A novel cucumber albino mutant caused by chloroplast development deficiency

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Abstract

Background Photosynthesis is a fundamental process for plant growth and development dependent on a precise network, including formation of chloroplast and chlorophyll synthesis. Chloroplast development deficiency could lead to albinism in higher plant.

Results Here, we report a cucumber albino recessive mutant that processed white cotyledons under light condition and is unable to produce first true leaf. Meanwhile, albino mutant could grow out creamy green cotyledons under dark condition but died after exposing to light. Using fluorescence microscopy and transmission electron microscope (TEM), impaired chloroplasts were observed. We identified 7 and 3 differentially expressed genes (DEG) involved in Chlorophyll metabolism and Methylerythritol 4-phosphate (MEP) pathway through transcriptome analysis, respectively. We also examined the reported homologous genes for albino mutants from other plants. Two of 12 genes, TOC159 and DXS1, were up-regulated in cucumber albino mutants as well. The reliability of RNA sequencing results were further confirmed by real-time quantitative PCR (qPCR).

Conclusions Taken together, we elaborate the differences between albino mutant and normal seedlings from a single cucumber progeny. This mutant is a new material to study protoplast development.

Background

As a DNA containing organelle, Chloroplast plays crucial roles in attuning plant development and plant interaction with environmental cues. Chloroplast is originated from proplastids in cotyledons at the time of illumination via the process of photomorphogenesis (1,2). Chloroplast, consisted with thylakoids stacked into defined grana, is the site of photosynthesis, production of hormones (i.e., abscisic acid, jasmonic acid and salicylic acid), and major metabolism (3). Its abnormal development or accumulation of pigment content could affect photosynthesis thus disrupt plant growth and biomass yield (4–7).

Leaf color mutation has been widely reported in many plant species, among which natural or induced albino mutants were frequently identified and characterized, especially in Arabidopsis and rice. Some mutants were environmental dependent albino but conditionally green-revertible. A mutation in OsABCI8 caused plants showing albino leaves under continuous rainy weather with defective chloroplast, but the leaves could turn green gradually after rainy season (8). Mutation in gene OsTCD5 that encodes a monooxygenase, or OsTCD11 that encodes the ribosomal small subunit protein S6 in chloroplasts (RPS6) were temperature sensitive albino mutant, presenting albino leaves at low temperature and normal green leaves at high temperature (9). Gene FLN2 encoding fructokinase-like protein2 showing opposite performance, with mutation in this gene were with albino leaves at high temperature (10). Many mutations were found only showing albino at certain development stage. Disruption of a pentatricopeptide repeat protein caused albino during seedling stage but the plants were able to turn green accompanying plant development (11). A mutation in SEEDLING PLASTID DEVELOPMENT1 resulted in albino cotyledon but the plants had similar appearance once initial green true leaves developed and transferred to soil (12).

Somatic albino mutants were also described with genes expression related to chlorophyll biosynthesis and chloroplast development affected in mutated branch leaf (13, 14). Still some mutations were described lethal mainly caused by deficiency in chloroplast development. A single T-DNA insertion causing fragment deletion affecting the function of gene DXS1 led to albino phenotype in tomato with premature lethal performance (15). A single nucleotide mutation in the plastid ribosomal protein produced abnormal chloroplast and caused lethal rice seedlings (16).

Cucumber (Cucumis sativus L.) is a worldwide important vegetable crop of Cucurbitacea family. Cucumber albino mutation was only reported by Iida and Amano in 1991 (17), which was induced by irradiation. However, no any further studies were carried out since then. Here, we reported a spontaneous mutation of albino mutant from inbred line “g32”, which exhibited white cotyledons and hypocotyl, dying before developing any true leaves. Cytological analyses revealed that chlorophyll signal was absent and chloroplast development was disrupted in the mutant. In the present study, we performed the high-throughput RNA sequencing to examine the differentially expressed genes between albino mutant and wild type.

Results

Phenotypic characterization of a cucumber *albino* mutant

We observed a few natural occurring albino seedlings during the seed increase of cucumber inbred line “g32” which is a south china type cucumber. The cotyledons of these albino seedlings are small and absolute white, and of which hypocotyl is short and white and primary root length is short (Fig.1). The albino mutants dried out and died in a few days after emerging from substrate without growing any true leaves.

Inheritance model of *albino* mutant

To investigate the inheritance nature of the albino mutant, we planted a total of 123 seeds from the self-pollinated g23 cucumber, of which 116 were germinated (germination rate 94.3%). Among the germinated seeds, 32 and 84 seedlings present albino and wild type phenotypes which fit the Mendelian ratio for single recessive gene ($\chi^2 = 0.414$, $p = 0.520$, Additional file 2: Table S2).

Short-lived chloroplast recovery in *albino* mutant under dark condition

Occasionally, we observed some subtle greenish cotyledons in few albino mutants that were grown under the canopy. It draws our attention that albino mutation might be affected by light. We therefore performed three sets of experiments to validate this hypothesis (Fig. 2a). In the first experiment, all the seeds were grown under dark condition all the time. After emerging from substrate, *albino* mutant presented creamy green cotyledon and completely white hypocotyl and wild type seedlings show yellowish green cotyledon and hypocotyl (Fig. 2b). As continuedly dark environment, both *albino* mutant and wild type spindled and died without growing first true leaf eventually. In the second experiment, after the seeds emerged from

substrate, we first let them grow under light condition until we are able to distinguish *albino* mutant and wild type. Later on, we transferred them under dark condition and found that *albino* mutant maintained whitish tissues without turning to cream green (Fig. 2c1-2). In the last experiment, the seeds grew under dark condition until seedlings emerging from substrate, and then they were exposed to light (Fig. 2a3). Upwards 5 hours after exposed to light, the green color in the cotyledon of *albino* mutant started to degrade (Fig. 2d1). The cotyledon contracted and dry out after exposed to light for 30 hours (Fig. 2d2). Therefore, we may conclude that light is a lethal factor for *albino* mutant and this damage is irreversible.

Most of the albino phenotypes in other plants are lack of chlorophyll. Thus, we examined the chlorophyll content of cotyledon from wild type, albino mutants that grow under light and dark condition using fluorescence microscopy. Much intensive chlorophyll signals were observed in wild type compared with albino mutant that grown under dark conditions (Fig. 2e; Presented in red). As expect, no chlorophyll signals were caught in albino mutants that grow under light condition.

