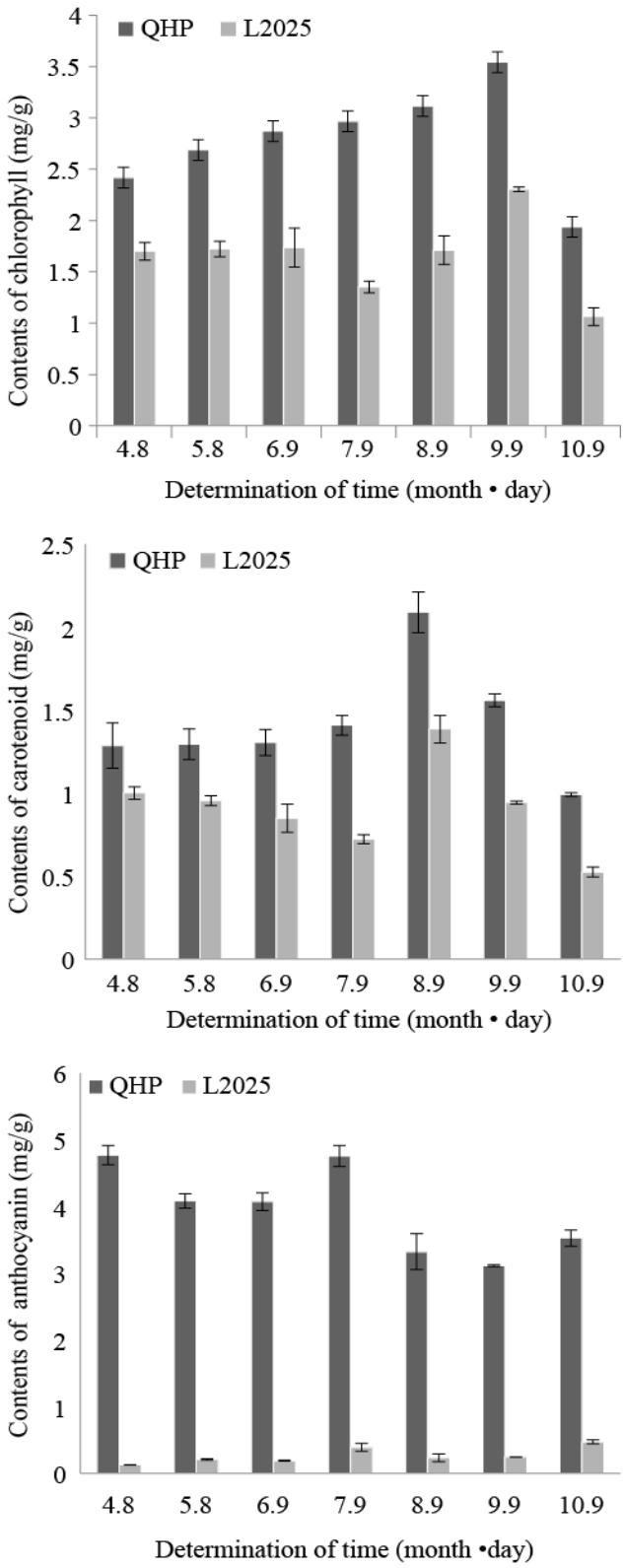

Fig. 2. Seasonal changes of chlorophyll, anthocyanidin and carotenoid contents in the leaf of two poplar varieties.

Discussion

The QHP phenotype is distinct with red-colored bark, petioles and leaf blades compared to the wild type L2025. This red appearance has become an attractive trait for landscape use. This study indicated that the growth and the photosynthetic rate of QHP were weaker, the phenophase of QHP was significantly later and the photosynthetic pigment content of QHP was higher than that of L2025. What do affect the growth of the mutant QHP? It is generally accepted that the growth and photosynthetic capacity is positively related to the content of the photosynthetic pigment (Ghirardi & Melis 1988; Masuda *et al*., 1996; Zhao *et al*., 2011). However, some researches showed that the mutant have higher photosynthetic efficiency than the wild type. Zhou *et al*. (2006) indicated that the photosynthesis capacity of the *Xantha* mutant (yellow plant) was unexpectlly enhanced while it had less content of all photosynthesis pigments compared to the wide type. Therefore, the photosynthesis capacity is not always linearly positivly related to the content of photosynthetic pigments in the mutants (Dai *et al*., 2000; Kiran *et al*., 2013).

