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PGL3 is required for chlorophyll synthesis and impacts leaf senescence in rice*#

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Abstract: Rice leaf color mutants play a great role in research about the formation and development of chloroplasts and the genetic mechanism of the chlorophyll (Chl) metabolism pathway. *pgl3* is a rice leaf color mutant derived from Xiushui11 (*Oryza sativa* L. spp. *japonica*), treated with ethyl methane sulfonate (EMS). The mutant exhibited a pale-green leaf (*pgl*) phenotype throughout the whole development as well as reduced grain quality. Map-based cloning of *PGL3* revealed that it encodes the chloroplast signal recognition particle 43 kDa protein (cpSRP43). *PGL3* affected the Chl synthesis by regulating the expression levels of the Chl synthesis-associated genes. Considerable reactive oxygen species were accumulated in the leaves of *pgl3*, and the transcription levels of its scavenging genes were down-regulated, indicating that *pgl3* can accelerate senescence. In addition, high temperatures could inhibit the plant's growth and facilitate the process of senescence in *pgl3*.

Key words: Pale-green leaf; Chlorophyll synthesis; Reactive oxygen species; Senescence; Rice
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1 Introduction

The production yield is largely determined by the process of photosynthesis, which provides the raw materials for all plant products (Richards, 2000). Chlorophyll (Chl) is pivotal to photosynthesis as it captures light energy, transforms it into chemical energy, and plays an important role in plant life activities (Lee et al., 2005). For almost all plants, photosynthesis is the main energy source. Thus, increasing Chl content (Huang et al., 2013) and extending the duration of photosynthesis (Long et al., 2006) could substantially enlarge photosynthetic products, and thereby increase crop yields.

Chl-deficient genes usually directly or indirectly affect chloroplast development and Chl metabolism, then alter Chl content and finally diversify the leaf color (Ramundo et al., 2014). The considerable variation of Chl content regularly leads to the alteration of leaf color, so Chl-deficient mutation is the same as leaf color mutation (Zhang et al., 2013). Leaf color mutants are very useful and are regarded as important genetic materials in the exploration of Chl synthesis and degradation mechanisms (Deng et al., 2014). Leaf color mutants have very extensive sources and mainly originate from natural and artificial mutation. Leaf color mutants can be distinguished by

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ORCID: Jing YE, https://orcid.org/0000-0003-2354-0571 their pale green, yellow, and albino color (Carol et al., 1999). In recent years, more and more leaf color mutants have not only provided us many genetic materials, but also facilitated the understanding of the mechanisms of leaf color formation, chloroplast development, and Chl biosynthesis and degradation.

Chl synthesis is a complex reaction process involving many enzymes in rice. *OsGluRS* encodes glutamyl-tRNA synthetase, which is the key enzyme in the initial step of Chl synthesis, and its mutant is characterized by yellow green leaves and is sensitive to temperature (Liu WZ et al., 2007). *OsChlH*, *OsChlI*, and *OsChlD* encode H, I, and D subunits of magnesium chelatase, respectively, and any variation of them will cause the color to change (Goh et al., 2004; Zhang et al., 2006). *OsDVR* encodes divinyl reductase, which is essential for Chl biosynthesis, as well as its mutant exhibiting a yellow-green leaf phenotype, which reduces Chl level and arrests chloroplast development (Wang P et al., 2010, 2013). A characteristic of leaf senescence is Chl degradation, which is not as complicated as Chl synthesis, but only a few related genes have been cloned up to now (Zhang et al., 2013). *NYC1* catalyzes Chl b to 7-hydroxymethyl Chl a and is necessary for the first step of Chl b degradation (Kusaba et al., 2007). *NOL*, as the highly homologous gene of *NYC1*, also encodes a Chl b reductase, and its mutant shows a stay-green phenotype and is very similar to the *nyc1* mutant. *NYC1* and *NOL* are localized on the thylakoid membrane and form a complex, which exercises the function of Chl b reductase (Sato et al., 2009).

In autumn, plant leaves generally change from green to yellow or red because of Chl degradation combined with carotenoid retention or anthocyanin accumulation, which is one of the important signs of leaf senescence (Park et al., 2007). The slow Chl degradation in the senescence process can lead to a late-green phenotype, which can delay the senescence process, prolong the photosynthesis time, and increase the crop yield to some extent (Wu et al., 2012). The process of senescence will produce large amounts of reactive oxygen species (ROS), mainly due to the process of cell death and cell membrane rupture (Piquery et al., 2000). ROS control many different processes in plants, whereas, being toxic molecules, they are also capable of injuring cells (Mittler et al., 2004).

