The Chlorophyll Biosynthesis and Degradation and Chloroplast Structure in Tomato Yellow Mutant

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Abstract

Yellow mutant is an important material for studying chlorophyll biosynthesis, degradation and the genetics and development of chloroplasts. The chlorophyll biosynthesis and degradation pathways of tomato (Solanum lycopersicum) mutant ym (Yellow mutant) and control ZS4 (Zhongshu 4) were studied. Photosynthetic parameters and chlorophyll fluorescence parameters of the variety ym and ZS4 were measured. The results showed that ym was more sensitive than the variety ZS4, while the variety ym has more tolerant to low light. The chloroplasts in ym exhibited onset of degradation compared with ZS4 under the transmission electron microscopy (TEM). The lack of chlorophyll-protecting material or the chloroplast grana degradation causes the leaf fade.

Keywords

Biosynthesis and Degradation, TEM, Chlorophyll, Leaf Color Mutant, Tomato

Abbreviations

Pn: Net photosynthetic rate; Gs: Stomatal conductance; CI: Intercellular CO2 concentrations; Tr: Transpiration rate; Fv/Fm: Maximum quantum efficiency of PSII; Y(II): Actual photosynthetic efficiency; qP: Photochemical quenching; qN: Non-photochemical quenching; ETR: Absolute electron transfer rate; Chl: Chlorophyll; Ccar: Carotenoids; SOD: Superoxide dismutase; POD: Peroxidase

Introduction

Most plants are mainly green in nature. The chlorophyll content is one of the important determinants of leaf color. Leaf color variation usually affects photosynthetic efficiency and causes crop failure. In 1991, Parks proved the biliverdin IX [alpha] is an indispensable substance for the synthesis of pigment groups, which used the yellow mutant hy1 and hy2 [1]. In recent years, people realized the value of leaf color mutation. Leaf color mutants have become a valuable material for studying photosynthesis.

Yellow mutantation have been reported in many crops such as rice, barley, pea, cotton, tobacco, and tomato (Solanum lycopersicum) [2,3]. Similarly, leaf color mutants have been found in various cereal crops [4-6] and most of these mutations are nuclear recessive traits. The mutant ylc, yg17, chl1 and chl9 are all controlled by single recessive nuclear genes [7-9]. The leaf color mutant of rice has been studied comprehensively. The leaf color phenotypic could be used as marker. Meanwhile, it also could be used to study photosynthesis [10,11].

Chlorophyll (Chl) is one of the important photosynthetic pigments. The content of chlorophyll could directly affect the plant photosynthesis. The reduce of Chl (b) (chlorophyll b) content could directly lead the photosynthetic less [12,13]. Low levels of chlorophyll may lead to unhealthy plants. But some leaf color mutants could allow plants normal survive. Most of these mutants do not change all leaves to yellow. The green tissue could provide enough carbohydrates [14]. Leaf color fade is caused by inhibition of chlorophyll biosynthesis or rapid degradation. In previous study, most of the main reason of Chl reductions was a partial block in the Chl synthesis

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pathway [13,15]. Many leaf color mutants such as the mutant CAO Oster, et al. [16] and CHLH play an important role in the study of chlorophyll biosynthesis. A variety of factors can affect the biosynthesis of chlorophyll. The excess heme could control chlorophyll synthesis via negative feedback to form the leaf color mutant [17]. δ-amino levulinic acid (ALA) also plays an important role in chlorophyll bio-synthesis. Martin Muller, et al. [18] provided exogenous ALA to the barley leaf color mutant in dark conditions, and the leaves still could change to green. Muller indicated the chlorophyll biosynthesis was not controlled by light [18]. The accumulation of protoporphyrin (Proto) and Mg-porphyrins were demonstrated playing the important role in Chi biosynthesis used the Chlorella mutants by Granic, et al. [19,20]. The silencing or inactivation of major genes role in chlorophyll bio-synthesis also could cause the plant leaves color faded green [21].

