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Original Research

Natural Albino Mutant of Daylily (*Hemerocallis* spp.) Reveals a Link between Drought Sensitivity and Photosynthetic Pigments Metabolism

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Abstract

Background: Mutant analysis remains one of the main genetic tools for characterising unclarified gene functions in plants, especially in non-model plants. Daylily (*Hemerocallis* spp.) is a popular perennial ornamental plant grown worldwide. Analysis of daylily mutants can enhance understanding of genes regulating the albino phenotype and improve the cultivar quality of daylily. **Methods**: The natural albino mutant ($Alb^{-/-}$) was isolated by screening a self-pollinated progeny of daylily cultivar 'black-eyed stella'. Transmission electron microscopy was used in analysing the structure of plastids between mutant and wild-type seedlings. The content of chlorophyll, carotenoids and chlorophyll precursors in plants was measured by ultraviolet spectrophotometry. RNA sequencing and physiological measurements were performed to explore the association between drought tolerance and mutation. **Results**: All the seedlings of the daylily albino mutants died spontaneously within fifteen days after germination when grown in soil. The carotenoid and chlorophyll content in the leaves of the mutant plants significantly decreased compared with those of the wild-type control. The mutant plants displayed stunted growth, and their leaves were white or light yellow in color. Abnormal plastids such as those showing endomembrane vesiculation and lacking stacking were discovered in the leaves of mutant plants. Furthermore, genetic analysis revealed that a single recessive nuclear gene mutation led to the albino trait, RNA sequencing and real-time quantitative PCR validation showed extensive differences in gene expression between the mutant plants and the wild-type control, and most of the genes related to chlorophyll metabolism were down-regulated, with foldchange ranging from 0.20–0.49. Additionally, the surviving homozygous plants ($Alb^{+/+}$), which do not contain this mutation, were also isolated by analysing the phenotype of their self-pollinated progeny. The net photosynthesis rate and light saturation point of $Alb^{+/+}$ were higher than those of heterozygous ($Alb^{+/-}$) plants. Additionally, the $Alb^{+/+}$ plants were more tolerant to drought conditions than the $Alb^{+/-}$ plants, suggesting that a heterozygous Alb^{-} mutation is sufficient to negatively affect photosynthetic efficiency and drought tolerance. **Conclusions**: The albino mutation negatively affects photosynthetic efficiency and drought tolerance, and homozygous mutation is required for the characteristic albino phenotype. This work highlights the link between albino mutation, photosynthetic pigment metabolism and drought sensitivity in daylily.

Keywords: carotenoid; chlorophyll; daylily (*Hemerocallis* spp.); drought tolerance; natural albino mutant; light saturation point; RNA sequencing

1. Introduction

Photosynthesis converts solar energy into chemical energy and relies on photosynthetic pigments that absorb light energy. Albino mutations in plants affect photosynthesis rate by reducing the content of chlorophyll and carotenoids, which are the main photosynthetic pigments. Chlorophyll deficiency is generally caused by the mutations of genes encoding enzymes that catalyse biochemical reactions involved in chlorophyll metabolism or genes responsible for changes in chloroplast ultrastructure [1–3]. Carotenoids belong to a large pigment group and are categorised into carotenes, like α-carotene and β-carotene [4] or xanthophylls, such as zeaxanthins, violaxanthins, indicaxanthins, and lutein, *etc*. [5]. Most carotenoids are involved not only in plant photosynthesis, but also in photoprotection of photosynthetic organs [6]. Chloroplasts, as the primary organelles involved in photosynthesis, play an important role in plant stress responses [7]. Furthermore, it was suggested that high levels of photosynthetic pigments confer tolerance to drought conditions in plants [8]. Albino mutants lack plant photosynthetic pigments. They have been identified and characterised in *Arabidopsis* [9,10], cotton [11], maize [12], pepper [13], tomato [14], and tobacco [15]. Moreover, the photosynthetic pigment content in apple and potato was negatively affected by drought stress [16,17]. The visible phenotype of albino mutants is leaf chlorosis, which is often accompanied by reduced yield or even death. Therefore, the albino pheno-

type is considered a non-adaptive mutation [11]. Mutant analysis remains one of the main genetic tools for characterising gene functions in plants, especially non-model plants, such as daylily (*Hemerocallis* spp.) [18]. Great progress has been made by studies on the metabolism of chlorophyll and carotenoids in the analysed albino mutants in recent years. For example, the precursors of carotenoid biosynthesis are isopentenyl pyrophosphate and its isomer dimethylallyl diphosphate [19]. These compounds are not only the precursors of carotenoids, but also of chlorophyll, abscisic acid, cytokinin, and gibberellin [20]. Thus, the intermediate products of the methyl-D-erythritol 4-phosphate (MEP) pathway play an important role in chlorophyll and carotenoid biosynthesis. In fact, the disruption of enzymes encoded by genes in the MEP pathway may reduce the chlorophyll and carotenoid content of plants. For example, *Arabidopsis* loss-of-function mutations in genes involved in the MEP pathway (*dxs*, *dxr*, *IspD*, *IspE*, *IspF*, *IspH* and *gps1*) result in an albino leaf phenotype with reduced chlorophyll and carotenoid levels [9,10,21–23]. The disruption of the *pds3* gene impairs chlorophyll, carotenoid and gibberellin biosynthesis in *Arabidopsis* [24]. Furthermore, silencing the expression of *IspG* and *IspH* in tobacco results in an albino leaf phenotype, whereas silencing the *idi* gene in tobacco results in mottled white-pale green leaves [25]. The *Arabidopsis* double mutant of the *ipi* genes exhibited reduced chlorophyll and lutein content in leaves [26]. These data indicate that genes involved in the MEP pathway are important factors in the regulation of chlorophyll and carotenoid biosynthesis. In addition to the obstruction of chloroplast synthesis and metabolism, mutations in genes related to chloroplast development lead to an albino phenotype [27–29].

Daylilies (*Hemerocallis* spp.) are popular perennial ornamental plants. Most daylily cultivars are developed by sexual hybridisation [30], and their population amplification depends on division propagation. The American Daylily Association (https://www.daylilies.org/DaylilyDB/) has collected approximately 90,000 daylily cultivars. Mutant analysis is a widely used tool for characterising gene functions in plants including non-model plants, such as daylily, and great progress has been made in cloning genes from plant mutants in recent decades. Several studies have reported gene cloning using daylily [31–37]. Mapping-based cloning is an effective strategy for identifying causal mutations responsible for variations in a trait. In addition to mapping-based cloning, microarray [38], RNA sequencing (RNA-seq) [39], and mapping by sequencing [18,40] are used in analysing the gene functions of mutants. Giving that the vegetative growth period of daylily is longer than 2 years, mapping-based cloning is not an ideal method for analysing the natural mutants of daylily. Thus, RNA-seq was performed to compare gene expression between wild-type and albino mutant plants of daylily in the present study.

To understand the genetic and physiological mechanisms underlying daylily albino mutations, we performed its functional characterization and determined the most likely genetic and physiological causes. Moreover, impacts on the physiological functions of the plant, such as photosynthesis, metabolism and tolerance to drought, were explored.

2. Materials and Methods

2.1 Plant Materials and Growth Conditions

Hemerocallis Middebdorffii Trautv. & C. A. Mey and *Hemerocallis* 'black-eyed stella' and 'stella de oro' were cultured in experimental fields (Shanghai Institute of Technology, N: 30°50′33.98″ E: 121°30′38.34″), and each plant was grown in a single pot. The cross and self-pollinated progeny were cultured in chambers at 25 °C and 16 h light and 8 h dark photoperiod with cool white illumination (46.8 µmol photons·m^{-2}·s^{-1}). Plants were grown in soil and cultured in a medium containing half-strength Murashige and Skoog (MS) salts [41] and 3% sucrose (v/v), MS medium, sucrose, and other drugs were purchased at Shanghai Titan Technology Co., Ltd. (Shanghai, China).

2.2 Microscopic Analysis

The sections of daylily leaf tissue from albino mutants and wild-type plants were observed and recorded under a light microscope (ECLIPSE E200, NIKON, Japan).

Transmission electron microscopy analysis was performed according to the methods described by Hashimoto *et al*. [42] with some modifications. In detail, the leaf tissues of mutant and wild-type plant leaves were sampled, fixed and pumped with 4% glutaraldehyde at 4 °C, stored at 4 °C overnight and washed three times with 0.1 mol·L^{-1} phosphate buffer (pH = 7). Then, the leaf samples were fixed with 1% osmic acid for 2 h and washed three times with phosphate buffer. The leaf samples were then dehydrated in a series of ethyl alcohol solution gradually followed by acetone, embedded in resin, and sectioned by slicing in an ultrathin slicing machine (EM UC7, LEICA, German). The samples were stained and then observed by transmission electron microscope.

