Lipidomics Analysis Reveals the Effect of Light Intensity on Lipid Metabolism and Leaf Color in Light-Sensitive Albino Tea Plant (*Camellia Sinensis* cv. *Baijiguan*)

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Abstract

Background: *Camellia sinensis* cv. *Baijiguan* is a light-sensitive albino tea germplasm. Under light stress, the development of thylakoid membrane structure is not complete, leading to the white color of leaves. Previous studies have shown that the change of lipid can cause the change of thylakoid membrane structure. However, there is no study on the effect of lipid on the leaf color of light-sensitive albino tea germplasm. Thus, we hypothesized that the changes of lipid composition in *Baijiguan* could affect the leaf color.

Results: In order to understand the mechanisms of its light sensitivity, *Baijiguan* and *Rougui* were grown under three different conditions: normal light, shading, shading followed by the resuming of light. The total lipids were isolated from the second leaf. 156 lipid species were identified and analyzed by lipidomics. We found that under normal light condition, the newly-developed leaves showed the yellow color with incomplete development of thylakoid membrane, the defense enzyme activity was maintained at a high level, accelerated degradation of chlorophyll. The ratio of MGDG to DGDG of *Baijiguan* was lower than that of *Rougui* which kept normal green leaf color. The shading treatment, reduced the content of MGDG and DGDG; meanwhile chlorophyll accumulated, and the thylakoid membrane formed, the leaves turned into green color. When shade-treated leaves were reexposed to light, the MGDG to DGDG ratio increased significantly, the lipid content decreased significantly, and the albinism emerged again.

Conclusion: Our data demonstrated that the effect of light intensity on *Baijiguan* leaf color was realized by changing the lipid content and components in the leaves, and offered a new insight about the mechanisms of its leaf albinism.

Background

The tea plant (*Camellia sinensis*) is a perennial evergreen woody plant, of which shoots are often processed into various tea products, such as green tea, black tea, and oolong tea[1]. To date, tea cultivars and their products in China show the 'colorful' trend[2]. Albino tea cultivars are special mutants of the tea plant with white or yellow leaf color under certain environmental conditions, such as low temperature or high light intensity[3–5]. Compared to normal green cultivars, they are deficient in chlorophyll. Albino tea germplasm is precious because of their special flavor, distinct leaf color and scarcity[4]. The sun-light is one of the necessary conditions for photosynthesis which plays an important role in the chlorophyll synthesis[6]. *Baijiguan*, a light-sensitive albino tea cultivar, the new shoots display white color under high light intensity, and turn green under low light intensity[7]. Currently, the research for the formation of albino leaves are mainly focused on the metabolism of chlorophyll and carotenoid[8–11]. Under sun-light, the chlorophyll synthesis in *Huangjinya* was blocked and the degradation was accelerated. The content of antenna protein, PSII and PSI related proteins were significantly reduced, resulting in the photosynthetic electron transportation arrestation, and reduction of the photosynthetic efficiency [6]. Transcriptomic analysis found that the affected genes were enriched in fatty acid metabolic pathway and unsaturated fatty acid metabolic pathway[12].

Chlorophyll degradation is an important step in plant self-regulation and prevention of photosynthetic damage. Its degradation products have antioxidant effect and can maintain or alleviate cell activity. Li[13] discussed the gene expression of chlorophyll degradation pathway in *Huangjinya*. She found that *CLH*, *RCCR* and *SGR* were significantly high expression in the golden bud, and their chlorophyll synthesis genes were inhibited, resulting in the insufficient chlorophyll content after 40 days of shading. The degradation of chlorophyll in *Anji Baicha* and *Huabai 1* was accelerated and the leaves became white[14, 15].

When plants are stressed by living and non living things, cells produce a series of comprehensive reactions. Superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD) are important active oxygen defense enzymes in plants, which respond to the damage caused by stress and protect plant cells. In the process of turning white and green of *Anji Baicha*, the activity of defense enzyme changes in the opposite direction. When turning white, the activity of POD increases, while the activity of SOD and CAT decreases[16]. The continuous increase of active oxygen can cause lipid peroxidation and directly cause plant death. Therefore, it is necessary to reduce the active oxygen in vivo to maintain chloroplast photosynthesis[17].