The only way of plants getting carbon is photosynthesis. Leaf color mutants are widely used to improve photospiration efficiency and yield of crops. Coschigano, et al. [22] identified the GLUI gene from the Arabidopsis leaf color mutant gls and proved the GLUI was associated with photospiration [22]. This discovery provides a new way to improve crop yield by inhibiting the photospiration of C3 crops to increase photosynthetic efficiency. In addition Gan and Amasino, et al. [23] identified a tobacco evergreen mutant which could obtain an apparent delay in aging, meanwhile the biomass and seed yield increased by 40% and 52% compared to wild-type tobacco [23]. The tomato mutant nv was a common leaf color mutant. The study of leaf color mutants can improve the understanding of plant photosynthesis and the role of chlorophyll in the presence of plants.

Redox reactions play an important role in plant photosynthesis. The reactive oxygen species (ROS) come from photosynthesis and respiration in plants, and chloroplasts are the main organelles produced ROS [24]. If C3 plants grow under the high-light, drought and low-temperature conditions, ROS will accumulate with the photorespiration increasing [25]. ROS is considered unfavorable products in aerobic condition. Excess ROS production could cause the plant death. If more light was assimilated, the plant will cause the photo-oxidative [26]. Superfluous light energy is used to synthesize ROS or some toxic free radicals [27,28]. To avoid the occurrence of this phenomenon, plants utilize a range of self-protective mechanisms [28-32]. Carotenoids Adams, et al., Frank, et al. [33,34] alpha-tocopherol, ascorbic acid (ASA) Nicholas, et al. [35] and glutathione (GSH) could keep the dynamic balancing process in plant photosynthesis. Keeping balancing requires the collective function of multiplication protective mechanisms. Here we also focus on the chlorophyll biosynthesis or degradation pathways which cause the leaf color change to yellow in tomato mutant ym.

Except the pigment content the balance of photosystem I (PSI) and photosystem II (PSII) is crucial in yellow leaf mutants [36]. The plant reduces to the imbalance in light absorption usually used to change distribution of PSI and PSII [37]. Non-chemical quenching (NPQ) is the key process in PSII, by harmlessly dissipated excess light energy [38]. It is induced by a pH difference value in thylakoid lumen which is generated by photosynthetic electron transport under excess of light [39]. Meanwhile the PSI and PSII may linear electron transport under stress conditions too [40].

There are many contentious issues in the field of photoprotective responses, and still need further research. Many leaf color mutants were used in the study of plant photosynthesis. Here we used the transmission electron microscopy to obtain the structure of the mutant ym and control zs4’s chloroplast. The gas exchanges parameters and fluorescence parameters of ym and zs4 were measured. We measured the photosynthetic prerequisite, chlorophyll-degrading enzyme and the key enzyme of redox in plant. It is interesting to note most leaf color mutants have weak growth potential, but ym had similar growth vigor and biomass compared with the control zs4. The yellow mutant is used to simplify identification of hybrid purity and improve the efficiency of field purity identification in the hybrid production. The study of yellow mutant was necessary and valuable.

Materials and Methods

Plant materials

The genetically stable, yellow-leaves mutant tomato variety ym, the green-leaves tomato variety zs4 were provided as gifts by the Tomato Research Institute of Northeast Agricultural University in China (Figure 1). All plants were raised in the greenhouse of Northeast Agricultural University (25 ℃, 16 h light and 8 h dark), Harbin, China, in 2017-2019. The mutant ym was a natural mutation from zs4. It was crossed with zs4 to produce F1, F2 progeny for agronomic trait analyses.

The content of the plant photosynthetic pigment

0.1g leaves of mutant ym and control zs4 (After sowing 9, 12, 15 and 60 days) were grind into a homogenate in 10 mL of 95% (v/v) ethanol in dark until became white. Absorbances were measured at 663, 645, and 470 nm. Chlorophyll a (Chla) and chlorophyll b (Chlb) content were measured by the spectrophotometric. The experiment followed three biological iterations. Triplicate of experiments were performed.