In this study, we characterized a rice mutant, *pgl3*, which displayed pale-green leaves (*pgl*) and an insensitivity to heat and darkness treatments. Map-based cloning showed that *PGL3* encodes a chloroplast signal recognition particle, *OscpSRP43*. Here, we present the results of phenotypic and physiological characterizations and the expression analysis of associated genes, which indicate that *PGL3* plays an important role in Chl synthesis and leaf senescence in rice.

2 Materials and methods

2.1 Plant materials and growing conditions

The *pgl3* mutant was derived from Xiushui11 (*Oryza sativa* L. spp. *japonica*) and treated by ethyl methane sulfonate (EMS). The plants were grown in a paddy field during natural seasons at the China National Rice Research Institute (Hangzhou, Zhejiang Province and Lingshui, Hainan Province, China). The chamber conditions of etiolated seedlings were as follows: the rice seeds were pre-soaked for 2 d and continued to germinate for 5 d in the dark. They were then transferred to light for 24 h. The mutant and wild-type (WT) Xushui11 in the heat treatment group were grown in an artificial climate chest under the growth condition of 42 °C for 16 h (day) and 35 °C for 8 h (night). The growing conditions were measured as previously described by Yang et al. (2016).

2.2 Map-based cloning of *PGL3* and complementation assay

The *pgl3* mutant was crossed with CH113 (*O. sativa* L. spp. *indica*) to generate an F_2 mapping population. One was mixed with 20 individuals with the pale-green leaf phenotype of *pgl3*, and the other contained 20 individuals with a normal green phenotype of WT. A total of 181 simple sequence repeat (SSR) markers covering 12 chromosomes of rice were used for initial localization. For further mapping, 34 insertion-deletion (InDel) markers were designed using the Primer 5.0 software after comparison of the sequences between the *japonica* cultivar Nipponbare and the *indica* cultivar 9311. Sequences of primer pairs for mapping are listed in Table S1.

Target DNA fragments in this region were amplified from *pgl3* and WT plants, then sequenced and compared using DNAstar. A 6.2-kb genomic region, including 1.3-kb entire open reading frame (ORF) of *PGL3*, a 4.1-kb upstream region, and a 0.8-kb downstream region (Lv et al., 2015). The 6.2-kb fragment was cloned into the binary vector pCAMBIA1300 to generate the transformation construct, *PGL3*-COM. The resulting construct was introduced into the embryogenic calli generated from the mature seed embryos of *pgl3* using the *Agrobacterium*-mediated transformation method (Liu XQ et al., 2007).

2.3 RNA extraction and qRT-PCR

Total RNA was extracted from rice issues using a Total RNA Extraction kit (Axygen, cat No. AP-MN-MS-RNA-250, USA). The RNA was pre-treated with DNase I and used for cDNA synthesis using an RT-PCR kit (Promega, http://www.promega.com). Quantitative real-time polymerase chain reaction (qRT-PCR) was performed using a SYBR Premix Ex Taq kit (TaKaRa Bio Inc., China) in an Applied Biosystems® 7500 Real-Time PCR System (Invitrogen, USA). Each analysis was repeated three times. The rice *Actin 1* gene was used as a normalization control (Zhu et al., 2006). Sequences of primer pairs for RT-PCR analysis are listed in Table S2.

2.4 Transmission electron microscopy analysis

Leaves, 10 d after flowering, were fixed in a solution of 2.5% glutaraldehyde in phosphate buffer (pH 7.2) for 4 h at 4 °C. The polymerization and staining of the leaf samples were based on the method of Li et al. (2011). The chloroplast ultrastructure of samples was observed by transmission electron microscope at Zhejiang University, Hangzhou, China.

2.5 Chl content measurement and grain quality analysis

The total Chl from 0.4 g fresh leaves was extracted with 80% acetone in the dark at 28 °C for 24 h. The extract was analyzed using a spectrophotometer with light absorption values of 470, 645, and 663 nm (Wellburn, 1994). The percentage of grains with chalkiness and the degree of endosperm chalkiness were measured as previously described by Zhou et al. (2015). Three biological repeats were performed for each analysis.