2.3 Determination of the Chlorophyll and Carotenoid Content

The contents of chlorophyll and carotenoids were determined according to the methods described by Chen [43] with some modifications. The leaf samples of 20d daylily seedlings were ground using a pestle and mortar. Chlorophyll and carotenoids were extracted using a prepared extraction solution containing ethanol, acetone, and water in a ratio of 5:4:1 (v/v/v). The absorbance was measured by a microplate reader (Spark readers, TECAN, Swiss) at wavelengths of 470, 645 and 663 nm. The content of total chlorophyll, chlorophyll a (Chla), chlorophyll b (Chlb) and carotenoids was calculated according to the following formula:

$$\text{Chl (total Chlorophyll)} = 6.63 \times \text{D\_663} + 18.08 \times \text{D\_645} \quad (1)$$

$$\text{Chla} = 13.95 \times \text{D\_663} - 6.88 \times \text{D\_645} \quad (2)$$

$$\text{Chlb} = 24.96 \times \text{D\_645} - 7.32 \times \text{D\_663} \quad (3)$$

$$\text{Car} = (1000 \times \text{D\_470} - 2.05 \times \text{Chla} - 114.8 \times \text{Chlb})/245 \quad (4)$$

2.4 Determination of the 5-Aminolevulinic Acid

The content of 5-aminolevulinic acid (ALA) was determined according to the methods described by Dei [44] with some modifications [45]. Leaf samples of 12-day-old daylily seedlings were ground and ALA was extracted by 4% trichloroacetic acid (m/v). Approximately 5 mL of extraction solution, 2.35 mL of sodium acetate (1 mol·L^{-1}), 0.15 mL of acetylacetone, and 2.5 mL acetate buffer (1 mol·L^{-1}, pH 4.6) were mixed and placed in boiling water for 10 min. The coloration of the mixture was performed by adding an equal volume of Ehrlich-Hg reagent after cooling. The absorbance was then measured by a microplate reader at 553 nm. The ALA content was then calculated according to the formula $7.2 \times 10^4 \times D_{553}$.

2.5 Determination of Porphobilinogen

The content of porphobilinogen (PBG) was determined according to the methods described by Peng *et al.* [46] with some modifications. Leaf samples from 12 d old daylily seedlings were ground, and PBG was extracted by buffer containing 0.1 mol·L^{-1} EDTA and 0.6 mol·L^{-1} Tris-HCL (pH 8.2). Coloration of the extraction was performed by adding an equal volume of Ehrlich-Hg reagent. Then the absorbance was measured by a microplate reader at 553 nm. The content of PBG was calculated according to the formula: $6.1 \times 10^4 \times D_{553}$.

2.6 Determination of Uroporphyrinogen III and Coproporphyrinogen III

The contents of uroporphyrinogen III (urogen III) and coproporphyrinogen III (coprogen III) were determined according to the methods described by Bogorad *et al.* [47] with some modifications. Leaf samples of 12 d old daylily seedlings were ground, and PBG was extracted by 0.067 mol·L^{-1} phosphoric acid buffer (pH 6.8). Then, 5 mL of extraction solution and 0.25 mL sodium thiosulfate were mixed and illuminated with intense light for 20 min. The pH of the mixture was adjusted to 3.5 by adding 1 mol·L^{-1} glacial acetic acid. The absorbance of the aqueous phase, which was combined after chloroform extraction, was measured by a microplate reader at 405.5 nm. The urogen III content was calculated using the formula: $5.48 \times 10^5 \times D_{405.5}$. The absorbance of the aqueous phase, which was combined after ether extraction, was measured by microplate reader at 399.5 nm. The content of coprogen III was calculated according to the formula: $4.89 \times 10^5 \times D_{399}$.

2.7 Determination of Protoporphyrin IX, Mg-Protoporphyrin IX and Pchlide

The content of protoporphyrin IX (proto IX), Mg-protoporphyrin IX (Mg-proto IX), and pchlide were determined according to the methods described by Hodgins *et al.* [48] with some modifications [46]. Leaf samples from 12 d old daylily seedlings were ground after the addition of 25 mL of 80% alkaline acetone. The extraction was centrifuged at 15000 ×g and 4 °C for 15 min. The absorbance of the supernatant was measured by a microplate reader at wavelengths of 575, 590, and 628 nm. The content of proto IX, Mg-proto IX, and pchlide were calculated according to the following formula:

$$\text{proto IX} = 0.18016 \times \text{A\_575} - 0.04036 \times \text{A\_628} - 0.04515 \times \text{A\_590} \quad (5)$$

$$\text{Mg – Proto IX} = 0.06077 \times \text{A\_590} - 0.01937 \times \text{A\_575} - 0.003423 \times \text{A\_628} \quad (6)$$

$$\text{Pchlide} = 0.03563 \times \text{A\_628} + 0.007225 \times \text{A\_590} - 0.02955 \times \text{A\_575} \quad (7)$$

2.8 Determination of Photosynthetic Parameters

Photosynthetic parameters were determined according to the methods described by Iqbal *et al.* [45] with some modifications [49]. A portable photosynthesis tester (CIRAS-3, PP SYSTEMS, USA) was used to determine photosynthetic parameters. The fifth leaves from apical meristem of daylily were selected for measurement in September and October 2022 (10:00–12:00 am). Photosynthetic parameters including net photosynthetic rate (Pn), water use efficiency (WUE), transpiration rate (Tr) and intercellular CO_2 concentration (Ci) were recorded after that the absolute value of the change amplitude of Pn was less than 0.2.

2.9 Determination of the Relative Water Content of Leaves

The relative water content of the leaves was determined according to the methods described by Chen *et al.* [50] with some modifications. Daylily leaves with the same physiological status were treated under drought conditions, and weighed. Fresh weight was recorded as the initial weight. The saturated weights (Wt) were recorded after the samples being placed in distilled water for 2 hours to absorb water. After that, the dry weights (Wd) were recorded after the samples being dried at 80 °C for 48 h. The water content (RWC) was calculated according to the following formula:

$$\text{RWC} = (\text{Wf} - \text{Wd})/(\text{Wt} - \text{Wd}) \quad (8)$$

2.10 Determination of Soil Moisture Content

Soil moisture content was determined according to the methods described by Wang *et al.* [51] with some modifications. The wet weight (M1) of the soil was acquired by randomly weighing the soil from three different positions in the pot. Dry weight (M2) was recorded after the samples

being dried at 80 °C for 48 h. The soil moisture (W) content was calculated according to the following formula: (M1 – M2)/M2 × 100%.

2.11 RNA Sequencing (RNA-seq)

Leaf samples from 12 d old albino mutant and wild-type plants were ground, and five sets of biological replicates of each sample were randomly sampled. Three sets with the good-quality samples were obtained for analysis. Total RNA was extracted after digestion by DNase. The mRNA was enriched by magnetic beads with oligo dT. Used mirVana™ miRNA ISOlation Kit, Ambion-1561 kit and Refrigerated centrifuge (ST16R, Thermo, USA), Gel imaging system (Tanon 2500, Biotanon Co., Ltd., Shanghai, China), UV spectrophotometer (NanoDrop 2000, Thermo, USA).

cDNA libraries were constructed using a VAHTS Universal V6 RNA-seq Library Prep Kit for Illumina sequencing according to the manufacturer's instructions. The libraries were finally sequenced using an Illumina Novaseq 6000 platform. A total of 150 bp paired-end reads were generated. The transcriptome sequencing and analysis were conducted by OE Biotech Co., Ltd. (Shanghai, China). Oligonucleotide adapters were removed using Trimmomatic software (version 0.36) [52], and clean reads were obtained after low quality bases and N bases were filtering out. Clean reads were then spliced through the paired-end method performed using Trinity software (version 2.4) to obtain transcript sequences [53]. The most extended sequence was selected as a unigene. The unigenes were annotated to the NR, COG/KOG, and Swissprot databases. GO annotation was acquired by mapping Swissprot ID to the GO term, and pathway information was acquired by comparing unigenes with the KEGG database [54].

Fragments per kilobase of exon model per million mapped fragments (FPKM) and unigene count were analysed in bowtie2 (version 2.3.3.1) [55] and eXpress software (version 1.5.1) [56]. The number of unigene reads in the samples were acquired by eXpress software. All the data were standardised by estimating SizeFactors, a function of DESeq package (version 1.18.0) [57]. The *p*-value and foldchange of the difference in gene expression between mutant and wild-type plants were calculated using nbinomTest software. The unigenes with difference value greater than 2 and *p* value less than 0.05 were selected for further analysis.

RNA-seq analysis was performed using Bioedit, NCBI, and Tair (https://www.arabidopsis.org/).

2.12 Quantitative Real-Time PCR (qPCR)

Six differentially expressed genes (DEGs) from RNA-seq data related to terpene, carotenoids, and chlorophyll metabolism were randomly selected for quantitative real-time PCR (qPCR) validation. Primers were designed using premier5 software (**Supplementary Table 1**). The Prime ScriptTM RT reagent kit with gDNA Eraser (TaKaRa) was applied to synthesise cDNA. qPCR was then performed according to the methods described by Zuo [58]. *HfUBQ* and *HfEF-1a* were used as internal reference genes [59]. The $2^{-\Delta\Delta Ct}$ method was used for data analysis [50].

2.13 Drought Stress

Two-week-old sterile cultured self-pollinated progeny of daylily 'Black-eyed stella' were transplanted to a medium containing half-strength MS salts, 3% sucrose (v/v), and 10% PEG2000 (v/v) [60]. One-year-old daylily plants, which were cultured in pot with soil, were left unwatered for two weeks, whereas the control plants were regularly watered every two days. Relative soil water content was determined at the same time.