Lipids are the structural material of cell membranes that play a number of key roles in plant growth, development, and responses to environmental factors[18, 19]. The comprehensive classification system organizes lipids contain eight well-defined categories, they are: Fatty acyls (FA), Glycerolipids (GL), Glycerophospholipids (GP), Sphingolipids (SP), Sterol Lipids (ST), Prenol Lipids (PR), Saccharolipids (SL) and Polyketides (PK)[20]. The membranes of plant cells containing 5–10% lipids (dry weight) are able to distinguish the cells and compartments where many key processes make up, including light harvesting and electron transport reactions of photosynthesis[21]. Chloroplast thylakoid membrane is the place where plants perform photosynthesis. The lipid bilayer of thylakoid membrane is mainly composed of monogalactosyldiacylglycerol (MGDG), digalactosyldiacylglycerol (DGDG), sulfoquinovosyldiacylglycerol (SQDG) and phosphatidylglycerol (PG), MGDG and DGDG account for a large proportion. The photosynthetic protein complex in chloroplast is embedded in polar lipids which play critical role in photosynthesis with regular arrangement. The changes of lipid content and proportion often changes of thylakoid membrane structure which has been studied in other plants. Under low nitrogen, phosphorus deficiency or low temperature, the thylakoid membrane structure become unstable, the development is not perfect or the membrane disintegrates, the stacking of basal grains is reduced, the lamellar structure is fuzzy, and the starch grains are increased. The content of MGDG, DGDG, PG and other species of lipids, the MGDG to DGDG ratio, the content of unsaturated fatty acids, and the membrane fluidity all are decreased, which affected the photosynthetic efficiency[22–24].

We hypothesized that the changes of lipid composition in *Baijiguan* is an important factor for its leaf color changes. Lipidomics is dedicated to identifying changes in lipid metabolism and lipid-mediated signaling processes[25, 26]. Due to the wide variety of lipids, complex structures and large differences in content, a good analytical method for the analysis of all lipids in plants is needed. The development of liquid chromatography-mass spectrometry provides a strong technical support for the qualitative and

quantitative determination of lipids[27–29]. At present, ESI-MS / MS technology is widely used for lipid analysis because it can obtain a wealth of information on the structure fragments of compounds[30, 31]. Therefore, lipids were comprehensively analyzed from *Baijiguan* which were grown under various light conditions, and our data offered new insights about the relationship between the changes of leaf color and lipids in this light-sensitive albino tea germplasm.

Results

Phenotypic characterization of *Baijiguan* and *Rougui*

In *Baijiguan*, the SPAD value of BS was increased significantly in 6 days, while those of BC and BRL were quite stable during this period (Fig. 2). Under normal conditions, the second leaf of *Baijiguan* was still yellow (Fig. 1). In *Rougui*, the SPAD value of RS showed the increasing trend but not so significant compared with those of BS. (Fig. 2). After shading treatment, the *Baijiguan* leaf turned into green within one day, and this process can be reverted by reexposing the leaves to high light. In contrast, *Rougui* remained green in all treatments (Figs. 1 and 2). The results showed that the shading treatment turned the leaves of *Baijiguan* into green and increased the chlorophyll content.

Effect of different light treatments on defense enzyme activity of tea

Defense enzyme activities of SOD, POD and CAT in different treatments were analyzed. On the whole, the activities of SOD, POD and CAT in *Baijiguan* were higher than that in *Rougui*. The activity of SOD was higher than POD and CAT. The activity of enzyme in *Baijiguan* fluctuated more rapidly than in *Rougui*, especially in normal light. After shading, the enzyme activity of *Baijiguan* decreased to similar to that of *Rougui*. After the recovery of light, the activities of SOD and POD increased gradually, while the activities of CAT decreased.

Total lipids of tea plants

Glycerolipids are the most abundant lipids in plants, including phospholipids, glycolipids and neutral lipids. In this study, 156 lipid species were detected, including 16 digalactosyl glycerol (DGDG), 16 monogalactosyldiglycerol (MGDG), 13 phosphatidylglycerol (PG), 5 lysophosphatidyl glycerol (LPG), 6 lysophosphatidylcholine (LPC), 5 lysophosphatidylethanolamine (LPE), 20 phosphatidylcholine (PC), 23 phosphatidylethanolamine (PE), 14 phosphatidylinositol (PI), 26 phosphatidylserine (PS), and 12 phosphatidylacids (PA) (Additional file 1: Table S1).