Since most chlorophyll content in plants are presented in chloroplast, we further investigate the chloroplast ultrastructure in cotyledon of wild type and *albino* mutant that grew under both light and dark condition using transmission electron microscopy. In wild type cotyledon, we observed numerous well-developed, crescent-shaped chloroplast with stroma thylakoids, grana thylakoids, starch granules and plastoglobuli within the membranes (Fig. 2f1,4,7). In contrast, the chloroplast in albino mutant decreased dramatically in number and showed abnormal shape that lack of stroma thylakoids and grana thylakoids structure but contained osmiophilic plastoglobulis in inner membrane system (Fig. 2f2,5,8). The albino mutant that grow under dark condition comprised stroma thylakoid as well as osmiophilic plastoglobulis (Fig. 2f3,6,9).

To summarize, the above results indicated that the chloroplast development is impaired in the *albino* mutant under light condition during seedling development. Moreover, light probably acts as a lethal factor to the *albino* mutant by interrupting thylakoid biogenesis, as we could observe the presence of thylakoid in the *albino* mutant grown in dark but not under light condition.

Transcriptome profiling and identifying differentially expressed genes (DEGs) between *albino* mutant and wild type

The transcriptomes of cotyledons from *albino* mutant and wild type were examined using RNA-seq, each with three biological replicates. Overall, 97,869,948 to 123,516,412 clean reads were obtained after filtering low quality reads (Table 1). After mapping to the cucumber reference genome 9930 v2(17, 18), in total, 21,664 transcripts were detected. High coefficients among the replicates demonstrated the consistency of the transcriptional changes within each type (Fig. 3a). In total, 1,256 genes were found up-regulated and 1,584 were down-regulated in *albino* mutant compared with wild type ($|\log2FC| \geq 2$) (Fig. 3b, Additional file 3: Table S3). Based on the annotation, DEGs were categorized to GO and KEGG pathway to deeply understand the significant biological processes and pathways between *albino* mutant and wild type. 2,175 DEGs were classified into 814 GO processes involved in three categories, biological process, cellular component and molecular function (Additional file 4: Table S4), with biological process being

dominant category. Cellular carbohydrate biosynthetic process (GO:0034637, p=0.00010) and Cellular carbohydrate metabolic process (GO:0044262, p=0.00017) are the most significantly enriched processes (Additional file 8: Figure S1). As shown in Fig. 4, transmembrane transport (GO:0055085) and transporter activity (GO:0005215) accounted for the majority among the enriched GO terms, including 171 and 196 DEGs, respectively (Additional file 4: Table S4). Totally, 957 DEGs were assigned to 110 KEGG pathways (Additional file 5: Table S5), among which top 20 enriched KEGG pathways were illustrated in Fig. 4. Carbon metabolism (KEGG: csv01200), phenylpropanoid biosynthesis (KEGG: csv00940) and starch and sucrose metabolism (KEGG: csv00500) pathways occupied large proportion, with 69, 50 and 44 DEGs included, respectively (Additional file 5: Table S5).

Differentially expressed genes involved in chlorophyll metabolism

Porphyrin and chlorophyll metabolism (KEGG: csv00860) was a significantly enriched pathway in KEGG analysis. Many key enzymes involved in this pathway showed distinct expression profile between *albino* mutant and wild type. Most of genes, including *HEMB* (Csa2G401270), *HEME* (Csa4G082410, Csa5G218840), *HEMF* (Csa4G056670), *HEMG* (Csa6G007980), CHLG (Csa4G311220) and CAO (Csa6G385090) were slightly higher expressed in *albino* mutant than in wild type (Fig. 5a, Additional file 6: Table S6). However, POR (Csa4G638340) was down-regulated in *albino* mutant, presenting opposite pattern to other genes (Fig. 5a, Additional file 6: Table S6).

Differentially expressed genes involved in Methylerythritol 4-phosphate (MEP) pathway

The methylerythritol 4-phosphate (MEP) pathway is a source for the production of isoprenoid precursors, isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP) in photosynthetic eukaryotes(19). As shown in Fig. 5b, there are at least seven key enzymes involved in the MEP pathway, four candidate genes encoded *CMS* (Csa3G113320, Csa4G049620), *CMK* (Csa1G600780) and *MCS* (Csa4G049620) were found down-regulated in *albino* mutant (Additional file 6: Table S6).

Thylakoid related genes are affected in *albino* mutant

Nineteen DEGs belonging to thylakoid related functional activities, i.e., thylakoid (GO:0009579), thylakoid part (GO:0044436) and thylakoid membrane (GO:0042651) were highlighted (Fig. 6, Additional file 3: Table S3). Three *PsbPs* (Csa2G030040, Csa1G088470 and Csa1G181310) were found up-regulated in *albino* mutant while other 16 genes *PsbR* (Csa4G064020), *PsaG/PsaK* (Csa3G060980, Csa6G525340), *PsbO* (Csa6G488340), *PsaD* (Csa3G147780), *PsaH* (Csa3G483830), *PsbP* (Csa4G063440), *PsaE* (Csa2G079660), *PsbY* (Csa5G592810), *PsbW* (Csa7G378440), *PsaN* (Csa6G483300), *PsbQ* (Csa1G066480, Csa3G414060), *PsbX* (Csa1G595840), *PsaF* (Csa1G714680) and *PetM* (Csa7G075020) were down-regulated in *albino* mutant (Additional file 3: Table S3).

Homologous genes from *albino* mutants in other species

Mutation of *FLN1* (fructokinase-like 1) from barley and rice(20-22), *RPL21c* (chloroplast 50S ribosome protein L21) from arabidopsis and rice(23, 24), *EMB* (embryo-specific) from arabidopsis(25-28), *PDS3*

(phytoene desaturase) from arabidopsis(29), TOC159 (Translocase of chloroplast 159) from arabidopsis(30, 31), DXS1 (1-deoxy-D-xylulose-5-phosphate synthase 1) from tomato(15), and PurD (phosphoribosylamine-glycine ligase) from rice(32) could cause albinism in higher plant. The CDS of homologous genes of *albino* mutant and wild type were compared based on our transcriptome results. No variant was found among these genes between mutant and wild type. Nearly no gene, except two, *TOC159* (Csa4G001670) and DXS1 (Csa3G114510) were up-regulated in the mutant (Additional file 7: Table S7).