2.14 Statistical Analysis

At least three independent biological replicates were performed for each experiment. Statistical analysis was performed with Excel 2010 and SPSS26. Figures were made using Origin8 software.

3. Results

3.1 Isolation of a Natural Albino Mutant of Daylily

Albino seedlings were observed during the selection and development of new varieties from self-pollinated progeny of daylily 'Black-eyed stella' and one of its parents, 'Stella de oro' in May 2020. The leaf colour of the albino seedlings was light yellow (Fig. 1A). The albino seedlings displayed a dwarf phenotype (Fig. 1B), and survived for less than 15 days in soil. However, these seedlings survived longer when grown on the medium containing half MS salts and 3% sucrose under sterile culture conditions (Fig. 1C–F). The chlorophyll and carotenoid content of albino seedlings was determined and compared with those of wild-type plants. The results showed that the content of chlorophyll a (Chla), chlorophyll b (Chlb) and carotenoids decreased significantly compared with that of the wild-type plants (**Supplementary Fig. 1**). Albino phenotypes are generally attributed to changes in the metabolism of photosynthetic pigments and chloroplast development. Therefore, a microscopic analysis was performed to verify whether the chloroplast development of the mutant plants was altered. Extremely few chloroplasts were found in the albino mutant leaf cells (Fig. 1G,H). To better understand chloroplast development, transmission electron microscopy was used for investigating the morphology of mesophyll plastids. Both the number of thylakoids and the extent of stacking in the grana were significantly reduced in albino mutant plants (Fig. 2).

To understand the mechanism of the decrease in chlorophyll content, the precursors of chlorophyll and carotenoids biosynthetic pathways were measured. Except PBG, the content of the precursors of chlorophyll biosynthesis significantly decreased in the leaves of albino mutants compared with that of wild-type plants (**Supplementary Fig. 3**).

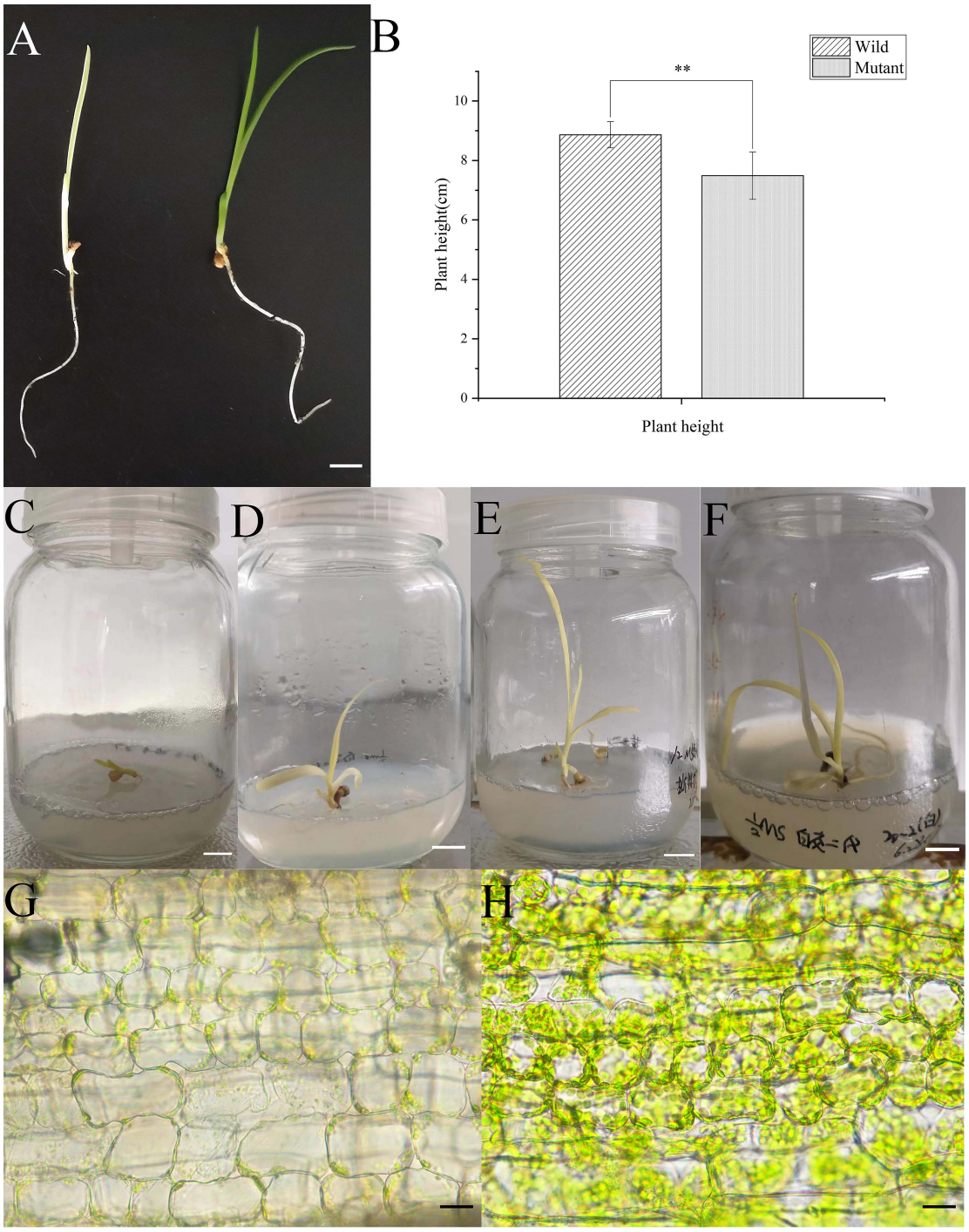

Fig. 1. Comparison of the morphological and characterization of the plastid between albino and wild-type seedlings of daylily. (A) Seedlings of 15 d old wild-type (right) and albino mutant (left) seedlings cultured in the soil. (B) Height of seedlings of 15 d wild-type (left) and albino mutant (right) seedlings cultured in the soil. (C–E) Seedlings of albino mutant culture on the media containing half MS salts and sucrose under sterile conditions. (G,H) Light microscopy of the chloroplast of the leaf cells of albino mutant (G) and wild-type seedlings (H). The bars in (A–F) represent 1 centimeter, and in (G,H) represent 20 micrometers. Mean values ± SE (n = 3); significant values $^{**}p < 0.01$.

3.2 Genetic Analysis of the Albino Phenotype

Daylily 'Black-eyed stella' is a cross between two diploid parents 'Stella de oro' and 'Little celena' (https://www.daylilies.org/DaylilyDB/). Albino mutants were isolated from the self-pollinated offspring population of 'Black eyed stella' and one of its parents, 'Stella de oro'. The F2 population of 'Black-eyed stella' was used for further analysis, and the segregation ratio was in accordance with the expected Mendelian ratio of 3:1 (Table 1). Furthermore, all cross-pollinated offspring between *H*. 'black-eyed stella'