The total lipid contents of *Baijiguan* and *Rougui* at day zero were 164.323 and 45.008 nmol/mg dry weight, which showed that BJJ has much more total lipids than RG (Fig. 6B). In all treatments, the total lipid content of *Baijiguan* decreased sharply. But all the total lipids detected in RG kept stable during the period, which showed that the lipid metabolism was different between BJJ and RG.

Glycolipid changes of Baijiguan and Rougui

MGDG and DGDG are the main components of thylakoid membrane in plants. The content of MGDG and DGDG in *Baijiguan* and *Rougui* showed the same trends as those of total lipids. (Fig. 7A and B). MGDG and DGDG occupied around the 1/5 and 1/7 of the total lipids in BJG, respectively. But they were about 1/4 and 1/6 in RG, which showed that these two components had more portion in RG than in BJG.

As shown in Figs. 8 and 9, the content of DGDG and MGDG was higher in *Baijiguan* than that in *Rougui*. DGDG (34:3), DGDG (36:6) and MGDG (36:6) are the main lipids in tea plants, their contents were higher in *Rougui* than those in *Baijiguan* before shading. After shading for one day, their contents in *Baijiguan* declined abruptly.

Lysophospholipid changes of Baijiguan and Rougui

Lysophospholipid which can be used as a signal molecule is produced by the partial hydrolysis of phospholipids. The contents of LPC and LPE in *Baijiguan* decreased dramatically to approximate 0.05 nmol/ mg DT after shading for one day. It is also showed that they decreased abruptly in BRL3 compared to BRL1 (Fig. 13B and C), while remained steady in *Rougui* (Fig. 13E and F). The LPG contents kept stable in *Baijiguan* and *Rougui* (Fig. 13A and D). At BC0, BC6, BS2, BS4 and BRL1, the contents of LPC in *Baijiguan* were significantly higher than that of *Rougui*. Like LPC, the LPE content of *Baijiguan* in BC0, BS4, BS6 and BRL3 was higher than that in *Rougui* in the same period.

Phospholipid changes of Baijiguan and Rougui

The content of PG, PC, PE, PI and PS in *Baijiguan* were significantly higher than those in *Rougui*. These lipids contents in *Baijiguan* declined obviously during the three treatments period, while did not show the same trend in *Rougui*. In the light resuming treatment, they increased remarkably instead of decrease. After shading for 1 day, the content of phospholipid in *Baijiguan* was decreased significantly. In addition, the content of phospholipid in the first day of recovery was significantly higher than that in the third day (Fig. 14A-F). But *Rougui* did not show the same trend in the this treatment. (Fig. 14G-L), and there was no significant change in the all treatments.

qRT-PCR analysis of lipid metabolism and chlorophyll degradation genes in Baijiguan

PLD, *plc*, *DGK*, *MGD* and *DGD* genes are some of the key genes in the lipid synthesis of the plant thylakoid membrane. In this study, the expression of these genes in the second leaves of *Baijiguan* treated with different light intensity was verified. The results showed that the expression of key genes of lipid synthesis after shading was generally higher than those expressed during the period of albinism and restoration of light, which indicated that membrane lipid synthesis was accelerated after shading (Fig. 15).

At the same time, we also verified the relative expression of chlorophyll degradation pathway. The results showed that the relative expression of chlorophyll degradation genes was generally lower after shading

than that under normal light and light recovery period, indicating that chlorophyll degradation accelerated in albinism period (Fig. 16).

Discussion

The mechanisms of plant leaf albino remains unclear, likely involve multiple regulatory pathways and metabolic processes, and affected by the interactions with internal and external factors[34]. *Baijiguan* is more sensitive to high light intensity, of which chloroplast development is retarded, and its chlorophyll content is lower than other green-leaf varieties at the same developmental stage[7]. Chlorophyll plays an essential role in photosynthesis by absorbing and transferring the light energy to other molecules of the photosynthetic electron transport chain[35]. The chlorophyll accumulation in plants is dynamic[36, 37], which is a comprehensive process of synthesis and decomposition. Light plays a key role in the regulation of chlorophyll synthesis. Previous studies have shown that the SPAD value of *Baijiguan* leaves basically didn't change in the natural environment, while increased linearly by shading treatment. The leaves obviously turn green after shading treatment, while return yellow if the light resumed. Our study showed that the chlorophyll in the albino leaves maintained at a low level under high light conditions, and significantly increased by the shading treatment. Shading treatment also significantly increased the chlorophyll content in another tea cultivar *Huangjinya*[6, 13].