Validation of DEG expression by RT-qPCR

Eighteen DEGs (gene names were listed in Additional file 1: Table S1) with absolute log2(FoldChange) > 3 were randomly selected for RT-qPCR verification. Genes encoding peroxidase, isocitrate lyase, Glutathione S-transferase, malate synthase and many others were included. The expression of each gene was presented by relative expression ($-\Delta\Delta Ct$) using normal green seedling as control. The correlation between relative expression value of *albino* mutant and RNA-seq result (log2FoldChange) was calculated in EXCEL using CORREL function. The correlation factor between RT-qPCR and RNA-seq data was 0.9899, indicating a strong correlation. Overall, RT-qPCR validation indicates the reliability of RNA-seq result.

Discussion

Albinism happens among different living things varying from human beings, animals as well as higher plants (33–35). In this study, some albino seedlings were observed in the progenies from a self-pollinated cucumber fruit during the breeding selection period of an inbred line named g32. This cucumber albino mutant presented white hypocotyl finally presented lethal performance without developing first true leaf under light condition. In most studies, happening of albino phenotype in plants were caused by absence of chlorophyll and abnormal chloroplast development (27, 36, 37). Absent chlorophyll signal and defective chloroplast development in albino mutant were in consistent with most albino mutant in this respect. In normal seedlings, ultrastructure of chloroplast is well presented with compactly arranged chloroplasts while few or even no chloroplast structure was seen in albino seedlings. Also, an abnormal chloroplast ultrastructure in albino mutant were observed lacking starch granules and thylakoids, but with osmiophilic plastoglobuli (Fig. 4). Osmiophilic plastoglobuli generally appears as a result of the degradation of thylakoid membranes under stresses (38), therefore we propose that light might act as an abiotic stress cue for albino mutant, leading to the degradation of thylakoid membranes.

Interestingly, we found that this cucumber albino mutant is different to fln1, rpl21c, emb, pds3, toc159, dxs1 and purd albino mutants [15, 20–32] since it could recover white cotyledon to green under dark condition. Similar phenotype was reported in Arabidopsis albino pap7-1 mutant (39, 40). Pap7-1 mutant was with albino cotyledon and finally died grown under light condition but undistinguishable with wild type grown in darkness (40). From the TEM analysis, chloroplasts with thylakoids were observed. Since pap7-1 mutant could be arrested under very dim light (40), we tried to culture the mutant to dark to let it

develop but finally failed. Still it needs to be proved whether our mutant could produce plant architecture or even flower as pap7-1 grown under sucrose supplemented medium and dim light.

Differences in transcriptional level between albino mutant and wild type were also determined. Key genes involved in chlorophyll metabolism and MEP pathway and thylakoid related genes were differentially accumulated. Genes in chlorophyll metabolism were mainly up-regulated in the albino mutant, except POR. This increased expression of most genes might be a feedback regulation. POR is a light dependent key enzyme required for chlorophyll biosynthesis by catalyzing protochlorophyllide to chlorophyllide (41, 42). It has been reported that POR is crucial for plant growth and development since nonfunctional plants displayed reduced chlorophyll content and severe photoautotrophic growth defects (43, 44). The expression level of POR is lower in albino mutant than in wild type, indicating that albino mutant might be incapable in chlorophyll biosynthesis. MEP pathway is essential for the biosynthesis of photosynthesis-related compounds, such as carotenoids, chlorophylls, gibberellins and abscisic acid, which is vital important for plant development and metabolism(45). The mutation of genes in the pathway impaired biosynthesis of these compounds, disrupted chloroplast development, resulted in abnormal plant architecture, especially in leaf color(46, 47). IspD (CMS), IspE (CMK) and IspF (MCS) are the third, fourth and fifth enzyme in MEP pathway respectively (Fig. 5b). Related mutants possessed yellow or albino leaves and arrested development of chloroplasts(48–50). In this study, expression of CMS, CMK and MCS in albino mutant was lower than in wild type. The low-level expression may contribute to disruption of chloroplast development. Thylakoid related genes corresponded to reaction centers of PS I and PS II where photochemical reactions occur and convert light energy into chemical energy 31. Almost all genes related with thylakoid were down-regulated in albino mutant, demonstrating the decrease of photosynthesis viability.

Isocitrate lyase and malate synthase are two unique enzymes to glyoxylate cycle, which was considered essential for postgerminative growth and seedling establishment (51). Under normal condition, glyoxylate cycle enzyme activities decreased rapidly as seedlings become photosynthetic but maintained a continuously high level when grown in dark (52–54). In this study, the isocitrate lyase (Csa2G420990) and malate synthase (Csa1G050360) are abundant in mutant than in wild type (Fig. 6). Wild type started autotrophic growth at this timepoint already, with a high activity of peroxidase (Csa4G285740) and chlorophyll A-B binding protein (Csa3G664560), while albino mutant still needed to utilize the storage because it is still not able to photosynthesize resulted from a lack of functional chloroplast. Glutathione S-transferase have been reported mainly function in response to biotic and abiotic stresses, such as oxidative stress (55), high/low temperature stress (56, 57) and different pathogen invasion (58–60). As well, a high concentration of glutathione S-transferase resulted in decreased chlorophyll content (61). When albino mutant is exposed to light, a lethal factor rather than a gift, the mutant presented (Csa4G304240, Csa4G303170) (Fig. 6) more glutathione S-transferase than wild type, this might alsopromote chlorophyll degradation.

Since all the seeds were planted under the same culture condition, we could exclude the possibility of environmental but genetic determination of this albino phenotype. The genetic analysis revealed that this

albino phenotype was recessively controlled by a single locus. To trace the mutation origin, the previous generation of g32 was also evaluated. Phenotypic evaluation presented no albino performance in all the tested seedlings (data not shown), indicating that the mutation most probably occurred in previous generation in heterozygosis and albinism emerged in this generation. Albinism is not a desired phenomenon in plant breeding since it could affect plant growth as well as production. However, this mutant is of great importance for us to detect new gene that involve in protoplast development.