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\text{Chl (a)} = 13.95A_{663} - 6.8A_{645} \\
\text{Chl (b)} = 24.96A_{645} - 7.32A_{663} \\
\text{C}_{1} = 18.16A_{663} + 6.63A_{645}
\]

Transmission electron microscopy (TEM) of the chloroplast

The mutant (ym) was changed yellow when it cotyledon period. Two time points were chose (5 leaves and 10 leaves) to observe by using the transmission electron microscopy. Cut the leaves of these 2 points ym and zs4 into 1 mm x 2 mm segments, fixed with 2% (v/v) glutaraldehyde, washed with 1% (w/v) PBS (0.1 M each Na2HPO4,12H2O and NaH2PO4,2H2O in saline), and fixed in 1% (w/v) osmic acid (pH 7.2) for 12 h at 4 °C. The samples were then washed with 1% (w/v) PBS; sequentially dehydrated with 50%, 70%, 80%, 90%, and 100% (v/v) acetone then join in epoxy resin. Sectioned and viewed under the H-7700 canning TEM (Hitachi Ltd., Tokyo, Japan).
Gas exchange measurements

Portable photosynthetic rate tester (Li-6400) was used in here. The growth well fruiting period tomatoes *ym* and it control *zs4* were choice. We measure the gas exchange at 5 time points. Repeat three times at each group. The method of using the Li-6400 was reference [41].

Chlorophyll fluorescence measurements

The growth well fruiting period tomatoes *ym* and it control *zs4* were choice for measurement. Pulse fluorometer FMS2...
leaves were weighed out [42]. Then, 4 ml·g⁻¹ FW standard extract I was added (50 mM Tris-HCl (pH 8) and 0.4 M sucrose). The mixture was ground in an ice bath and filtered through a nylon cloth. Then, the filtrate was centrifuged at 12000 × g for 10 min at 4 °C, and 5 ml of 50 mM Tris-HCl (pH 8) was added. The suspended sediment was then centrifuged again and resuspended in acetone (-20 °C). The sediment was dried to obtain the acetone powder, which was stored at -20 °C until further application. Thirty milligrams of the acetone powder were weighed out, and 0.167 ml·mg⁻¹ standard extract II was added (50 mM PBS (pH 7.5), 50 mM KCl and 0.24% Triton-X 100). The mixture was ground in ice; the grinding fluid was incubated for 1h at 30 °C and then centrifuged at 12,000 × g for 10 min at 4 °C, and the supernatant was extracted (Minguez-Mosquera). Petroleum ether was used to extract Chl from fresh green spinach, and the substrate concentration was calculated (Fernandez-Lopez). Then, 0.5 ml of 50 mM PBS (pH 7.5), 1 ml of chlase and 2 ml of a Chl solution in acetone (0.2 mM Chl) were mixed in a water bath at 45 °C for 30 min. Then, 0.5 ml of the reagent and 4.5 ml of extracting solution III (Vacetone : Vpetroleum ether = 1:2) were mixed in a water bath at 45 °C for 30 min. The mixture was ground in ice; the grinding fluid was incubated for 1h at 30 °C and then centrifuged at 12,000 × g for 10 min at 4 °C, and the supernatant was extracted (Minguez-Mosquera). Petroleum ether was used to extract Chl from fresh green spinach, and the substrate concentration was calculated (Fernandez-Lopez). Then, 0.5 ml of 50 mM PBS (pH 7.5), 1 ml of chlase and 2 ml of a Chl solution in acetone (0.2 mM Chl) were mixed in a water bath at 45 °C for 30 min. Then, 0.5 ml of the reagent and 4.5 ml of extracting solution III (Vacetone : Vpetroleum ether = 1:2) were mixed in a water bath at 45 °C for 30 min. The mixture was ground in ice; the grinding fluid was incubated for 1h at 30 °C and then centrifuged at 12,000 × g for 10 min at 4 °C, and the supernatant was extracted (Minguez-Mosquera). Petroleum ether was used to extract Chl from fresh green spinach, and the substrate concentration was calculated (Fernandez-Lopez). Then, 0.5 ml of 50 mM PBS (pH 7.5), 1 ml of chlase and 2 ml of a Chl solution in acetone (0.2 mM Chl) were mixed in a water bath at 45 °C for 30 min. Then, 0.5 ml of the reagent and 4.5 ml of extracting solution III (Vacetone : Vpetroleum ether = 1:2) were mixed in a water bath at 45 °C for 30 min. The mixture was ground in ice; the grinding fluid was incubated for 1h at 30 °C and then centrifuged at 12,000 × g for 10 min at 4 °C, and the supernatant was extracted (Minguez-Mosquera). Petroleum ether was used to extract Chl from fresh green spinach, and the substrate concentration was calculated (Fernandez-Lopez). Then, 0.5 ml of 50 mM PBS (pH 7.5), 1 ml of chlase and 2 ml of a Chl solution in acetone (0.2 mM Chl) were mixed in a water bath at 45 °C for 30 min.
Chl content in mutant ym decreased sharply to only 39% of that in zs4 (CK). Sixty days later, in the leaves of the mutant ym, the total chlorophyll content was 36% that in the leaves of the control zs4. Total chlorophyll content of the mutant ym was always less than that of the zs4. After 15 days, the Chl (a)/Chl (b) levels were relatively higher than the initial values. At 60 days, the Chl (a)/Chl (b) levels in the leaves of both the mutant ym and zs4 were twice lower than it in 9 days (Figure 2B).