In this study, the lipid composition of *Baijiguan* and *Rougui* leaves was compared to explore the reason of albinism. The lipid composition of *Baijiguan* changed greatly by shading, and correlate with leaf color changes. MGDG/DGDG ratio plays an important role in maintaining the structure and function of photosynthetic organs, which affects the permeability of chloroplast membrane and the stability of membrane bilayer[38]. Plants maintain the balance between the physical state of membrane and the normal function of membrane protein by means of increasing the ratio of MGDG/DGDG[39]. Under normal sun-light, the ratio of MGDG/DGDG in *Baijiguan* was significantly lower than that in *Rougui* (Fig. 11). It led to the thylakoid developing uncompletely, lamellar structure fuzzy, and lipid droplets increasing during albinism, which led to the abnormal photosynthesis and energy shortage in *Baijiguan*[7]. Membrane lipid/chlorophyll is an indicator of the density of thylakoid assembly protein. The higher the ratio is, the lower the density of the assembly protein is. and it reflects the destroying of the fluidity, structure and function of the membrane[40, 41], The ratios of total lipid/chlorophyll, DGDG/chlorophyll and MGDG/chlorophyll in *Baijiguan* were higher than that in *Rougui* under different treatments (Fig. 12A, D and G). These results showed that the density of thylakoid assembly protein in *Baijiguan* was lower than that in *Rougui* under different treatments, and the fluidity, structure and function of thylakoid membrane were destroyed. Therefore, we speculate that the membrane structure of *Baijiguan* was unstable during the albinism period, which led to the increase of active oxygen in vivo, the increase of enzyme activity and the albinism of leaves.

The degradation of chlorophyll is closely related to the change of lipid morphology[42]. After shading for one day, the lipid content in *Baijiguan* decreased significantly, but no significant difference showed in *Rougui*. Wu found that the thylakoid membrane structure of *Baijiguan* was reconstructed gradually in the

shading treatment, but the membrane structure of *Rougui* was intact[7]. The relative expression of liposynthesis genes increased significantly after shading and the activity of defense enzymes decreased, which also indicated that the thylakoid membrane was reconstructed. Therefore, we speculate that the decreased total lipid was involved in the membrane reconstruction. Shading regulates the content of MGDG and DGDG, and results in thylakoid membrane remodeling, the stability of membrane maintaining, and the degradation of chlorophyll slowing down, which turns leaves green[13, 43]. The ratio of membrane lipid/chloroplast of *Baijiguan* decreased after shading, but it was still significantly higher than that of *Rougui* until the 6th day (Fig. 12B, E and H), the density of the assembly protein was still lower than that of *Rougui*, and the function and fluidity of the thylakoid membrane were not completely restored. Therefore, the SPAD value of *Baijiguan* was significantly higher than that of *Rougui*.

We also found that the color of *Baijiguan* returned yellow after resuming light, the activities of SOD and POD increased, and the lipid content decreased significantly after the restoration of light for three days compared to those just treated for one day. The proportion of MGDG/DGDG in *Baijiguan* increased significantly, but was still lower than that in *Rougui* (Fig. 13). However, the membrane lipid / chlorophyll in *Baijiguan* was significantly higher than that of *Rougui* treated at the same day (Fig. 12C, F and I). The relative expression of lipid synthesis genes was also lower than that after the restoration of light. This indicated that the structure and function of chloroplast membrane were destroyed, which affected the chlorophyll synthesis ability, and the degradation of chlorophyll accelerated[44, 45]. It is possible that receptor proteins and docking proteins in the membrane are unable to bind or form stacking structures on the chloroplast membrane due to the absence of DGDG[46].