Conclusions

In this study, a novel naturally occurring albino mutant was firstly reported in cucumber. We showed that the albino mutant had abnormal chloroplast and did not produce any chlorophyll, therefore, the whole seedling presented white color. Interestingly, we found that this mutant is different from the ones in other plant species, since this cucumber albino mutant could recover white cotyledon to green under dark condition. In addition, with transcriptome data, we detected that differentially expressed genes involved in chlorophyll metabolism, Methylerythritol 4-phosphate (MEP) pathway, as well as thylakoid development. Taken together, this mutant can be a new material to study protoplast development.

Methods

Plant materials

Cucumber inbred line g32 was used in this study. The inbred line was provied by Vegetable Research Institute, Guangdong Academy of Agricultural Sciences. The institute is also in responsible for the plant materials used in this study. Yu'e Lin undertakes the formal identification of the plant material. This material has not been deposited in any publicly herbarium. Seeds from a self-pollinated g32 cucumber fruit were soaked into water for 4 hours and then kept in the incubator with moderate humidity at 28℃ for germination. Thereafter, germinated seeds were planted in plug tray in the greenhouse of Vegetable Research Institute, Guangdong Academy of Agricultural Sciences, Guangzhou, China. Dark treatment was performed in a black plastic bag covered homemade growth chamber. Seven-day-old wild type and albino mutant seedlings were used for phenotypic evaluation, fluorescence microscopy, transmission electron microscopy analysis and high-throughput RNA sequencing.

Fluorescence microscopy

The abaxial epidermises of cotyledons were used for fluorescence observation. The autofluorescence (red) of chloroplasts were captured under Zeiss LSM710 confocal microscope at the following setting: excitation at 633 nm, emission at 647–721 nm. Data were analyzed using ZEN2010 software.

Transmission electron microscope(TEM)

Cotyledons of wild type and albino mutant seedlings grown under light condition, and cotyledons of albino mutant seedling grown in dark were analyzed for TEM. All the cotyledon samples were cut into 1–

2 mm^2 sections and fixed in 2.5% glutaraldehyde and 4% paraformaldehyde in phosphate buffer (pH 6.8–7.2) under a vacuum for 3 hours, followed by washing with phosphate buffer. Subsequently, samples were fixed in 1% osmium tetroxide for 3 hours, followed by washing with phosphate buffer, and dehydrated in a graduated ethanol series. The samples were infiltrated with an increasing ratio of Spurr's resin dilutions (25%, 50%, 75%, and 100% (v/v)) to substitute ethanol. Finally, samples were embedded on Spurr's resin. After cutting, the sections were viewed under a HitachiH-7700 (Hitachi) transmission electron microscope.

RNA extraction

Total RNA was extracted from cotyledons of 7-day-old wild type and albino mutant seedlings using Trizol Kit (Promega, USA) according to the manufacturer's instructions. Extracted RNA was treated with RNase-free DNase I (TaKaRa, Japan) to remove residual DNA. RNA degradation and contamination was monitored on 1% agarose gels. RNA purity was checked using the NanoPhotometer® spectrophotometer (IMPLEN, CA, USA) and RNA integrity was assessed using the RNA Nano 6000 Assay Kit of the Bioanalyzer 2100 system (Agilent Technologies, CA, USA).

cDNA library construction, high-throughput sequencing and mapping

A total amount of 1 μg RNA per sample was used as input material for the RNA sample preparations. Sequencing libraries were generated using NEBNext® UltraTM RNA Library Prep Kit for Illumina® (NEB, USA) following manufacturer's recommendations. Library preparations were sequenced on an Illumina Novaseq platform and 150 bp paired-end reads were generated. Thereafter, reads with adaptors, reads with unknown bases as well as low quality reads were removed to generate clean reads. The remaining high-quality clean reads were mapped to Cucumber (Chinese Long) Reference Genome v2 (http://www.cucurbitgenomics.org/organism/2).

Quantification of gene expression, gene ontology (GO), and KEGG pathway enrichment analysis

The mapped reads of each sample were assembled by StringTie (v1.3.3b) 17 in a reference-based approach, and featureCounts v1.5.0-p3 18 was used to count the reads numbers mapped to each gene. FPKM (Fragments Per Kilobase of transcript sequence per Millions base pairs) of each gene was calculated based on the length of the gene and reads count mapped to this gene. Differential expression analysis of two groups was performed using the DESeq2 R package (1.16.1) 19. The resulting P-values were adjusted using the Benjamini and Hochberg's approach for controlling the false discovery rate. Genes with an adjusted P-value (padj) < 0.05 and |log2(FoldChange)| > 2 were assigned as differentially expressed genes (DEGs). To functionally annotate detected DEGs, GO (Gene Ontology, http://www.geneontology.org/) and KEGG (Kyoto Encyclopedia of Genes and Genomes, http://www.genome.jp/kegg/) annotation of the unigenes were analyzed using clusterProfiler R package.

Quantitative real-time PCR validation

To confirm RNA-seq results, 18 DEGs with |log2(FoldChange)| > 3 were randomly selected for qRT-PCR validation. First strand cDNA synthesis was performed using TransScript All-in-One First-Strand cDNA Synthesis SuperMix for qPCR (Transgen, China) with 1 μg of RNA as that used for high-throughput sequencing. Quantitative RT-PCR was carried out using 0.2 μg cDNA using PerfectStart Green qPCR Supermix (Transgen, China) according to the instructions. Reaction was performed and analyzed on a CFX96 Real-Time PCR Detection System. Three biological replicates and three technical replicates per sample were performed for each gene. Gene expression was normalized against α-TUBULIN (TUA) gene(62). Primers used were listed in Additional file 1: Table S1.

Abbreviations

CDS: Coding domain sequence

DEG: Differentially expressed gene

GO: Gene ontology

MEP: Methylerythritol 4-phosphate

PSI: Photosystem I

PSII; Photosystem II

qPCR: Quantitative real-time PCR

TEM: Transmission electron microscope

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

The datasets supporting the conclusions of this article are included either within the manuscript or its additional files.

Competing interests

The authors declare that they have no conflict of interest.

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Authors' contributions

J.Y., B.L., X.H., Y.L. and B.J. conceived the research plans; J.Y., Z.C. and P.S. performed most of the experiments; Z.L., W.L., M.W., Q.P. and L.C performed rest of experiments; J.Y. and B.L. analyzed the data; J.Y. and B.L. drafted the paper, X.H., Y.L. and B.J. revised the paper. All authors have read and approved the manuscript.