Photosynthesis is a process used by plants and other organisms to convert light energy into electric energy, and further into chemical energy (Melis & Thielen, 1980). Photosynthesis needs many ingredients such as PSI, PSΠ, Cyt b6/f, ATPase et al. and is involved many steps such as light absorption, water photolysis, carbon dioxide, electron transport, ATP synthesis *et al*. (Bryant & Frigaard, 2006; Schreiber *et al*., 2012). Therefore, any step in the process that is inhibited will reduce the photosynthetic rate and further influence the growth of the plant.

In the study, the data from transcriptome sequencing indicated that DEGs (PetC, PetF and PetH) were significantly down-regulated in the mutant QHP when compared to L2025. The expression levels of PetC, PetF and PetH in QHP were 35.3%, 46.2%, and 37.1% lower than those in L2025, respectively. PetC is a subunit of Cyt b6/f. PetF is mainly involved in regulation the synthesis of Iron redox proteins (Fd) and PetH mainly regulated the synthesis of Iron redox proteins-NADP reductase (FNR). Cyt b6/f, Fd and FNR participated in the transportation of the photosynthetic electron. PetC, Pet F and Pet H were down-regulated in QHP. As a result, the photosynthetic electron transportation had been slowed down, so as to the production of ADP and NADPH was dropped.

On the other hand, *Delta* and *b* were subunits of F-ATPase (Amzel *et al*., 2003). The function of F-ATPase is to use a proton gradient to drive ATP synthesis (Gaspard & Gerritama, 2007). ATP can story the active chemical energy. The ATP production was decreased due to delta and b were down-regulated in QHP.

Conclusions

Based on the data of transcriptome sequencing, a reasonable conclusion can be obtained that the weaker growth and the lower photosynthetic rate which were found in the mutant QHP were clearly due to a direct consequence of the inhibition of the photosynthetic electron transportation. Similar research showed that the photosynthesis electron of green leaves have higher efficiency than red ones (Gould *et al*., 2002; Zivcak *et al*., 2013).

Table 2. Gas-exchange characteristics in QHP and L2025.

Accessions	P_{nmax} (μmolCO_2/m^2s)	R (μmolCO_2·/m^2s)	C_i (μmol CO_2/ mol)	AQE (molCO_2/ mol photon)	g_s (mmol/m^2s)
L2025	17.9 ± 0.4A	95.3 ± 0.02A	239.8 ± 5.3A	0.096 ± 0.01A	2.57 ± 0.05A
QHP	11.2 ± 0.2B	65.1 ± 0.01B	146.9 ± 3.4B	0.045 ± 0.01B	0.89 ± 0.02B

Each value is the mean ± SE of fifteen replicates, and the terms A and B within each column indicate significant differences between means ($p<0.01$)

Table 3. Chl fluorescence in the leaves of QHP and L2025.

Accessions	F_o	F_m	F_v/F_m	F_v'/F_m'	Φ_{PSII}	qP
L2025	96.5 ± 3.5B	628.4 ± 15.2A	0.85 ± 0.06A	0.72 ± 0.04A	0.58 ± 0.02A	0.82 ± 0.04A
QHP	138.3 ± 5.2A	615.3 ± 8.5 A	0.66 ± 0.02B	0.53 ± 0.02B	0.36 ± 0.01B	0.58 ± 0.03B

Each value is the mean ± SE of six replicates, and the terms A and B within each column indicate significant differences between means ($p<0.01$)

Table 4. The expression levels of the differentially expressed genes (DEGs) related to the photosynthesis capacity in QHP leaves when compared to L2025.

Gene name	Involved in Metabolic pathways	Differential expression
PetC	Synthesis of cytochrome complex subunit	35.3%
PetF	Synthesis of ferredoxin I	46.2%
PetH	Synthesis of ferredoxin-NADP oxidoreductase	37.1%
Delta	Synthesis of ATPase subunit	27.9%
Subunit *b* of F-ATPase	Synthesis of ATPase subunit	39.3%

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