Conclusion

In this study, the lipids of *Baijiguan* and *Rougui* tea leaves grown under normal light, shading and recovery light was analyzed, and the effects of light intensity on leaf color and lipid composition of *Baijiguan* were revealed. Under normal light, the ratio of MGDG/DGDG of *Baijiguan* was lower than that of *Rougui*, and the ratio of total lipid/chlorophyll, DGDG/chlorophyll and MGDG/chlorophyll in *Baijiguan* were higher than that in *Rougui*. The relative expression of lipid synthesis genes in *Baijiguan* were low. These resulted in incomplete development of thylakoid membrane, high level of defense enzyme activity, failure of photosynthesis, and accelerated degradation of chlorophyll, leading to leaf whitening. After shading, the content of MGDG and DGDG was reduced accompanied by the thylakoid membrane remodeling, the stability of membrane increased, the degradation of chlorophyll slowed down and leaves turned green. Compared with one day after light restoration, the lipid content of *Baijiguan* significantly decreased and the ratio of MGDG/DGDG significantly increased after three days of light resuming, but it was still lower than that of *Rougui*. The ratio of membrane lipid/chlorophyll in *Baijiguan* was significantly higher than that of *Rougui* treated at the same time, and the relative expression of lipid synthesis genes of *Baijiguan* were decreased. It was clear that the *Baijiguan* was sensitive to light after light resuming. The normal growth of *Baijiguan* was maintained by adjusting the lipid ratio, but the membrane structure was still unstable, photosynthesis could not be carried out normally, and the leaf color turned white. All

these confirmed that the effect of light intensity on *Baijiguan* leaf color was realized by changing the lipid content and components in the leaves.

Methods

Plant material and experiment

Camellia sinensis cv. *Baijiguan* and *Camellia sinensis* cv. *Rougui*, nationally released and commercially available clone optimum for oolong tea produce, are widely planted in the Wuyixing Tea Germplasm Resources Garden in Wuyixing tea co. LTD, Wuyi Mountain, China (27 ° 55 ' 15 " N, 118 ° 02 ' 50 " E). In the middle of September 2018, the second leaves of *Baijiguan*(BJG) and *Rougui*(RG) with similar growth and development status were taken for treatment. *Rougui*, a common green leaf variety, was used as the control. There were three treatments in the experiment: normal light (control,C), shading (S) and shading 3 days followed by the resuming of light (RL). The second leaf was covered with aluminum foil for shading treatment. After 3 days of shading, part of the aluminum foil was removed to restore the light to the blade. Samples for lipidomics measure were selected on the 4th and 6th days which equivalented to resume light 1 and 3days; the control leaves were taken from the normal growth of *Baijiguan* and *Rougui*. The total processing time was 6 days and the samples were taken every 24 hours. All samples were frozen with liquid nitrogen and stored in - 80 °C refrigerator for and lipid group detection.

Determination of chlorophyll content

SPAD-502PLUS Chl meter (Spectrum Technologies, Konica Minolta, Japan) was used to determine the chlorophyll SPAD value from the leaves[32]. We took 3 positions on each side of each leaf by avoiding the main leaf veins for testing and repeated 5 times.

Activity determination of SOD, POD and CAT

Grind the sample to be tested in a mortar with liquid nitrogen, and transfer the ground sample to a 10 ml centrifuge tube. According to the proportion of weight (g): Volume (mL) = 1: 9, add 9 times volume of pH = 7 phosphoric acid buffer solution, stand for 10 minutes, put it into a freezing centrifuge at 4 °C, centrifugation at 5000 rpm / min, centrifugation for 10 minutes, take the supernatant in a 2 mL centrifuge tube, the supernatant is a crude enzyme extraction solution, and the ice bath is to be tested. SOD, POD and CAT were determined by SOD assay kit (A001-1, Nanjing Jiancheng Bioengineering Institute), POD assay kit (A084-3, Nanjing Jiancheng Bioengineering Institute) and CAT assay kit (A007-2, Nanjing Jiancheng Bioengineering Institute). All indexes were repeated three times. The average value and standard error were calculated.

Lipid isolation from tea leaves

Glass tubes or vials with Tefon-lined caps were used in all methods. All solvents, including water, were HPLC-grade.