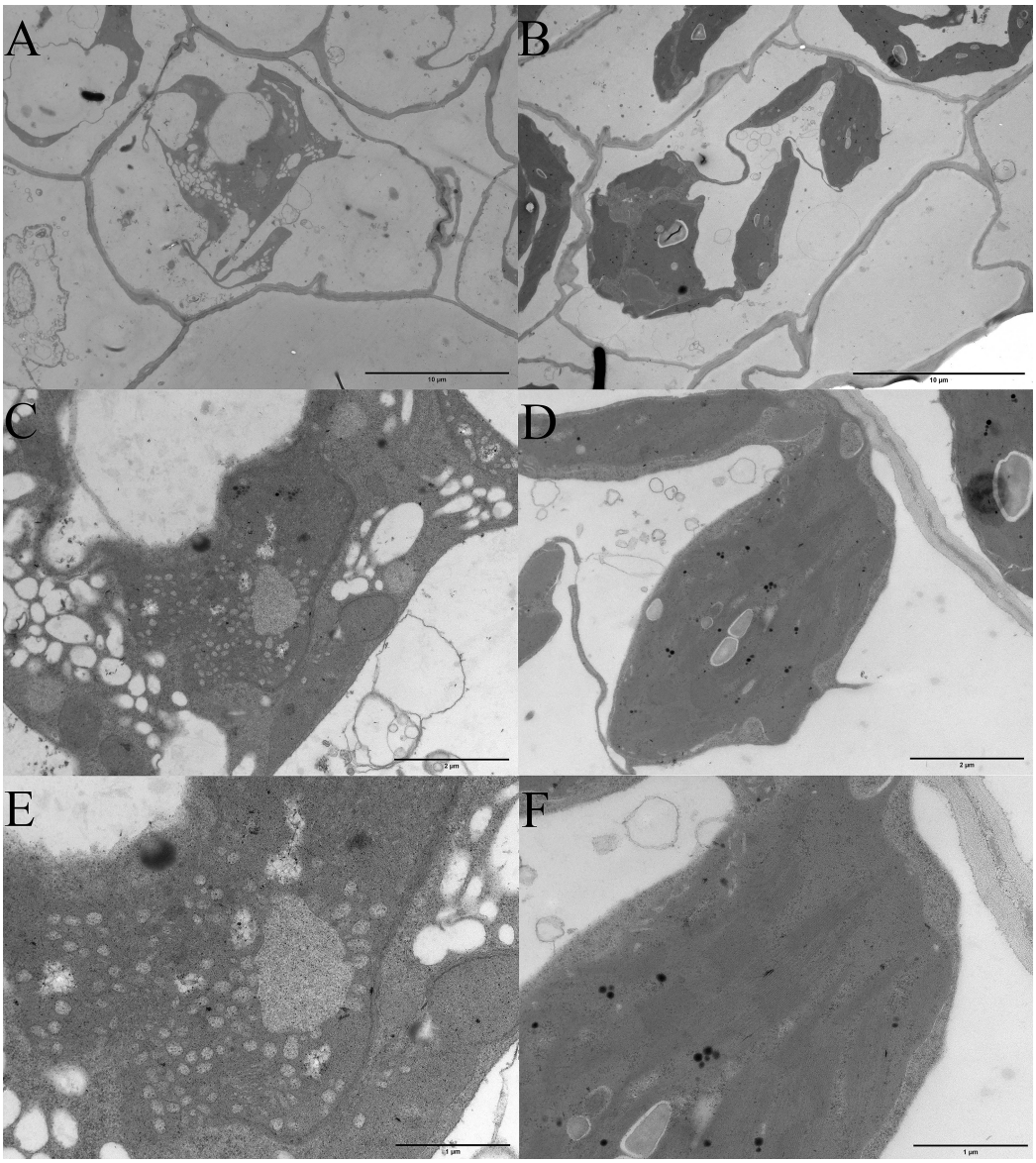

Fig. 2. Microscopic analysis of the plastids tissue between albino mutant and wild type seedlings of daylily. (A,C,E) Transmission electron microscopic examination of plastids of albino seedlings. (B,D,F) Transmission electron microscopic examination of plastids of wild-type. The bars in (A,B) represent 10 micrometers, (C,D) represent 2 micrometers, and (E,F) represent 1 micrometer.

and *H. Middebdorffii* Trautv. & C. A. Mey displayed a wild-type phenotype. However, albino seedlings accounted for approximately a quarter of the F2 population.

The surviving F2 plants should have consisted of two-thirds of heterozygous plants and one-third of homozygous plants, because the albino mutant plants died before flowering. The plants with self-pollination progeny that did not appear as albino seedlings were designated as homozygous plants ($Alb^{+/+}$), and the other F2 plants were then marked as heterozygous ($Alb^{+/-}$).

3.3 RNA Sequencing (RNA-seq) Analysis and Real-Time PCR Confirmation

RNA-seq was performed to compare gene expression patterns between the mutant and wild-type plants. Then, 41.96 G clean data were acquired. The effective data volume of each sample ranged from 6.74 G–7.17 G. The Q30 bases were distributed in 95.47–95.55%, and the average GC content was 46.59%. A total of 53479 unigenes were spliced and had a total length of 55899490 base pairs (bp) and an average length of 1045.26 bp (**Supplementary Table 3**). These results showed that the quality of the RNA-seq data was good enough for subsequent analysis. A total of 7952 DEGs, including 4069 up-regulated genes and 3883 down-regulated genes were identified between daylily albino mutant and wild-type plants.

Table 1. Genetic analysis of self-pollinated offspring from daylily 'black-eyed stella'.

Generations	Total	Wildtype	Albino	Expected ratio	χ^2
F2	1505	1137	368	3:1	0.001

Table 2. Differentially expressed genes (DEGs) involved in metabolism of terpenoids, carotenoids, and chlorophyll.

Gene id	Gene description	Fold Change	Regulation	Pathway
TRINITY_DN24954_c0_g1_i7_1	*lcyE*, lycopene epsilon-cyclase	2.40	Up	Carotenoids metabolism
TRINITY_DN6095_c0_g1_i1_2	*LUT1*, carotenoid epsilon hydroxylase	8.62	Up	Carotenoids metabolism
TRINITY_DN18331_c0_g1_i1_2	*CCS1*, capsanthin/capsorubin synthase	0.43	Down	Carotenoids metabolism
TRINITY_DN16727_c0_g1_i1_3	*NECD*, 9-cis-epoxycarotenoid dioxygenase	0.19	Down	Carotenoids metabolism
TRINITY_DN17994_c0_g1_i1_1	*ABA2*, xanthoxin dehydrogenase	0.38	Down	Carotenoids metabolism
TRINITY_DN16388_c0_g1_i1_1	*atoB*, acetyl-CoA C-acetyltransferase	2.00	Up	Terpenoid biosynthesis
TRINITY_DN24924_c0_g1_i1_3	*atoB*, acetyl-CoA C-acetyltransferase	0.23	Down	Terpenoid biosynthesis
TRINITY_DN24275_c0_g1_i1_3	*E2.3.3.10*, hydroxymethylglutaryl-CoA synthase	0.31	Down	Terpenoid biosynthesis
TRINITY_DN24659_c0_g1_i9_3	*HMGCR*, hydroxymethylglutaryl-CoA reductase (NADPH)	0.46	Down	Terpenoid biosynthesis
TRINITY_DN24770_c0_g1_i2_3	*dxs*, 1-deoxy-D-xylulose-5-phosphate synthase	0.29	Down	Terpenoid biosynthesis
TRINITY_DN22835_c2_g3_i1_1	*dxr*, 1-deoxy-D-xylulose-5-phosphate reductoisomerase	4.06	Up	Terpenoid biosynthesis
TRINITY_DN17066_c0_g1_i1_2	*ispS*, isoprene synthase	0.14	Down	Terpenoid biosynthesis
TRINITY_DN17405_c0_g1_i2_2	*ispS*, isoprene synthase	0.07	Down	Terpenoid biosynthesis
TRINITY_DN25913_c0_g2_i19_2	*GPS*, geranyl diphosphate synthase	2.62	Up	Terpenoid biosynthesis
TRINITY_DN27270_c0_g1_i15_1	*GPS*, geranyl diphosphate synthase	2.12	Up	Terpenoid biosynthesis
TRINITY_DN23792_c1_g1_i3_3	*hemB*, porphobilinogen synthase	0.49	Down	Chlorophyll metabolism
TRINITY_DN24157_c0_g1_i6_2	*por*, protochlorophyllide reductase	0.42	Down	Chlorophyll metabolism
TRINITY_DN24157_c0_g2_i1_2	*por*, protochlorophyllide reductase	0.23	Down	Chlorophyll metabolism
TRINITY_DN25035_c0_g3_i3_3	*por*, protochlorophyllide reductase	0.34	Down	Chlorophyll metabolism
TRINITY_DN17815_c1_g1_i1_3	*SGR*, magnesium dechelatase	0.21	Down	Chlorophyll metabolism
TRINITY_DN17815_c1_g2_i1_3	*SGR*, magnesium dechelatase	0.27	Down	Chlorophyll metabolism
TRINITY_DN24503_c1_g4_i2_1	*CAO*, chlorophyllide a oxygenase	2.41	Up	Chlorophyll metabolism
TRINITY_DN17158_c0_g1_i1_3	*E3.1.1.14*, chlorophyllase	0.47	Down	Chlorophyll metabolism
TRINITY_DN26828_c0_g4_i1_3	*HCAR*, 7-hydroxymethyl chlorophyll a reductase	0.20	Down	Chlorophyll metabolism

GO function enrichment analysis showed that many DEGs were involved in biological processes related to cellular components and molecular function, such as cellular process, metabolic process, cell part, organelle, membrane, membrane part, membrane-enclosed lumen, binding, catalytic activity, and transporter activity (**Supplementary Fig. 2**). KEGG significant enrichment analysis revealed that the DEGs were involved in 125 metabolic pathways. The metabolic pathways with the most DEGs were carbohydrate, lipid, amino acid, and energy metabolism. The down-regulated DEGs were pyruvate, amino sugar, nucleotide, starch and sucrose metabolism (**Supplementary Fig. 3**, **Supplementary Table 4**).

Six DEGs were randomly selected from genes encoding for terpene, carotenoid and chlorophyll metabolism and one transcription factor for quantitative real-time PCR (qPCR) validation, because all these genes were related to the albino trait (Fig. 3). The expression level of DEGs between qPCR and RNA-seq data was coincident (**Supplementary Table 5**). Therefore, the RNA-seq data described here can be considered reliable.

Given that the level of chlorophyll and carotenoid in albino mutants were much lower than those of wild-type plants (**Supplementary Table 2**), the DEGs related to chlorophyll biosynthesis were analysed with the RNA-seq data, showing that most of the DEGs related to chlorophyll biosynthesis were down regulated (Fig. 4, Ref. [61–63]). The fold change range was 0.20–0.49 (**Supplementary Table 6**). *CAO*, a gene necessary for transforming Chla to Chlb in chlorophyll oxygenase biosynthesis [64], was up-regulated (Fig. 4). This result might be responsible for the observed decrease in the Chlorophyll a/b ratio in the mutants.