Observation of chloroplast ultrastructure

The result suggested that the chloroplasts of the green cotyledons were normal (zs4) (Figure 3A and Figure 3C), but the chloroplasts of the mutant yellow cotyledons were very unevenly distributed in the cell (Figure 3B), and most chloroplast membranes had started to be degraded (ym) (Figure 3D). Observation of the green areas of the second leaves of ym and zs4 revealed that the mutant ym hardly retained the double membrane structure, and only some of the chloroplasts perfectly retained their lamellar structures (Figure 3F). However, the chloroplasts of the control zs4 had complete double membrane structures (Figure 3E and Figure 3G). The yellow leaves of the ym mutant exhibited completely degraded chloroplasts (Figure 3H).

Measurement of the photosynthetic parameters of the yellow mutant

The Pn (net photosynthetic rate) and Gs (stomatal conductance) trends were similar, and the Pn values for both the mutant ym and the control zs4 decreased markedly at high light.
**Figure 3:** Observation of chloroplast ultrastructure of the ym and zs4 under TEM. A&C) The zs4 green leaves’ whole cell chloroplast distribution; B&D) The ym mutant leaves’ whole cell chloroplast distribution; E&G) The zs4 green leaves’ chloroplast lamellar structure observation; F&H) The chloroplast lamellar structure observation of ym mutant leaves.

**Note:** CW: Cell wall; PM: plasma membrane; Chl: chloroplast; OS: Osmiophilic granule.
intensity. However, the $P_n$ of $ym$ decreased faster than that of $zs4$. $G_s$ showed a similar trend. The $P_{n}$ of $ym$ was higher than that of the control $zs4$ when the light intensity was under 200 $\mu$mol·m$^{-2}$·s$^{-1}$. Throughout the process, the trend observed for $C_i$ (internal carbon dioxide concentration) was the opposite of that observed for $P_n$ (Figure 4).