2.6 Detection of ROS

To monitor superoxide accumulation, leaves were excised and immediately placed in a 0.5 mg/ml nitroblue tetrazolium (NBT) solution in 10 mmol/L potassium phosphate buffer (pH 7.6) at 25 °C for 3 h in the dark. For hydrogen peroxide detection, excised leaves were treated with 1 mg/ml diaminobenzidine (DAB) in 50 mmol/L Tris acetate buffer (pH 5.0) at 25 °C for 24 h in the dark (Fukao et al., 2011). The staining and bleaching of the samples was performed as previously described by Wang YH et al. (2013).

3 Results

3.1 Characterization of *pgl3*

The leaf color mutation was isolated from the mutagenesis of cultivar "Xiushui11" using EMS, which exhibited a pale-green leaf phenotype throughout the entire developmental process (Figs. 1a–1d). The mutant was named *pgl3* according to the phenotype. Leaves of the *pgl3* mutant had a 68% and 70% reduction of Chl a and a 78% and 70% reduction of Chl b levels compared with those in WT at seedling and heading stages, respectively (Figs. 1e and 1f). Furthermore, the higher ratio of Chl a/Chl b may be due to the potential of Chl b synthesis in suffering a more severe decline than Chl a in the *pgl3* mutant at seedling stage (data not shown). In addition, the Chl and carotene (Car) levels were dramatically decreased in *pgl3* compared with WT. The *pgl3* phenotypic characterizations indicated that *PGL3* was essential for Chl synthesis. The low levels of Chl and Car may affect the growth and development of plant and further influence yield and quality of rice. As expected, *pgl3* displayed shorter plant height, less tillers, and lower grain yield per plant than those of the WT (data not shown). The percentage of grains with chalkiness and the degree of endosperm chalkiness in *pgl3* were higher than those in WT (Fig. S1).

3.2 Map-based cloning of *PGL3*

To determine the genetic control of the pale-green leaf phenotype, we used a map-based cloning strategy to isolate the *PGL3* gene. The *pgl3* mutant was crossed with *indica* cultivar CH113 to generate a segregation population for gene mapping. The F_1 plants exhibited the normal green phenotype, suggesting that the mutant phenotype was controlled by a recessive gene(s). Among the F_2 individuals, 2313 were green leaf phenotype plants and 736 were pale-green leaf phenotype plants, fitting to a 3:1 ratio

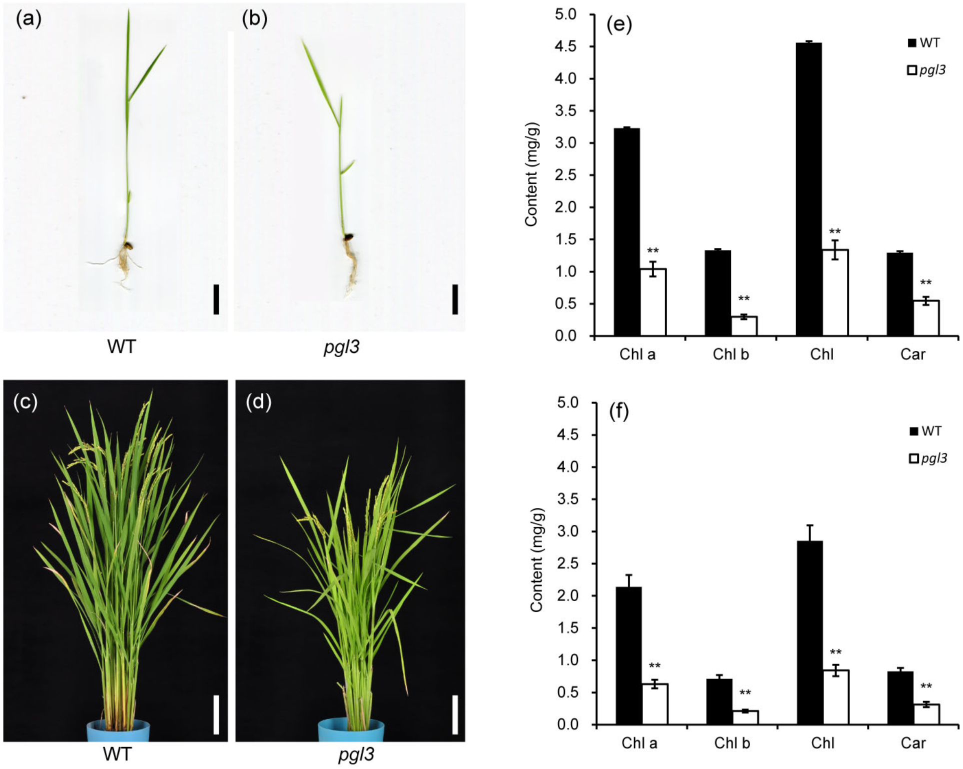

Fig. 1 Characterization of WT and *pgl3*

Plants of WT (a) and *pgl3* (b) at the seedling stage (bar, 2 cm) and plants of WT (c) and *pgl3* (d) at the heading stage (bar, 10 cm). Contents of photosynthetic pigments at the seedling stage (e) and the heading stage (f). The values are presented as the mean±standard deviation (SD) from three biological replicates. ** Highly significance at $P\leq0.01$ vs. WT