The direct precursor of carotenoids biosynthesis is geranylgeranyl pyrophosphate (GGPP), which is also a precursor of chlorophyll biosynthesis [24]. Therefore, the DEGs of terpene metabolism were analysed in detail. The results indicated that *HfDXS*, which encodes a key enzyme in the MEP pathway, was significantly negatively regulated (Figs. 3,4). The expression level of *HfDXS* in albino mutant plants was down-regulated 3.5 times compared with that in the wild-type control (**Supplementary Table 5**). Regarding the mevalonate pathway (MVA) path-

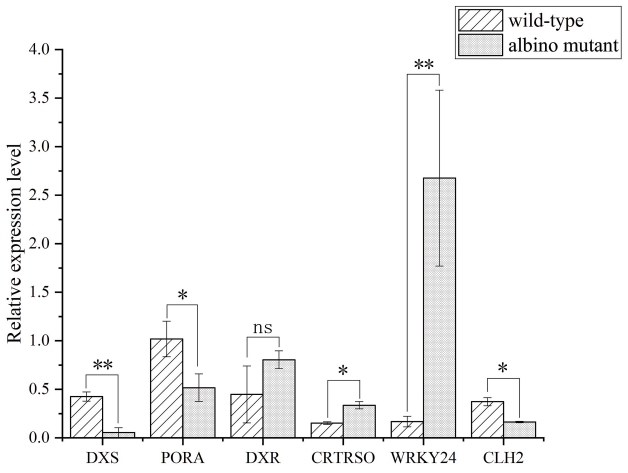

Fig. 3. qPCR validation of DEGs obtained from RNA-seq data. *DXS*, *PORA*, *DXR*, *CRTRSO*, *WRKY24* and *CLH2* stand for daylily 1-deoxy-D-xylulose-5-phosphate synthase, protochlorophyllide oxidoreductase A, 1-deoxy-D-xylulose 5-phosphate reductoisomerase, carotenoid isomerase, *WRKY* transcription factor 24 and chlorophyllase 2, respectively. Mean values ± SE (n = 3); significant values *$p < 0.05$, **$p < 0.01$; ns, no significance; qPCR, realtime quantitative polymerase chain reaction; RNA-seq, RNA sequencing.

way for terpene metabolism, the genes encoding acetyl-CoA C-acetyltransferase and hydroxymethylglutaryl-CoA synthase were down-regulated (Fig. 4). These DEGs act upstream of the metabolic pathways of MEP and MVA. Therefore, changes in these DEGs likely substantially contributed to decreases in chlorophyll and carotenoid content in the leaves of the mutant plants. Regarding other genes relevant to carotenoid biosynthesis, two up-regulated and three down-regulated DEGs were found (Table 2). The up-regulated DEGs were genes involved in the biosynthesis of ϵ- carotene and lutein. The foldchange values were 2.40 and 8.62, respectively (Table 2). The down-regulated DEGs were genes involved in biosynthesis of aldehydes, capsaicin and 2-cis- 4-trans-xanthin, and the fold change range was 0.38, 0.43 and 0.19, respectively (Table 2). The transcription factor *WRKY* genes regulate the expression of *DXS* genes [65,66]. Therefore, the bioinformatic analysis of the differential expression of the *HfWRKY* genes with RNA-seq was performed and followed by quantitative PCR confirmation, showing that *HfWRKY24* was remarkably up-regulated (Fig. 3 and **Supplementary Table 5**).

3.4 Analysis of the Photosynthetic Characteristics in the Homozygous (Alb$^{+/+}$) and Heterozygous (Alb$^{+/-}$) Plants

The photosynthetic pigment contents of Alb$^{+/+}$ and Alb$^{+/-}$ plants were measured after being identified by the albino phenotype that appeared in self-pollination-derived progeny. The content of chlorophyll and carotenoids in the leaves of Alb$^{+/-}$ plants was significantly lower than that of the Alb$^{+/+}$ plants (**Supplementary Table 7**). Measuring the photosynthetic characteristics of Alb$^{-/-}$ was difficult because of the small plant size and short surviving time. The results showed that not only the net photosynthetic rate (Pn), but also the transpiration rate (Tr), intercellular CO_2 (Ci), and water use efficiency (WUE) of Alb$^{+/+}$ were significantly higher than those of the Alb$^{+/-}$ plants (Fig. 5A–D). The photosynthetic response curves of Alb$^{+/-}$ and Alb$^{+/+}$ plants were further analysed because of the difference in Pn between the Alb$^{+/-}$ and Alb$^{+/+}$ plants. The Pn of the Alb$^{+/-}$ plants was significantly lower than that of the Alb$^{+/+}$ plants when the light intensity was higher than 100 µmol photons $m^{-2}{\cdot}s^{-1}$ (Fig. 5E), indicating that the light saturation point of Alb$^{+/+}$ plants was higher than that of the Alb$^{+/-}$ plants. qPCR was performed to confirm whether the above differences in phenotypes between Alb$^{+/+}$ and Alb$^{+/-}$ plants were accompanied by changes in the expression levels of *HfDXS* and *HfWRKY24*. The expression level of *HfDXS* in the Alb$^{+/+}$ leaves was slightly higher than that in the Alb$^{+/-}$ leaves, but the expression level of *HfWRKY24* in the Alb$^{+/+}$ leaves was significantly higher than that in the Alb$^{+/-}$ leaves (**Supplementary Table 8**). In terms of photosynthetic characteristics, the Pn of Alb$^{+/+}$ declined after drought treatment. However, the trend of decline slowed down after 4 d treatment. Meanwhile, the Pn of Alb$^{+/-}$ decreased continuously during the treatment period. The change trend of the stomatal conductance was consistent with that of Pn between Alb$^{+/+}$ and Alb$^{+/-}$ after drought treatment (Fig. 6).

3.5 Daylily Albino Mutant Plants are Sensitive to Drought Conditions

As photosynthetic efficiency affects the drought tolerance of plants [51], RWC of Alb$^{+/+}$ and Alb$^{+/-}$ plants were measured after drought treatment. The RWC of the leaves in Alb$^{+/-}$ plants declined immediately after drought treatment. Meanwhile, the RWC of the leaves in Alb$^{+/+}$ plants showed a significant reduction only after 1 week of treatment (Fig. 6), indicating that the Alb$^{+/-}$ plants were more sensitive to drought conditions than the Alb$^{+/+}$ plants. This result prompted us to investigate whether albino mutant seedlings were also sensitive to drought conditions. Owing to the short life of mutant seedlings cultured in soil, sterile culture with sucrose and PEG were used for drought stress treatment. Initially, the albino leaves grew similarly to the wild type (Fig. 7A), and as the number of days of stress increased, the albino leaves began to wilt and collapse (Fig. 7B). The mature leaves of albino seedlings almost completely wilted and were lodging after treatment (Fig. 7C), and dark brown spots appeared on the mature leaves (Fig. 7D). Meanwhile, the wild-type seedlings were almost unaffected by drought treatment (Fig. 7C). These results indicated that albino mutant is sensitive to drought, because high-MW PEG is considered the most suitable modelling system for modelling drought treatment in plants grown *in vitro* [67]. Furthermore, the 1-year-old plants cul-