Lipids were extracted from samples of control, shading treatment and light resuming as the method of Shiva[30]. 2.0 mL isopropanol/0.01% BHT (butylated hydroxytoluene) was added into 8 ml glass tube with screw cap (get each empty tube exact weight and make records), closed lid snugly. Then heat to 75 °C. Rapidly harvest 6–8 tea leaves and put into hot isopropanol very quickly to avoid lipolytic activity. Screwed lid on snugly to prevent evaporation. Heat for more than 15 minutes, and removed from heating block and allow cooling to room temp. Added 6.0 ml chloroform/methanol/water (30/41.5/3.5, v/v/v), closed lid snugly. Shook at room temperature for 24 h (50–100 rpm on shaker) until the green leaves become pale white, that's the indication that lipids were completely extracted. Intact, extracted tea leaves were transferred to a new vial using forceps, dried overnight at 105°C, equilibrated to room temp, and weighed to 6 decimal in grams. At the same time, using a glass, gas-tight syringe (or use glass pipette), transferred the solvent and lipids to 2.0 ml GC vial (clear glass, Teflon lined screw cap lid) dried down under nitrogen stream before taken to Kansas Lipidomics Research Center (KLRC, USA) for lipid testing.

Analysis of lipids by ESI-triple quadrupole MS Multiple reaction monitoring method for analysis of tea plants

With references to the methods of Sunitaha[30] and Vu[33]. ESI-triple quadrupole mass spectrometry multiple reactions monitoring analysis method was used to detect lipids in tea plants. The data was processed using Lipidome DB Data Calculation Environment (<http://129.237.137.125:8080/Lipidomics>) as described by Vu[33].

Validation of lipid synthesis and chlorophyll degradation genes by qRT-PCR

Combined with the previous transcriptome data, the expression of key genes in lipid synthesis and chlorophyll degradation was verified. Specific primers were designed by using Primer 5.0 (Table 1). First-strand cDNAs (10-fold dilution) were synthesized from RNA extracted from the second leaves were used as templates (TIANGEN RNAprep Pure Plant Kit). qRT-PCR was performed using a TB Green™ Premix EX Taq™ (Tli RNaseH Plus) Kit (TaKaRa, Dalian, China) according to the manufacturers' instructions. The glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*) gene was utilized as a loading control. Relative gene expression levels were calculated according to the $2^{-\Delta\Delta C_t}$ comparative CT method.

Table 1
Primer sequences

Primer name	Sequence(5'→3')	Function
q CLH -F	GCCGAAACCCAACCAACT	Real-time quantitative PCR
q CLH -R	CCACCCGAACAAGCAAGA	
q SGR -F	CCAAAGCCATGCCAAGAGAAC	
q SGR -R	GGAGTTGGGTTTGGTGGGTC	
q $CsPPH$ -F	TCAAGCATTGGCAGCGAAAC	
q $CsPPH$ -R	AATGAGCAACAGAGGCACCA	
q PAO -F	GGACACATGGGTAGCGGAAT	
q PAO -R	TGCATTGTCCCAATCCCTCC	
q $RCCR$ -F	CACATGGTTGGATTGGTGCG	
q $RCCR$ -R	TGGCAAGCTCGATCCCAAAT	
$GAPDH$ -F	TTGGCATCGTTGAGGGTCT	
$GAPDH$ -R	CAGTGGGAACACGGAAAGC	
q PLD -F	GAGAAACAAACGCACCCCAC	
q PLD -R	TCGCCGTGCAGATAGATGAC	
q plc -F	GGAAATCAACGGCGTAACCG	
q plc -R	CTTGTTCGAATATGGCGGCG	
q DGK -F	CGGGTGGCCCACTTTAGAAT	
q DGK -R	CTCGGGCCAAGTCATTTCT	
q MGD -F	TCAGAGAACGCGCTCAAAC	
q MGD -R	GGCTGGAAAACGACGATGTG	
q DGD -F	ATTCGAAGGGCGTACTCGTC	
q DGD -R	GCCAGTCCCACCTAAAGTCC	

Abbreviations

BJG

Baijiguan; RG:*Rougui*; SOD:Superoxide dismutase; CAT:catalase;

POD

peroxisome; FA:Fatty acyls; GL:Glycerolipids; GP:Glycerophospholipids;

SP

Sphingolipids; ST:Sterol Lipids; PR:Prenol Lipids; SL:Saccharolipids;
PK
Polyketides; MGDG:Monogalactosyldiacylglycerol;
DGDG
Digalactosyldiacylglycerol;
SQDG
sulfoquinovosyldiacylglycerol;PG:phosphatidylglycerol;
LPG
Lysophosphatidyl glycerol; LPC:Lysophosphatidylcholine;
LPE
Lysophosphatidylethanolamine; PC:Phosphatidylcholine;
PE
Phosphatidylethanolamine; PI:Phosphatidylinositol; PS:Phosphatidylserine;
PA
Phosphatidylacids; C:Normal light (control); S:Shading;
RL
Shading 3 days followed by the resuming of light.