(χ^2=1.205< $\chi^2_{0.05}$ =3.84). These results indicated that the mutation was controlled by a single recessive gene.

The *PGL3* locus was roughly mapped between markers RM22 and RM5474 on chromosome 3. The location of the *PGL3* locus was ultimately limited to an 85-kb region between the markers A1 and A2, which included eleven ORFs (Fig. 2a). A sequencing analysis revealed that *pgl3* had C-to-G and A-to-G nucleotide substitutions at positions 113 and 999 of the gene LOC_Os03g03990, resulting in Leu-to-Phe and Asp-to-Asn changes at 32nd and 328th amino acids, respectively (Fig. 2b). This gene encodes the chloroplast signal recognition particle 43 kDa protein (cpSRP43), which had been previously reported by Lv et al. (2015) and Wang et al. (2016). In the remainder of this paper, *pgl3* will continue to use the mutant line name *PGL3* as the gene name. For these reasons, this gene was identified as the candidate gene of *pgl3* that caused the phenotype of pale-green leaves during the whole growth stage.

To further confirm the identity of the candidate gene as *PGL3*, we performed a genetic complementation test by transforming the WT gene (LOC_Os03g03990) into the *pgl3* mutant. All the positive transgenic lines completely rescued the *pgl3* phenotypes, including leaf color and Chl and Car content (Figs. 2c and 2d). These results confirmed that *PGL3* was indeed LOC_Os03g03990.

3.3 *PGL3* is required for Chl synthesis

We first compared *PGL3* transcript levels in the *pgl3* mutant and WT plants using RT-PCR. The transcript levels of *PGL3* were always significantly decreased in *pgl3* compared with that of WT (Fig. 3a). Next, we addressed the question of whether the *pgl3* mutation affected the transcript of other genes associated with Chl synthesis. Compared with the WT, the expression of *DVR* (encoding divinyl reductase) was down-regulated and *PORA* (encoding NADPH-dependent protochlorophyllide oxidoreductase) was