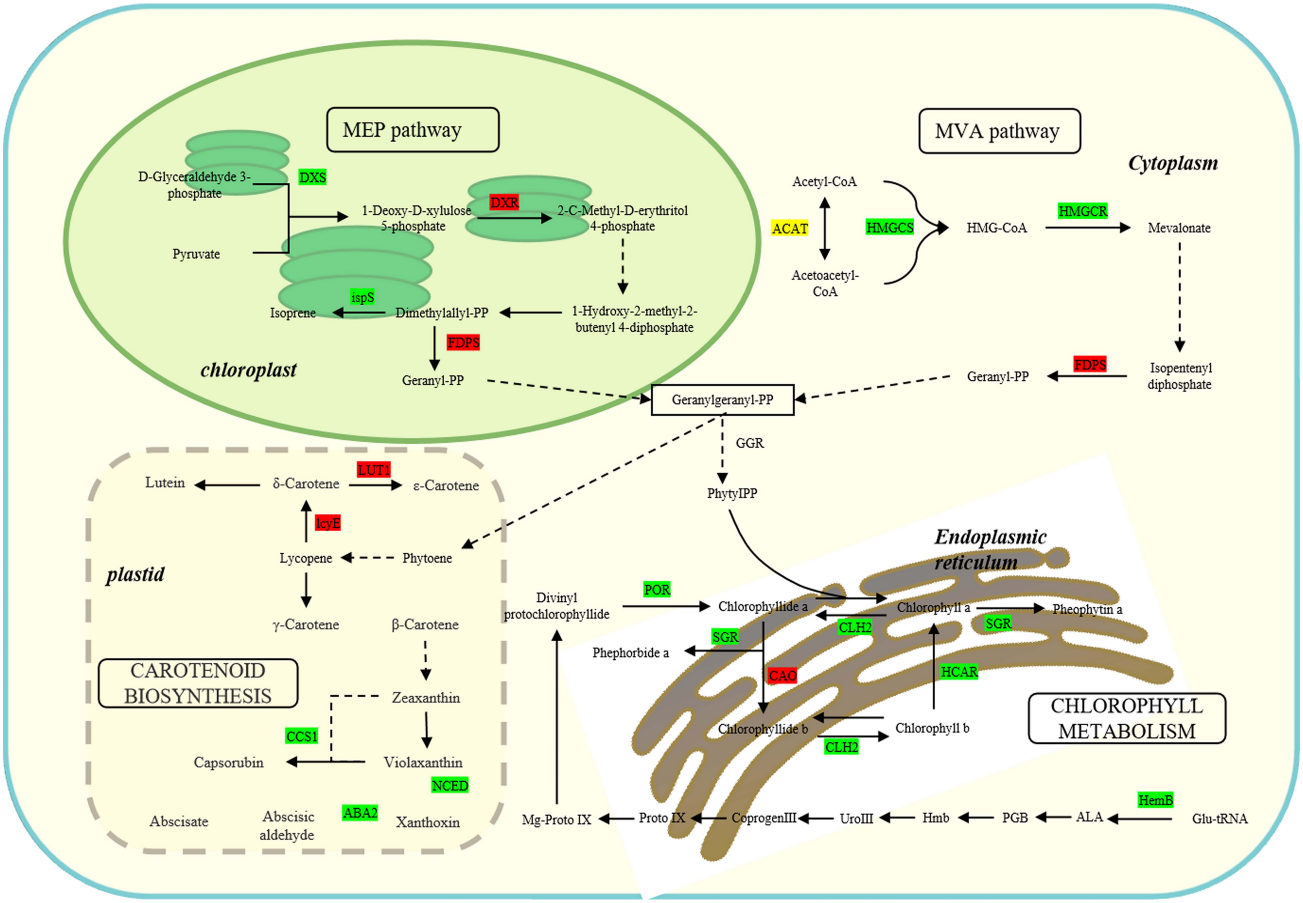

Fig. 4. DEGs in MEP, MVA, and metabolism of carotenoids and chlorophylls. Green represents down-regulated genes, red represents up-regulated genes. The MEP, MVA, and metabolism of carotenoids and chlorophyll map are summarized according to previous reports [61–63]. DXS, 1-deoxy-D-xylulose-5-phosphate synthase; DXR, 1-deoxy-D-xylulose 5-phosphate reductoisomerase; ISPS, isoprene synthase; FDPS, farnesyl diphosphate synthase; ACAT, acetyl-CoA C-acetyltransferase; HMGCS, 3-hydroxy-3-methylglutaryl-CoA synthase; HMGCR, 3-hydroxy-3-methylglutaryl-CoA reductase; GCR, unclear receptor subfamily 3, group C, member 1; LUT1, Cytochrome P450 superfamily protein; ICYE, lycopene epsilon cyclase; CCS1, copper chaperone CCS1; NCED, putative 9-cis-epoxycarotenoid dioxygenase 3; ABA2, NAD(P)-binding Rossmann-fold superfamily protein; POR, cytochrome p450 oxidoreductase; SGR, AP2-domain transcription factor SGR; CLH2, chlorophyllase 2; HCAR, coenzyme F420 hydrogenase family / dehydrogenase, beta subunit family; CAO, chloroplast signal recognition particle component; HemB, porphobilinogen synthase; PGB, porphobilinogen; Hmb, hydroxymethylbiliin; UroIII, uroporphyrinogen III; CoprogenIII, Coproporphyrin III; Proto IX, protoporphyrin IX; Mg-Proto IX, Mg-Protoporphyrin IX.

tured in pots with soil were exposed to drought conditions. The results showed that the leaves of $Alb^{+/-}$ plants curled downward and wilted after 1 week of drought treatment (Fig. 7F–K). However, the $Alb^{+/+}$ plants were almost unaffected after the first week of drought treatment (Fig. 7M–R). The leaves of $Alb^{+/-}$ and $Alb^{+/+}$ plants were smooth and flat before drought treatment (Fig. 7E,L). In addition, the degree of curling of $Alb^{+/-}$ leaves was higher than that of the $Alb^{+/+}$ leaves (Fig. 7K,R). These results indicated that $Alb^{+/+}$ plants were more tolerant to drought treatment compared with $Alb^{+/-}$ plants.

4. Discussion

This study reports the isolation of a natural albino daylily mutant ($Alb^{-/-}$) and homozygous lines ($Alb^{+/+}$) based on the screening of self-pollinated progenies from a daylily heterozygous cultivar. Compared with the heterozygous cultivar, $Alb^{-/-}$ plants showed lower chlorophyll and carotenoid content, and Chl a/b ratio, while $Alb^{+/+}$ plants showed a higher chlorophyll and carotenoid content (**Supplementary Table 7**). Genetic analysis revealed that the albino trait can be attributed to a single recessive nuclear gene. Furthermore, the results indicated that the mutated gene is related to drought sensitivity.

The analysis of RNA-seq and quantitative PCR indicated that expression of many genes was altered in mutant

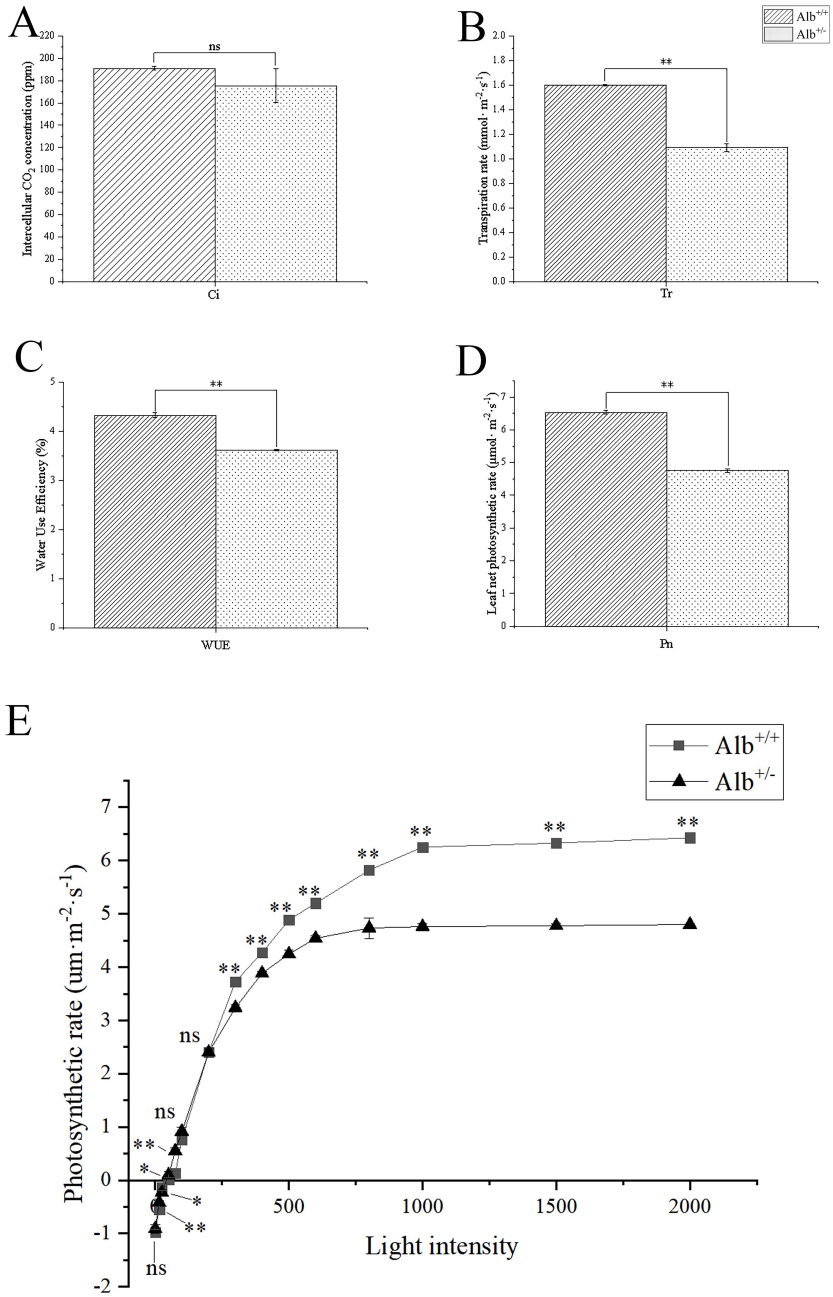

Fig. 5. Comparison of the net photosynthetic capacity, and light response curve between Alb$^{+/+}$ and Alb$^{+/-}$ plants of daylily. (A–D) Photosynthetic capacity of Alb$^{+/+}$ and Alb$^{+/-}$ plants of daylily was compared under laboratory conditions. (E) Light response curve of Alb$^{+/+}$ and Alb$^{+/-}$ plants. Mean values ± SE (n = 3); Significant values *p < 0.05, **p < 0.01; ns, no significance.