The PSII maximum photochemical quantum yield ($F_{v}/F_{m}$) and effective photochemical quantum yield ($F'_{v}/F'_{m}$) were constant. Notably, the effective photochemical quantum yield of $ym$ was slightly lower than that of $zs4$ at high light intensity, but at low light intensity, the trend was reversed. The PSII actual photochemical quantum yield ($\Phi_{PSII}$) had the same variation trend as $F'_{v}/F'_{m}$. Photochemical quenching ($q_{P}$) of the mutant $ym$ and the control $zs4$ showed a tendency to occur at higher light intensity. The photochemical quenching of $ym$ was slightly lower than that of $zs4$ at 200 $\mu$mol·m$^{-2}$·s$^{-1}$ light intensity. The non-photochemical quenching (NPQ) of mutant $ym$ changed steadily, while that of the control $zs4$ showed a tendency to decrease with increasing light intensity. The apparent electron transfer rate (ETR) of $zs4$ was significantly higher than that of $ym$ except at a light intensity of 940 $\mu$mol m$^{-2}$ s$^{-1}$ (Figure 5).

**Analysis of chlorophyll synthesis pathway**

The chlorophyll content of the mutant $ym$ was lower than $zs4$. The chlorophyll content of $ym$ decreased more than that of $zs4$. The carotenoid content of $ym$ was also significantly lower than that of $zs4$ (Table S2). The chlorophyll precursors of $ym$ and $zs4$ revealed that there was no precursor accumulation or absence throughout the process (Figure 6).

**The chlorophyll degradation pathway**

The activity of the chlorophyll-degrading enzyme in the $ym$ was higher than that in the control variety $zs4$ (Figure 7). More chlase could accelerate the decomposition of chlorophyll, which may cause the leaf lose green in the mutant $ym$.

**The key enzyme activity of redox**

Two time points (5 and 10 leaves) were used to measure SOD, POD, CAT, glutathione reductase (GR) and APX activities. The results showed the APX content in $ym$ variety was always higher than in $zs4$ (Figure 8). The chlorophyll/carotenoid contents of the $ym$ and $zs4$ decreased during the cotyledon period. The level of the $ym$ was higher than $zs4$ at 12 days. But at 15 days, the values for the $ym$ were lower than $zs4$ (Table 1).

**Discussion**

Photosynthetic efficiency has been identified as one of the key reason to improve the yield potential of the crops [46].

In this study, yellow leaf color mutant $ym$ was obtained. After cross-fertilization, the whole first filial generation exhibited green leaves. While the leaf color segregation ratio of the second filial generation was 3:1 (green:yellow), which

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**Figure 4:** Gas-exchange parameter of the leaves of the mutant $ym$ at different light intensities. A) netphotosynthetic rate; B) Stomatal conductance; C) Intercellular carbon dioxide concentration. 

**Note:** Significant differences were analysed by ANOVA, and the asterisk indicates significant differences in comparison with the $zs4$ at $P < 0.05$. Triplicate of experiments were performed.
Figure 5: Chlorophyll fluorescence parameters of the leaves at different light intensities. A) Maximum quantum yield of PSII photochemistry; B) Fv'/Fm'; C) Quantum efficiency of PSII; D) Photochemical quenching coefficient; E) Non-photochemical quenching; F) Apparent rate of electron transport at the PSII level.

Note: Significant differences were analysed by ANOVA, and the asterisk indicates significant differences in comparison with the zs4 at P < 0.05. Triplicate of experiments were performed.

Figure 6: The comparison of chlorophyll precursors in ym and zs4. Triplicate of experiments were performed.
Figure 7: Chlorophyll content and chlorophyllase activity of ym and zs4. Triplicate of experiments were performed.

Figure 8: Comparison of the key enzyme activity of redox in ym and zs4. Significant differences were analysed by ANOVA, and the asterisk indicates significant differences in comparison with the zs4 at P < 0.05. Triplicate of experiments were performed.