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Additional File Information

Additional file 1: Table S1. Primers used for qRT-PCR validation.

Additional file 2: Table S2. Segregation ratio of albino and wild type seedlings in progenies of g32.

Additional file 3: Table S3. Up-regulated and down-regualted genes in albino mutant.

Additional file 4: Table S4. GO enrichment analysis of differentially expressed genes between albino mutant and wild type.

Additional file 5: Table S5. KEGG analysis of differentially expressed genes between albino mutant and wild type.

Additional file 6: Table S6. Differentially expressed genes in chlorophyll metabolism and MEP pathway between albino mutant and wild type.

Additional file 7: Table S7. Differentially expressed genes of reported albino genes between albino mutant and wild type.

Additional file 8: Figure S1. Histogram of GO enrichment analysis.

Table

Table 1. RNA sequencing reads and sequencing quality of all samples

Sample	Raw_reads	Clean_reads	Clean_bases	Error_rate	Q20	Q30	GC_pct
Albino-1	112,074,500	110,556,516	8.29G	0.03	97.37	92.56	43.31
Albino_2	115,086,664	113,466,224	8.51G	0.03	97.47	92.73	43.43
Albino_3	116,871,728	115,589,940	8.67G	0.03	97.47	92.77	43.45
Normal_1	102,760,028	101,241,304	7.59G	0.03	97.5	92.83	43.16
Normal_2	127,644,424	123,516,412	9.62G	0.03	97.43	92.68	43.43
Normal_3	100,197,308	97,869,948	7.34G	0.03	97.35	92.54	43.55

Figures

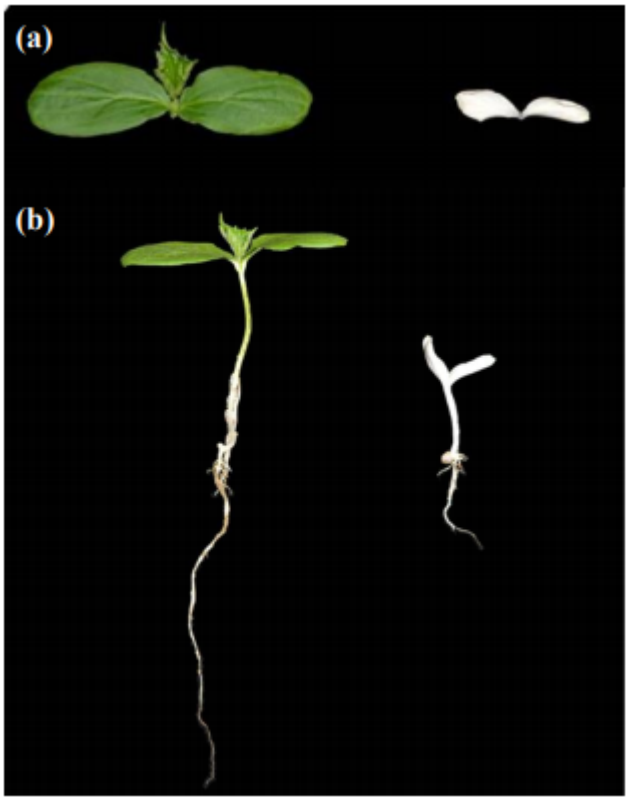

Figure 1

Growth phenotype of albino mutant and wild type from the progenies of a cucumber inbred line g32. a Differences in cotyledons of albino mutant and wild type seedlings. b Morphological difference between albino mutant and wild type seedlings.