plants compared with wild-type plants (Figs. 3,4,5, **Supplementary Fig. 1**; Table 2). To further understand the RNA-seq data, the top ten up-regulated and down-regulated DEGs have been analysed. Regarding the top 10 down-regulated genes, there were 4 genes encoding photosystem II Psb, 1 gene encoded for a heat shock protein, 2 genes encoded for cytochrome, 1 gene encoded for isoprene synthase, 1 gene encoded chalcone synthase, and 1 gene encoded for NAD (P) H-quinone oxidoreducase (**Supplementary Table 6**). *Psb* plays a role in protecting the PS II system [68]. So far no reports of downregulation of *psb* genes that affect leaf colour have been published. Regarding heat shock proteins, abnormal expression of genes encoding heat shock proteins was shown to cause abnormal chloroplast development, which in turn affects the colour of leaves [69,70]. The expression of a gene encoding cytochrome was showed altered in albino mutants, but its gene function is related to plant growth and development, as well as responding to biotic and abiotic stresses, and does not appear to be a key gene that causes leaf colour mutations in plants [71,72]. Isoprene synthase regulates an important reaction within the MEP

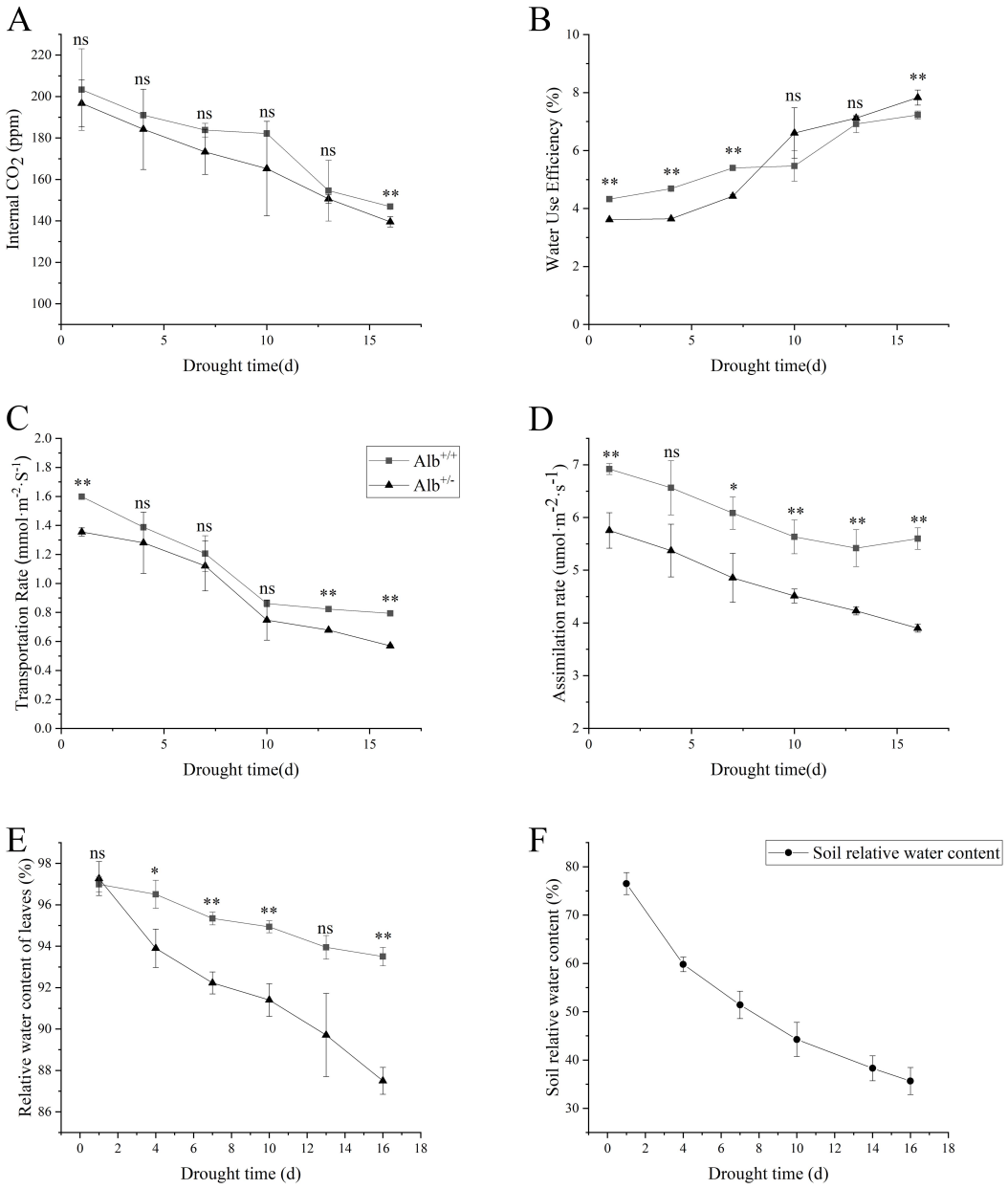

Fig. 6. Photosynthetic capacity and relative water content of Alb$^{+/+}$ and Alb$^{+/-}$ plants of daylily after drought treatment. (A–E) Photosynthetic capacity and relative water content of Alb$^{+/+}$ and Alb$^{+/-}$ plants of daylily were compared under progressive drought stress under laboratory conditions. Data were obtained from three two-year-old plants, and similar trends were observed in repeated experiments. (F) Soil moisture content during drought treatment. Mean values ± SE (n = 3); Significant values *$p < 0.05$, **$p < 0.01$; ns, no significance.

pathway [63–65]. However, no reports of downregulation of isoprene synthase-encoded genes to affect leaf colour have been published so far. Overexpression of the chalcone synthase-encoded gene increases the tolerance of plant leaves to strong light due to its high level of anthocyanin synthesis [73]. The top 10 genes upregulated were genes encoded beta-glucosidase, polygalacturonase, allene oxide synthase, autophagy-related protein, probable linoleate 9S-lipoxygenase, heat shock protein, ubiquitin-ribosomal protein, NADPH-dependent aldo-keto reductase, acetyl-CoA acyltransferase and DNA-directed RNA polymerase subunit beta (**Supplementary Table 6**). These genes are related to the activity of β-glucosidase and polygalacturonase, the induction of jasmonic acid by stress treatment, and cellular autophagy, respectively [74]. Regarding the DEGs related to the albino phenotype [14,23,29,75–87], 5 genes were down-regulated and 5 genes were upregulated (**Supplementary Table 9**). The seedlings of the LCYE mutant were white or light yellow, and can survive and grow until flowering [75]. *SGR* Over-expressing resulted in an

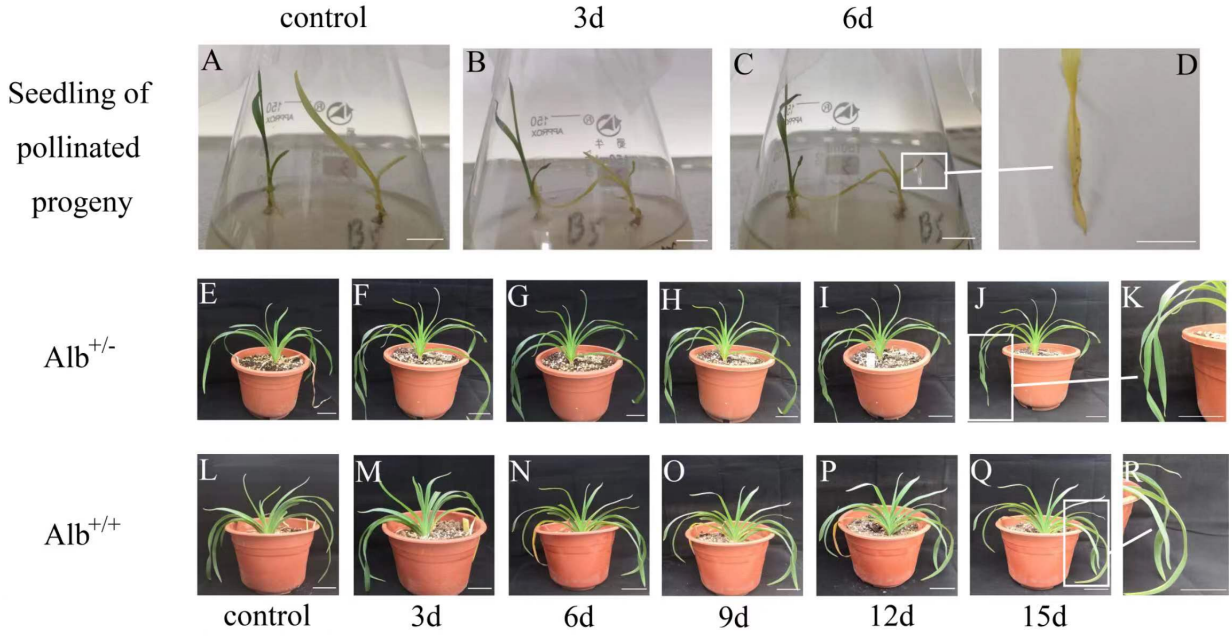

Fig. 7. Sensitivity of albino mutant plants of daylily to drought conditions. Seedlings of self-pollinated progeny of daylily cultivar 'black-eyed stella' were transplanted to a medium containing 10% PEG2000 after culturing on a PEG free medium for 2 weeks. (A) Seedlings before PEG-treatment. (B,C) Seedlings being treated for 3 and 6 days, respectively. (D) The albino leaves showing brown speckles after PEG-treatment. (E–R) One-year-old plants, grown in pot with soil, were treated with drought. (E,L) Potted $Alb^{+/-}$ and $Alb^{+/+}$ plants before drought-treatment. (F–J) $Alb^{+/-}$ plants exposed to drought for 3 d, 6 d, 9 d, 12 d, 15 d, respectively. (M–Q) $Alb^{+/+}$ plants exposed to drought for 3 d, 6 d, 9 d, 12 d, 15 d, respectively. (K) and (R) Leaves of potted $Alb^{+/-}$ and $Alb^{+/+}$ plants after drought treatment for 15 days.