Table 1: Carotenoid content and ratio total chlorophyll to carotenoid content.

| Time point   | 9d            | 12d           | 15d           | 60d          |
|--------------|---------------|---------------|---------------|--------------|
| YmC<sub>c</sub> | 1.227 ± 0.05<sup>b</sup> | 1.214 ± 0.00<sup>b</sup> | 1.334 ± 0.09<sup>b</sup> | 0.862 ± 0.01<sup>b</sup> |
| ymC<sub>car</sub>/C<sub>c</sub> | 7.012 ± 0.06<sup>a</sup> | 7.453 ± 0.02<sup>a</sup> | 2.786 ± 0.06<sup>a</sup> | 2.412 ± 0.09<sup>a</sup> |
| zs4 C<sub>c</sub> | 1.452 ± 0.12<sup>a</sup> | 1.324 ± 0.03<sup>a</sup> | 2.598 ± 0.35<sup>a</sup> | 2.512 ± 0.19<sup>a</sup> |
| zs4 C<sub>car</sub>/C<sub>c</sub> | 6.769 ± 0.26<sup>b</sup> | 6.36 ± 0.43<sup>b</sup> | 3.634 ± 0.15<sup>a</sup> | 2.247 ± 0.42<sup>a</sup> |

Data are presented as means (n = 3). Different lowercase letters (a, b, c) indicate statistically significant differences (P < 0.05) between treatments in the same phenotype using LSD tests.

Note: C<sub>car</sub>: Carotenoids, C<sub>c</sub>: Total chlorophyll content.

was consistent with Mendel’s law [47]. The result explained that this phenotype was regulated by a single recessive nuclear gene. 15 days after seeding the ym exhibited yellow. The chlorophyll deficiency caused the plant leaf change yellow. The maintained chlorophyll a-to-b ratio could affect the photosynthesis [48]. The low Chl (b) content is associated also with the pleiotropic effects on photosynthesis [49]. In previous studies, the lack of chlorophyll content may improve the
light distribution and light use efficiency in crops [50,51]. Here the ym total chlorophyll always lower than zs4, but the plant growth was not affected. The result in (Figure 2) and (Figure 4) shown the total Chl content in ym was gradually significantly lower than zs4. And with that the net photosynthetic rate of zs4 was higher than that of mutant ym at high light intensity. But when light intensity was low, the net photosynthetic rate of mutant ym was higher than that of ZS4. We suggest that the reason is the Chl content decrease may lead to significantly higher photosynthesis under the weak light [52].

Chloroplast protein mutations can cause defects in the structures of the inner membranes of chloroplasts [53]. We found that ym’s chloroplasts were detached from cell membranes under TEM. However, the zs4 chloroplasts were stable in cells. Meanwhile the TEM showed that the mutant ym chloroplasts’ double-layer membrane structure was absent, and the basal laminar structure began to degrade. The results showed that the chloroplast lamellar structure was indeed damaged and the chloroplast membrane structure was damaged in the mutant lines ym. Thylakoid plays an important role in photosynthesis [54,55]. Chlorophyll is widely found in thylakoid membranes. Thylakoid membranes damage could lead to the Chl free and out of action even rapid degradation. The chloroplasts loss was the main reason of the leaves change yellow. Chlorophyll is rapidly broken down due to the breakdown of chloroplasts. Finally, the yellow traits are formed. Chlorophyll biosynthesis and degradation pathways were mean effects. Only one biosynthesis pathway was involved [56,57]. Carotene is the major substances protecting chlorophyll from damage [58]. In this study, ym carotenoids content in the total pigment was significantly lower than these in zs4. We suggest that the lack of the protect substances cause the chlorophyll unstable, which leads to the high levels of chloroplast cystic membrane and chlorophyll degradation. Ten days after seeding, the chlorophyll content began to decrease meanwhile it was accompanied by chloroplast degradation.