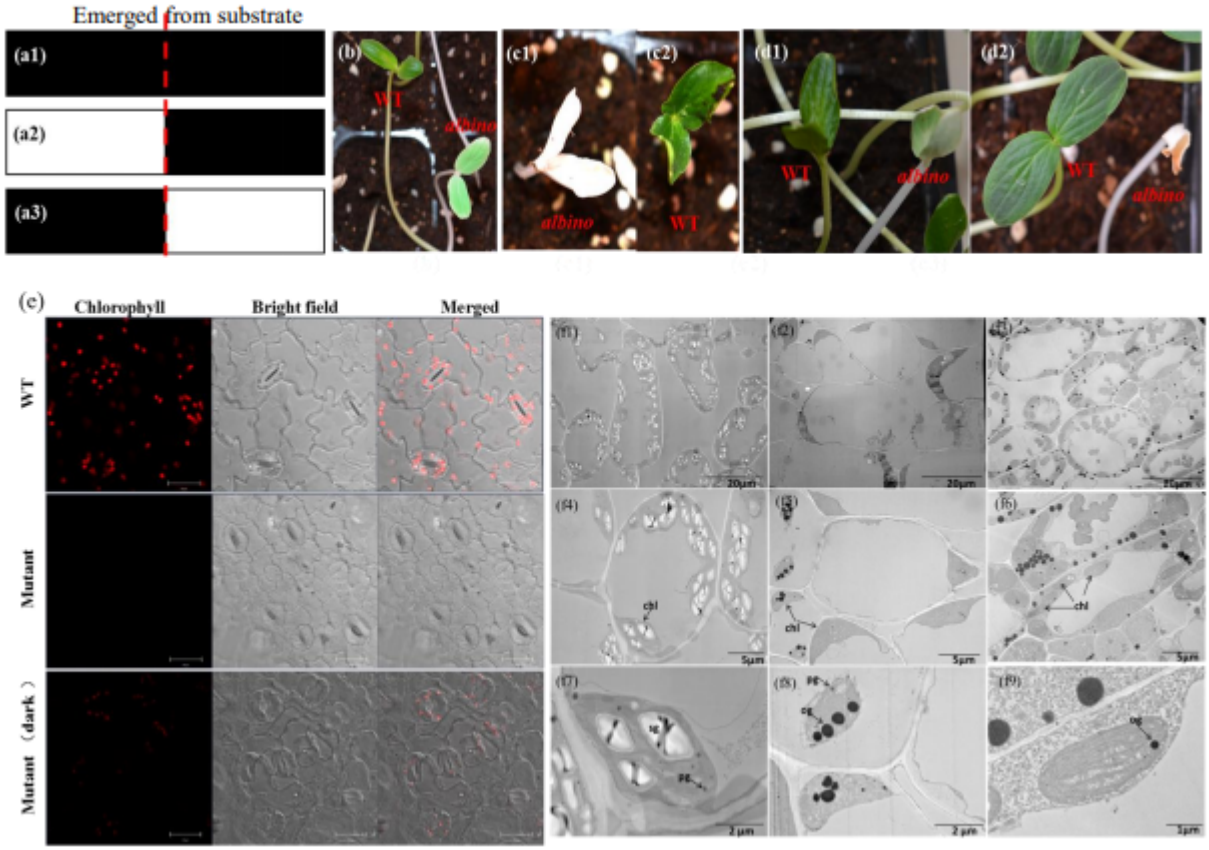

Figure 2

Short-lived chloroplast recovery in albino mutant under dark condition. a1-a3 Schematic illustration of light and dark treatment of WT and albino seedlings. a1 Plants were treated with continuous dark before and after they emerged from substrate. a2 First, plants were treated with light, then after emerging from substrate, they were moved to dark. a3 First, plants were treated with dark, then after emerging from substrate, they were moved to light condition. b Phenotype of WT and albino seedlings under indicated condition that described in a1. c Phenotype of WT (c1) and albino (c2) seedlings in the indicated conditions that described in a2. d Phenotype of WT and albino seedlings in the indicated conditions that described in a3. d1 Phenotype of WT and albino seedlings exposed to light after 5 hours. d2 Phenotype of WT and albino seedlings exposed to light after 30 hours. e Fluorescence microscopy images of cotyledon abaxial epidermis of wild type, albino mutant cucumber seedlings grown under light condition and albino mutant seedlings grown in dark. Bar: 20μm. f1-f9 Transmission electron microscopy of cotyledons from albino mutant and wild type seedlings. f1, f4 An overview of cotyledon cells of wild type grown under light condition. f2, f5 An overview of cotyledon cells of albino mutant grown under light condition. f3, f6 An overview of cotyledon cells of albino mutant grown in dark. f7, f8, f9 Enlarged views of chloroplast ultrastructure of wild type, albino mutant growon under light condition and albino mutant grown in dark, respectively. chl, chloroplast; sg, starch granules; pg, plastoglobuli; og, osmiophilic plastoglobuli.

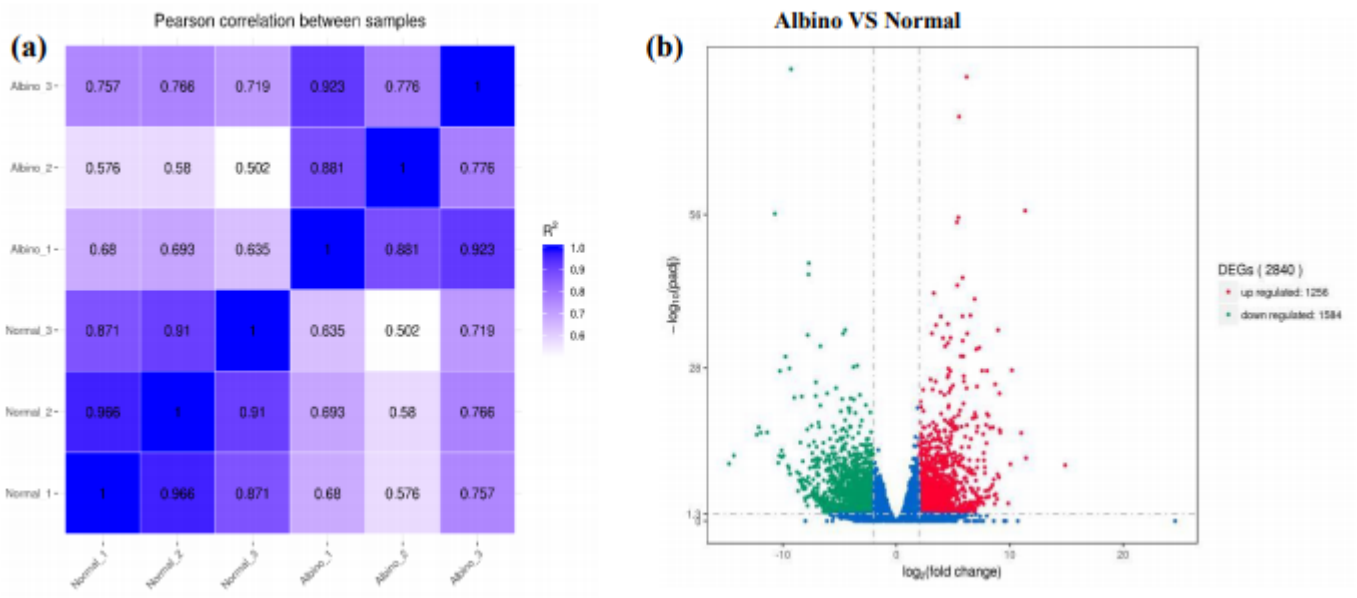

Figure 3

Diagrams illustrating correlations/distances among transcriptomes and the number of differentially expressed genes in albino mutant and wild type seedlings. a Correlation matrix and cluster dendrogram of the whole dataset of the mapped reads. The analysis was performed by comparing the values of the entire transcriptome in all two samples with three biological replicates. Correlation analysis (coefficients R2) and hierarchical cluster analysis were performed using R software. Dark blue color indicates a stronger correlation and light blue weaker (R2). b Volcano plot showing DEGs between albino mutant and wild type. X-axis represents log2 Fold Change and y-axis represents -log10 (padj). Red, green and blue dots represents up-regulated, down-regulated and not DEGs, respectively.