Declarations

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Availability of data and materials

All data generated or analysed during this study are included in this published article and its supplementary information files.

Author Contributions

W.S., M.C., Z.C. and Q.W. conceived and designed the experiments; Z.Z. performed the experiments; Z.Z. analyzed the data; Z.Z. wrote the manuscript; L.L., M.C. and Q.W. revised the manuscript critically. W.S. and Z.C. administrated the project. All the authors read and approved the final manuscript.

Ethics approval and consent to participate

No permission is needed for the collection of plant materials used in this study for the *Baijiguan* and *Rougui* tea cultivar is commercially available.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Figures

Figure 1

Leaves of Baijiguan and Rougui in shading and recovering light treatments. (A) The second leaves of Baijiguan in shading and recovering light treatments. (B) The second leaves of Rougui in shading and recovering light treatments.

Figure 2

The SPAD value of the second leaf in Baijiguan and Rougui in different treatments. The yellow and green lines with dots mean BC and RC. The yellow and green lines with triangles mean BS and RS. The yellow and green lines with squares mean BRL and RRL. Lowercase letters mean the significant difference of chlorophyll SPAD value in different days of the same treatment, $P < 0.05$. * indicated that the SPAD value of chlorophyll was significantly higher than that of other treatments on the same day, $P < 0.05$.

Figure 3

SOD enzyme activity of Baijiguan and Rougui under different light treatments. (A) SOD enzyme activity under natural conditions. (B) SOD enzyme activity under shading. (C) SOD activity after recovering light. The lines with dots mean BJG, and the squares lines with mean RG. The capital letters indicate the significant difference of SOD enzyme in Baijiguan under different days, $P < 0.05$; Lowercase letters indicate significant differences of SOD enzyme in Rougui under different days, $P < 0.05$; * indicates that the enzyme activity is significantly higher than that of another variety on the same day, $P < 0.05$.

Figure 4

POD enzyme activity of Baijiguan and Rougui under different light treatments. (A) POD enzyme activity under natural conditions. (B) POD enzyme activity under shading. (C) POD activity after recovering light. The lines with dots mean BJG, and the squares lines with mean RG. The capital letters indicate the significant difference of POD enzyme in Baijiguan under different days, $P < 0.05$; Lowercase letters indicate significant differences of POD enzyme in Rougui under different days, $P < 0.05$; * indicates that the enzyme activity is significantly higher than that of another variety on the same day, $P < 0.05$.

Figure 5

CAT enzyme activity of Baijiguan and Rougui under different light treatments. (A) CAT enzyme activity under natural conditions. (B) CAT enzyme activity under shading. (C) CAT activity after recovering light. The lines with dots mean BJG, and the squares lines with mean RG. The capital letters indicate the significant difference of CAT enzyme in Baijiguan under different days, $P < 0.05$; Lowercase letters indicate significant differences of CAT enzyme in Rougui under different days, $P < 0.05$; * indicates that the enzyme activity is significantly higher than that of another variety on the same day, $P < 0.05$.

Figure 6

Changes of total lipids content in tea plants. (A) Total lipids content of Baijiguan. (B) Total lipids content of Rougui. The lines with dots mean control, the lines with squares mean shading, and the lines with triangles mean recover light. Data are shown as mean $\pm$ SE (n = 5). Statistical significance was determined by one-way ANOVA. “*” indicates a significant difference in every treatment. (p < 0.05)

Figure 7

Changes of glycolipid in tea plants. (A) MGDG of Baijiguan. (B) DGDG of Baijiguan. (C) MGDG of Rougui. (D) DGDG of Rougui. The lines with dots mean control, the lines with squares mean shading, and the lines with triangles mean recover light. Data are shown as mean $\pm$ SE (n = 5). Statistical significance was determined by one-way ANOVA. “*” indicates a significant difference in every treatment (p < 0.05)

Figure 8

Bar charts showing the compounds of DGDG in Baijiguan and Rougui under different treatments. (A) The compounds of DGDG under normal growth. (B) The compounds of DGDG under shading. (C) The compounds of DGDG under recovering light.