Light is the most important factor in the change of ym mutant leaf color change. The photosynthetic coefficient and chlorophyll fluorescence parameters showed that under high light intensity (1290 μmol·m⁻²·s⁻¹), the Pn of ym is significantly higher than zs4. The stomatal conductance (Gₛ) also decreased severely. However, under low light intensity (200 μmol·m⁻²·s⁻¹), the Pn of ym was lower than that of the zs4. The trend of intercellular CO₂ concentration (Cᵢ) is basically opposite to that of net photosynthetic rate. The balance in light absorption between PSI and PSII may decrease photosynthetic electron flow. It could affect plant in some specific environmental conditions [59,60]. Under high light intensity, the effective photochemical quantum yield of ym PSII is slightly lower than zs4. At low light intensity, the effective photochemical quantum yield of ym PSII was significantly higher than zs4. Here shown that mutant ym was more inhibited under high light intensity, while more resistant in low light intensity. It may be a better low light tolerance tomato germplasm.

The chlorophyll precursor substance of the mutant ym was significantly lower than that of the control plant zs4. Meanwhile, the chlorophyll enzyme content in the mutant leaf was significantly higher than that in the control plant zs4. The results of REDOX activity showed that the REDOX ability of mutant ym leaves was lower than that of control zs4, and the ym plants were more prone to senescence and death compared with zs4. We suggest that the possible reason for this phenomenon is the decrease of chlorophyll content due to the reduction of chlorophyll synthesis and protective substances in the plants, which affects the REDOX ability of the plants and produces the phenomenon of yellow leaf.

The lack of chlorophyll-protecting substance and chloroplast cystic membrane degradation were the main aspects caused the yellow leaf phenotype in the mutant ym. The leaves change yellow at the cotyledon flattening stage. And it grows vigorously. The mutant variety ym, which lacks chlorophyll in the seedling stage, could provide a platform for the study of chlorophyll. The mutant can be used as a seedling marker trait.

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Author Contribution

MZC and HZ conceived the experiments, XNZ and XYW conducted the experiments, JGY, LZ, FLM, HNQ analysed the results, MZC wrote the manuscript. All authors reviewed the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

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Supplementary Tables:

Table S1: Changes in pigment content (mg·L⁻¹) during different developmental periods.

| Time points | 9d          | 12d         | 15d          | 60d         |
|-------------|-------------|-------------|--------------|-------------|
| ymC/Cₐ      | 2.867 ± 0.03a | 2.984 ± 0.02a | 3.214 ± 0.05a | 3.943 ± 0.23a |
| ymC         | 8.604 ± 0.13a | 9.048 ± 0.32a | 3.717 ± 0.02b | 2.08 ± 0.43a |
| zs4C/Cₐ     | 2.81 ± 0.77a  | 2.823 ± 0.04a | 2.692 ± 0.09b | 2.20 ± 0.66a |
| zs4C        | 9.829 ± 0.02a | 9.421 ± 0.03a | 9.441 ± 0.21a | 5.645 ± 0.34a |

Data are presented as means (n = 3). Different lowercase letters (a, b, c) indicate statistically significant differences (P < 0.05) between treatments in the same phenotype using LSD tests. 

Note: Cₐ: Chlorophyll a content, Cₐ: Chlorophyll b content, Cₐ: Total chlorophyll content.

Table S2: Chlorophyll and Cₐ content (mg·L⁻¹).

| Material | pigment content Cₐ/Cₐ (mg·L⁻¹) | pigment content Cₐ (mg·L⁻¹) |
|----------|---------------------------------|----------------------------|
| ym       | 3.869 ± 0.09a                   | 2.537 ± 0.01b              |
| zs4      | 2.135 ± 0.39a                   | 7.623 ± 0.54b              |

Data are presented as means (n = 3). Different lowercase letters (a, b) indicate statistically significant differences (P < 0.05) between treatments in the same phenotype using LSD tests. 

Note: Cₐ: Chlorophyll a content, Cₐ: Chlorophyll b content, Cₐ: Total chlorophyll content.

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