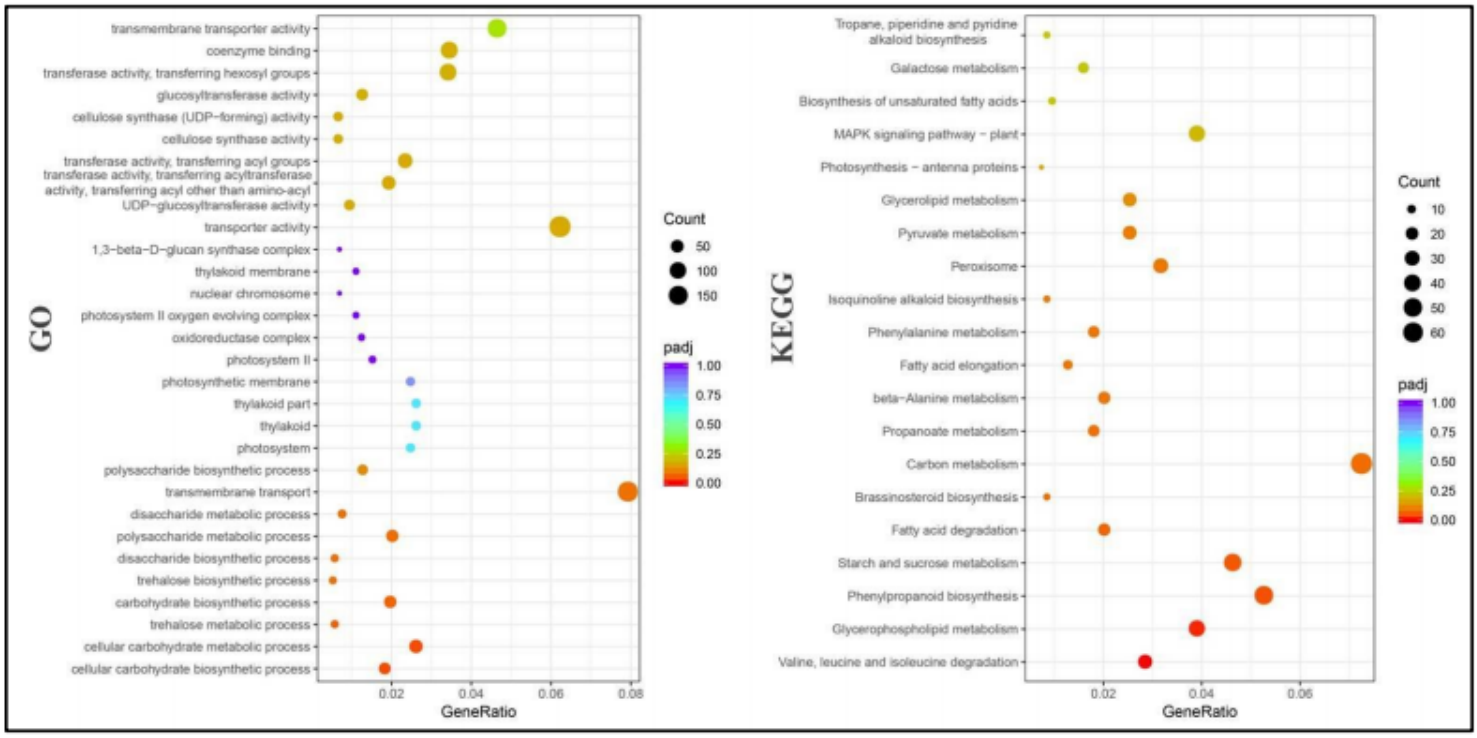

Figure 4

Genes enriched in different categories in the GO (left) and KEGG (right) analysis. X-axis represents the gene ratio of enriched genes among the background genes in different categories and while y-axis represents the GO or KEGG terms. A high padj-value is represented by red, and a low value is represented by purple. The size of the bubble represents the number of genes annotated to the terms.