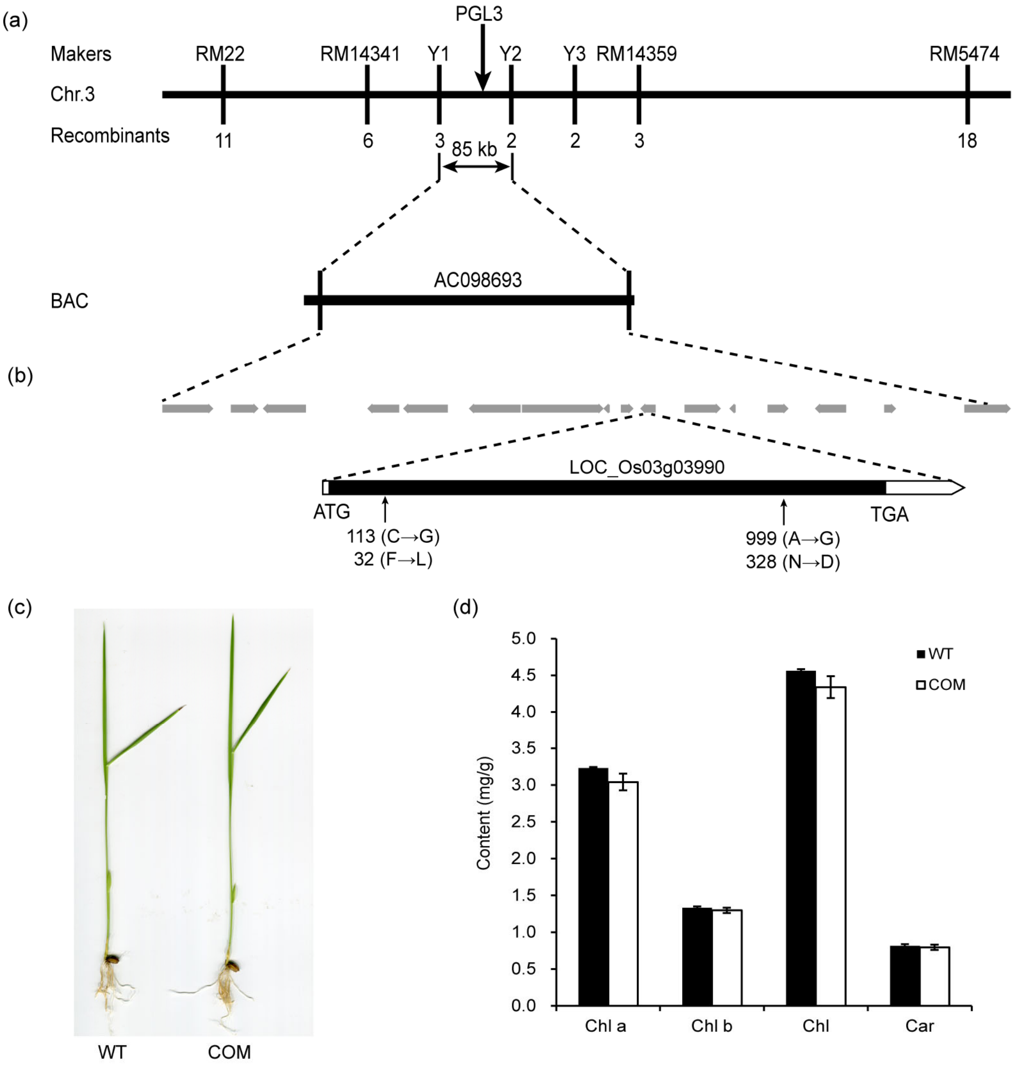

Fig. 2 Map-based cloning and identification of *PGL3*

(a) *PGL3* locus was mapped to a region between markers RM22 and RM5474 on chromosome 3 (Chr.3). Fine-mapping of *PGL3* to an 85-kb genomic DNA region between two makers Y1 and Y2. A bacterial artificial chromosome (BAC) contig (AC098693) cover the *PGL3* locus. (b) Sixteen ORFs were predicted in the mapped region and sequence comparison revealed two substitutions of C to G and A to G in LOC_Os03g03990 in the *pgl3* mutant. (c) Phenotype comparisons between WT and complemented plant (COM). (d) Chl a, Chl b, total Chl, and Car content of leaf in WT and complemented plant. The values are presented as the mean±SD from three biological replicates

obviously down-regulated while the expression of others (glutamyl-tRNA reductase (*HEMA*), protochlorophyllide oxidoreductase B (*PORB*), and yellow green leaf 1 (*YGL1*)) was similar to that of the WT (data not shown). The changes in expression levels of these genes may lead to lower Chl content in *pgl3* than in WT.

To further clarify the effects of *PGL3* on Chl synthesis, we subjected the WT and *pgl3* etiolated seedlings to light for 24 h. The seedlings began to synthesize Chl after being exposed to light. Over 24 h of light growth, the WT had turned normal green early, and *pgl3* was still albino (Fig. 3b). Comparing with that of its WT, the Chl synthesis rates of Chl a, Chl b, and Chl decreased remarkably in the mutant (Figs. 3c–3e). At the same time, the expression of Chl synthesis-related genes was also detected in different periods. The expression levels of *DVR* and *PGL3* were down-regulated and especially *PGL3* was almost entirely unexpressed in *pgl3* while the expression level of *PORA* decreased sharply after being exposed to light, revealing that it was very high in the dark and began