albino phenotype [76], and albinism causes up-regulated expression of the PPR gene [77]. The loss-of-function of *ALB3*, *CAO*, *DXR*, and *NUS1* resulted in an albino phenotype [14,29,82–86]. However, the expression changes of these DEGs in the albino mutant of daylily described here were completely opposite to those of the above-mentioned mutants. Downregulation of *PORA* caused dwarfism with a light green leaf colour [78]. Downregulated gene encoded for δ-aminolevulinic acid dehydratase causing yellow spots on leaves [79]. Therefore, the phenotype of the daylily mutant described here was different from these two mutants in surviving time and leaf colour. In particular, *HfDXS*, which encodes the first key enzyme of the MEP pathway, was down-regulated approximately five times in the mutant plants and increased slightly in $Alb^{+/+}$ plants compared with the heterozygous plants. GGPP, an intermediate of MEP pathway, is a common precursor of chlorophyll and carotenoids biosynthesis [24]. A similar phenotype with decreased chlorophyll and carotenoid content has been reported in *Arabidopsis* and tomato *DXS* mutants [9,10,88].

The results showed changes in the precursor contents of chlorophyll biosynthesis and a decrease in the Chl a/b ratio in mutant plants compared with wild-type plants. The possible reason was that Chla may not be as stable as Chlb. The decrease in the Chl a/b ratio can be attributed to the increased expression of *CAO* (Fig. 4), which encodes the synthesis of chlorophyll oxygenase. Changes in the content of chlorophyll biosynthesis precursors between albino and wild-type seedlings showed that urogen III synthase is the key enzyme for the decreasing chlorophyll content (**Supplementary Fig. 2**). However, the expression level of this gene revealed from the RNA-seq data did not change significantly. Changes in urogen III synthase activity may be a side-effect of a reduction in the expression level of *HfDXS* in the albino seedlings. Moreover, the chloroplast development of the mutant was impeded, indicating that the mutated gene of daylily affected not only photosynthetic pigment biosynthesis, but also chloroplast development. The results were consistent with the *DXS* loss-of-function mutants of *Arabidopsis* and tomato [9,10,88], supporting the notion that the albino phenotype described here might be influenced by the down-regulation of *HfDXS*. The higher expression level of *HfWRKY24* in both the $Alb^{+/+}$ and $Alb^{-/-}$ plants compared with the $Alb^{+/-}$ indicated that *HfWRKY24* is likely not a major factor that induced the albino phenotype of daylily.

The present results also showed that the light saturation point and the net photosynthesis rate of the $Alb^{+/+}$ plants were significantly higher than those of the $Alb^{+/-}$ plants. The higher value of light saturation points of

$Alb^{+/+}$ plants compared with the $Alb^{+/-}$ plants should be attributed to the high chlorophyll and carotenoid content of the $Alb^{+/+}$ plants, because carotenoids protect chlorophyll from oxidative decomposition by light. In addition, studying the shade tolerance of $Alb^{+/-}$ plants will be valuable for the understory cultivation of daylily, because shade tolerant daylily is relatively scarce in the market.

Our results showed that the RWC of the $Alb^{+/+}$ plants was higher than that of the $Alb^{+/-}$ plants after drought treatment, indicating that the $Alb^{+/+}$ plants were more tolerant to drought conditions compared with the $Alb^{+/-}$ plantsas RWC is considered an important indicator of plant drought tolerance [50]. Indeed, drought treatment experiments indicated that the $Alb^{+/+}$ plants were tolerant to drought conditions, and the albino mutant plants were more sensitive to drought conditions compared with the $Alb^{+/-}$ plants. This finding can be due to the higher photosynthetic efficiency of $Alb^{+/+}$ plants compared to that of $Alb^{+/-}$ plants, consistent with the study on genetic variation in a vacuolar H+-PPase encoded genes [51]. The high value of net photosynthesis rate of the $Alb^{+/+}$ plants compared with the $Alb^{+/-}$ plants can be due to the high concentration of photosynthetic pigments of $Alb^{+/+}$ plants compared with the $Alb^{+/-}$ plants, because a high concentration of photosynthetic pigments can capture more light energy to form a developed root system. Conversely, difference between the albino mutant and heterozygous plants in drought tolerance can be the consequence of direct regulation of the drought sensitivity trait by the mutated gene. In addition, the $Alb^{+/+}$ plants will be a good resource for the future breeding of daylily because of its drought tolerance trait.

5. Conclusions

The first natural albino mutant of daylily was isolated in this study. According to Mendelian proportions of the phenotypes in the progeny of heterozygous plants, the albino phenotype is caused by a single recessive mutation. The homozygous mutant plants suffered the pronounced downregulation of *HfDXS1*, causing a dramatic decline in chlorophyll and carotenoid levels and were unable to live in the soil for longer than 15 days. Heterozygous $Alb^{+/-}$ plants can be different from their homozygous $Alb^{+/+}$ counterparts in terms of the occurrence of albino mutants in self-pollination-derived progeny. These heterozygous $Alb^{+/-}$ plants showed a reduced light saturation point, net photosynthetic rate, and water use efficiency, and reduced tolerance to drought compared with homozygous $Alb^{+/+}$ plants, indicating that the presence of a mutated allele affected the viability of the heterozygous plants even in the absence of a visible albino phenotype typical of homozygous mutants. Further research will be needed for the exact mapping of the mutation described in this study, and for establishing a clear mechanism for its effects on plant viability, photosynthetic parameters, and drought tolerance. In addition, this study provided important resources for daylily breeding.

Abbreviations

ALA, 5-aminolevulinic acid; Car, carotenoids; ALB3, 63 kDa inner membrane family protein; CAO, chlorophyllide a oxygenase; Coprogen III, coproporphyrinogen III; Chla, chlorophyll a; Chlb, chlorophyll b; Ci, intercellular CO_2 concentration; DEGs, differentially expressed genes; DMAPP, dimethylallyl diphosphate; DXR, 1-deoxy-D-xylulose 5-phosphate reductoisomerase; FPKM, fragments per kilobase of exon model per million mapped fragments; GGPP, Geranylgeranyl pyrophosphate; HEMB, porphobilinogen synthase; IPP, isopentenyl pyrophosphate; ispS, isoprene synthase; LCYE, lycopene epsilon-cyclase; MEP, methyl-D-erythritol 4phosphate pathway; Mg-Proto IX, Mg-Protoporphyrin IX; MVA, mevalonate pathway; NUS1, Dehydrodolichyl diphosphate synthase; PBG, porphobilinogen; Pn, net photosynthetic rate; POR, protochlorophyllide oxidoreductase; Proto IX, Protoporphyrin IX; psbK, photosystem II PsbK protein; psbA, photosystem II P680 reaction center D1 protein; qPCR, Quantitative real-time PCR; RNA-seq, RNA sequencing; RWC, relative water content; TEM, transmission electron microscopy; Tr, transpiration rate; Urogen III, uroporphyrinogen III; WUE, water use efficiency.

Availability of Data and Materials

Sequence data from this article can be found in the NCBI database (https://www.ncbi.nlm.nih.gov) with the following accession numbers: Hf EF-1a (MT096368), Hf UBQ (MT096370), HfDXS (OP913381), HfPORA (OP913382), HfDXR (OP913380), HfCLH2 (OP913378), HfCRTRSO (OP913379), HfWRKY24 (OP913383).

Author Contributions

Conceptualisation: SD, MR and DN. Methodology: DN, QQ and KD. Resources: DN and ZZ. Funding acquisition: DN and MR. Investigation: SD and MF. Data curation: SD, ZZ and DN. Formal analysis: SD. Visualisation: SD. Validation: TĆ, MR, ZZ and DN. Writing - original draft preparation: SD. Writing - review and editing: MF, QQ, ZZ, KD, TĆ, MR and DN. Supervision: TĆ, MR and DN. Project administration: DN. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

The plants used in this study were Hemerocallis Middebdorffii Trautv. & C. A. Mey and Hemerocallis 'black-eyed stella' and 'stella de oro'. Hemerocallis 'black-eyed stella' and 'stella de oro' were popular. These two varieties were purchased from the local flower market. And their information could be obtained by the American Daylily Association (https://www.daylilies.org/DaylilyDB/). Hemerocallis Middebdorffii Trautv was a wild-type specie, which was collected from Northeast China.

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Conflict of Interest

The authors declare no conflict of interest.

Supplementary Material

Supplementary material associated with this article can be found, in the online version, at https://doi.org/10.31083/j.fbl2902060.

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