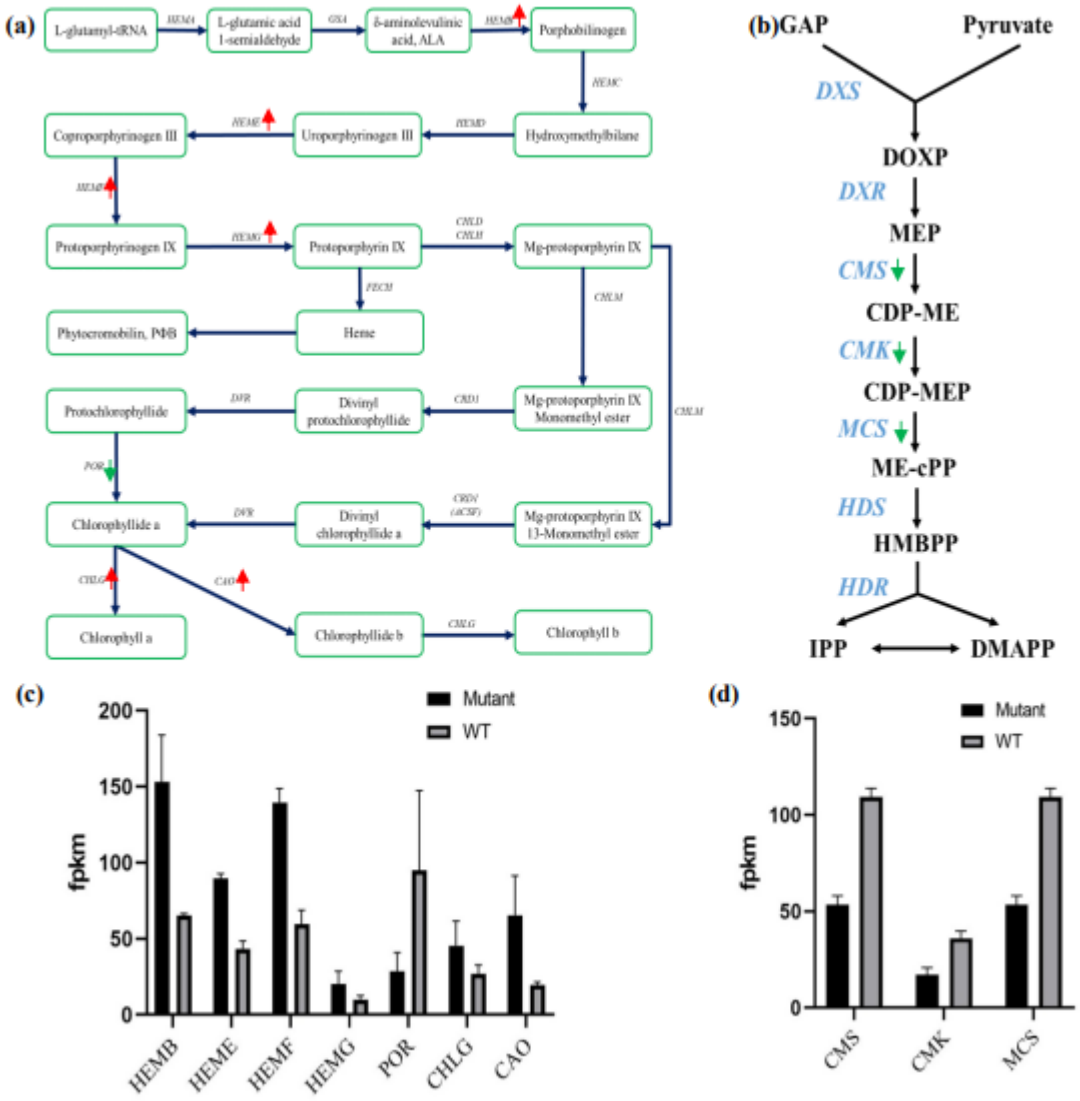

Figure 5

Differentially expression of genes associated with chlorophyll metabolism and Methylerythritol 4-phosphate (MEP) pathway. a, b Diagram showing major genes in chlorophyll metabolism and MEP pathway, respectively. Red arrows indicate up-regulated genes while green arrows indicate down-regulated genes in albino mutant. c, d Expression profile of DEGs in chlorophyll metabolism and MEP pathway achieved by RNA-seq. The mean FPKM (fragments per kilobase of transcript per million mapped reads) values for the DEGs were calculated from three biological replicates for each genotype. The error bars indicate the standard deviations. GAP, glyceraldehyde-3-phosphate; DOXP, 1-deoxy-D-xylulose-5-phosphate; MEP, 2-C-methyl-D-erythritol-4-phosphate; CDP-ME, 4-diphosphocytidyl-2-C-methyl-D-erythritol; CDP-MEP, 4-diphosphocytidyl-2-C-methyl-D-erythritol-2-phosphate; ME-cPP, 2-C-methyl-D-erythritol-2,4-C-cyclodiphosphate; HMBPP, 1-hydroxy-2-methyl-2-(E)-butenyl-4-diphosphate; IPP, isopentenyl diphosphate; DMAPP, dimethylallyl diphosphate.

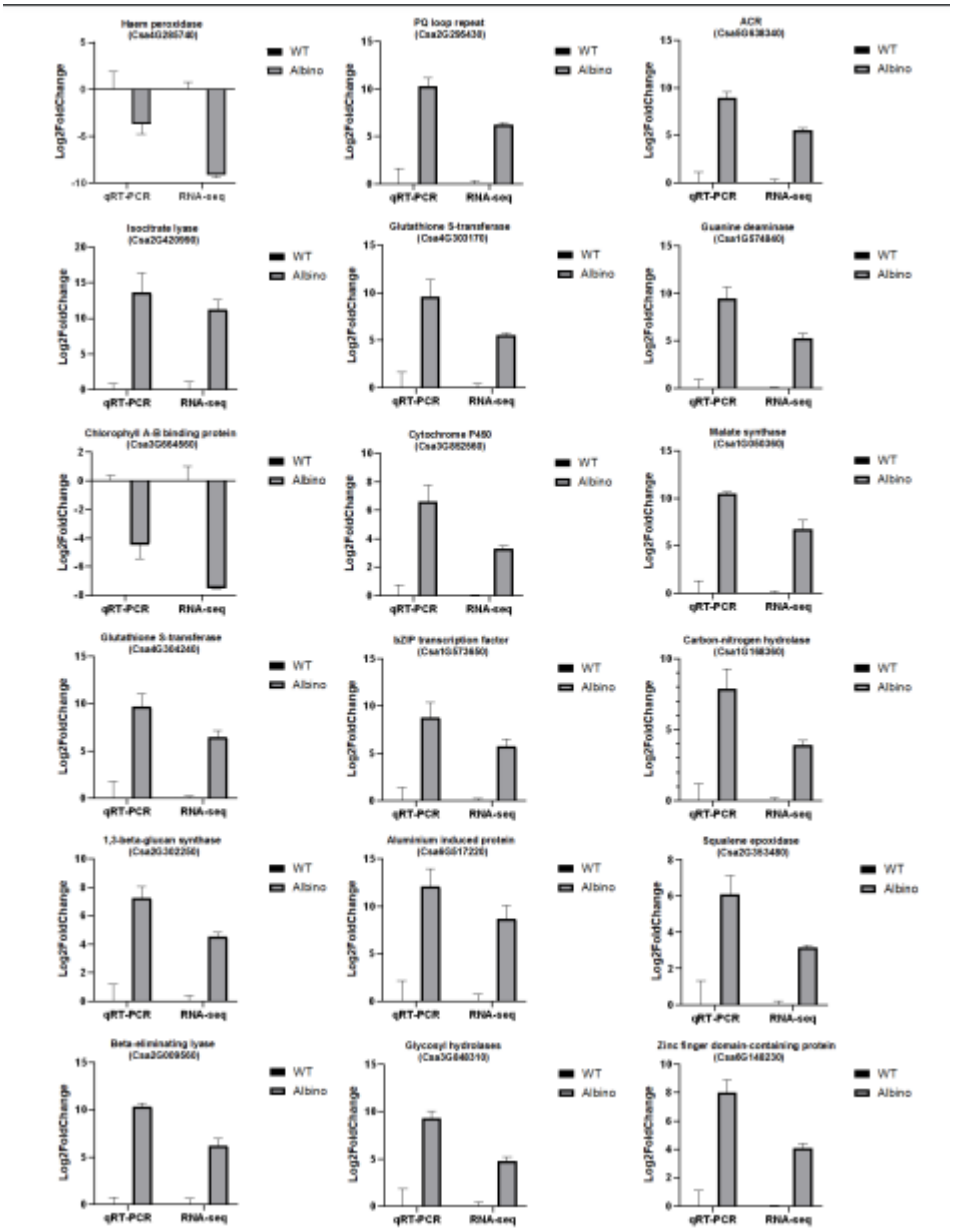

Figure 6

Expression profile of selected differentially expressed genes between albino mutant and wild type from RNA-seq result achieved by qRT-PCR. X-axis represents gene name while y-axis represents relative expression (−ΔΔCt) value of each gene. Data are shown as means (n=3). The error bars indicate the standard deviations

Supplementary Files

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