Figure 9

Bar charts showing the compounds of MGDG in Baijiguan and Rougui under different treatments. (A) The compounds of MGDG under normal growth. (B) The compounds of MGDG under shading. (C) The compounds of MGDG under recovering light.

Figure 10

MGDG/DGDG ratio of Baijiguan and Rougui under normal light treatment. The black bar means BJJ, and the gray bar means RG. Data are shown as mean $\pm$ SE (n = 5). Statistical significance was determined by one-way ANOVA. "*" indicates a significant difference in every treatment. (p < 0.05)

Figure 11

MGDG/DGDG ratio of Baijiguan and Rougui under recovering light treatment. The black bar means BJG, and the gray bar means RG. Data are shown as mean $\pm$ SE (n = 5). Statistical significance was determined by one-way ANOVA. “*” indicates a significant difference in every treatment. (p < 0.05)

Figure 12

The changes of total lipid / chlorophyll, DGDG / chlorophyll, MGDG / chlorophyll in Baijiguan and Rougui under different light treatment. (A) The changes of total lipid / chlorophyll in Baijiguan and Rougui under normal light intensity; (B) The changes of total lipid / chlorophyll in Baijiguan and Rougui after shading; (C) The changes of total lipid / chlorophyll in Baijiguan and Rougui after recovering light; (D) The changes of DGDG / chlorophyll in Baijiguan and Rougui under normal light intensity; (E) The changes of DGDG / chlorophyll in Baijiguan and Rougui after shading; (F) The changes of DGDG / chlorophyll in Baijiguan and Rougui after recovering light; (G) The changes of MGDG / chlorophyll in Baijiguan and Rougui under normal light intensity; (H) The changes of MGDG / chlorophyll in Baijiguan and Rougui after shading; (I) The changes of MGDG / chlorophyll in Baijiguan and Rougui after recovering light. The black bar means BJG, and the gray bar means RG. Data are shown as mean $\pm$ SE (n = 5). Statistical

significance was determined by one-way ANOVA. * indicates a significant difference in the same treatment, $p < 0.05$.

Figure 13

Changes of lysophospholipid in tea plants. (A) LPG of Baijiguan. (B) LPC of Baijiguan. (C) LPE of Baijiguan. (D) LPG of Rougui. (E) LPC of Rougui. (F) LPE of Rougui. The lines with dots mean control, the lines with squares mean shading, and the lines with triangles mean recover light. Data are shown as mean $\pm$ SE ($n = 5$). Statistical significance was determined by one-way ANOVA. "*" indicates a significant difference in every treatment ($p < 0.05$)

Figure 14

Changes of phospholipid in tea plants. (A) PG of Baijiguan. (B) PC of Baijiguan. (C) PE of Baijiguan. (D) PI of Baijiguan. (E) PS of Baijiguan. (F) PA of Baijiguan. (G) PG of Rougui. (H) PC of Rougui. (I) PE of Rougui. (J) PI of Rougui. (K) PS of Rougui. (L) PA of Rougui. The lines with dots mean control, the lines with squares mean shading, and the lines with triangles mean recover light. Data are shown as mean $\pm$ SE (n = 5). Statistical significance was determined by one-way ANOVA. “*” indicates a significant difference in every treatment (p < 0.05).

Figure 15

Expression of key genes of lipid metabolism pathway in Baijiguan treated with different light treatment. (A) PLD of BJG. (B) plc of BJG. (C) DGK of BJG. (D) MGD of BJG. (E) DGD of BJG. The yellow bar means control, the green bar means shading, and the orange bar means recover light. Data are shown as mean $\pm$ SE (n = 3). Statistical significance was determined by one-way ANOVA. * indicates that there is significant difference between other treatments on the same day, $p < 0.05$.

Figure 16

Expression of key genes of chlorophyll degradation pathway in Baijiguan treated with different light treatment. (A) CLH of BJG. (B) SGR of BJG. (C) PAO of BJG. (D) PPH of BJG. (E) RCCR of BJG. The yellow bar means control, the green bar means shading, and the orange bar means recover light. Data are shown as mean $\pm$ SE (n = 3). Statistical significance was determined by one-way ANOVA. * indicates that there is significant difference between other treatments on the same day, $p < 0.05$.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [AlltheidentifiedlipidspeciesinBaijiguanandRougui.xlsx](#)