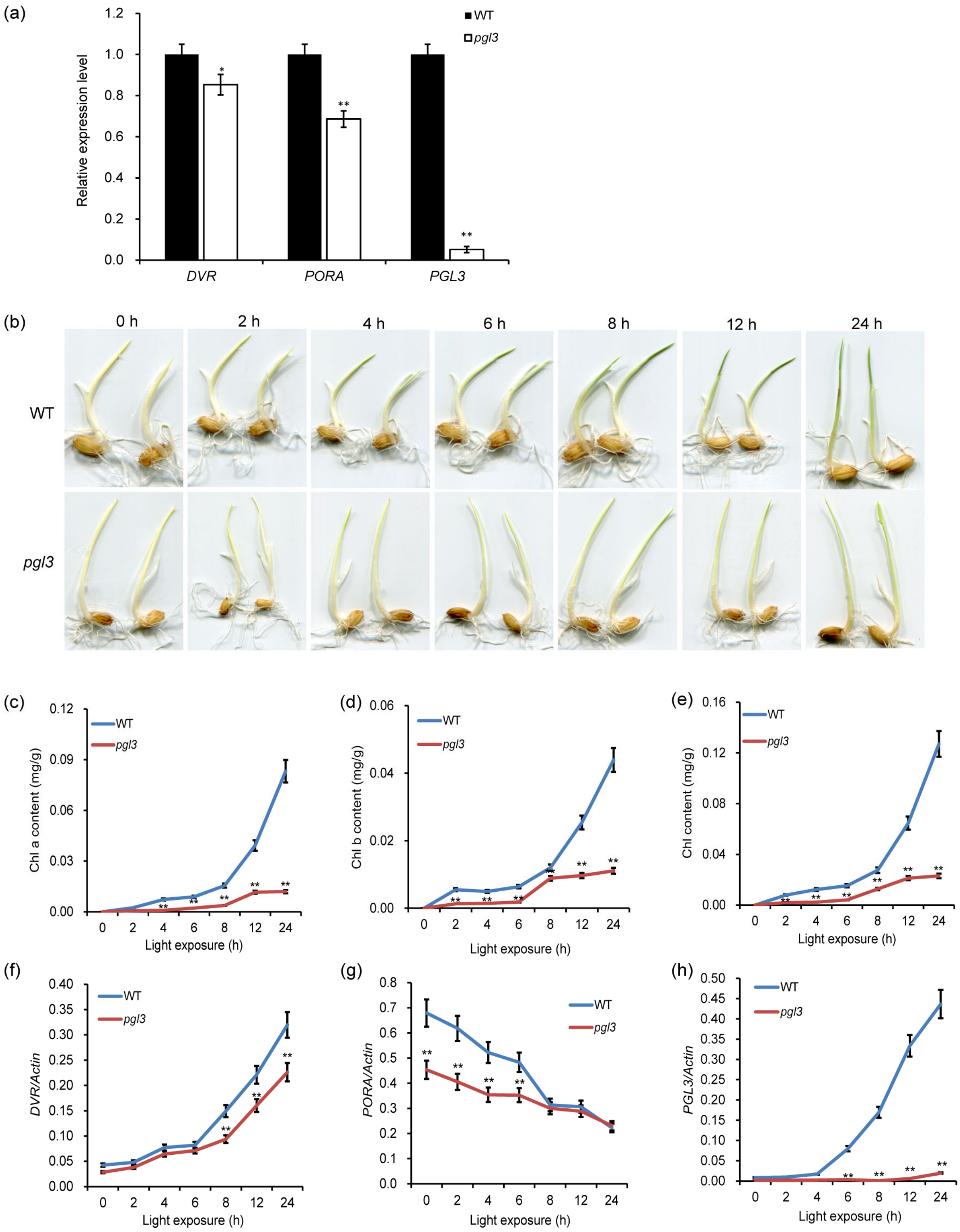

Fig. 3 Comparison of greening speed between WT and *pgl3*

(a) RT-PCR analysis of Chl synthesis-associated genes in WT and *pgl3*. (b) Comparison of greening speed in WT and *pgl3* etiolated seedlings that were exposed to light for 0–24 h. (c, d, e) Chl a, Chl b, and Chl content in the WT and *pgl3* seedlings during greening. (f, g, h) RT-PCR analysis of Chl synthesis-associated genes in WT and *pgl3* seedlings during greening: *DVR*, *PORA*, and *PGL3*. The values are presented as the mean±SD from three biological replicates. * $P\leq0.05$, ** $P\leq0.01$ vs. WT to decrease in the light (Figs. 3f–3h). This showed that *PORA* may have no effect on the greening process. These results demonstrated that *PGL3* was necessary for Chl synthesis under light.

3.4 *pgl3* accelerates leaf senescence

ROS can cause extensive cell injury or death and become a marker of senescence (Davletova et al., 2005). Superoxide anion (O_2^-) and hydrogen peroxide (H_2O_2) in the leaves of plants can be detected by NBT and DAB, respectively. In order to induce leaf senescence, the leaves of WT and *pgl3* were cut and placed in the dark at 28 °C for 5 d. NBT and DAB staining of WT and mutant leaves showed that the leaf color of *pgl3* was deeper than that of WT, indicating that the content of ROS in *pgl3* leaves was higher than that in WT during the process of senescence (Figs. 4a–4c).

Next, transmission electron microscopic analysis was performed to determine if high ROS content in the *pgl3* mutant affects the leaf senescence. The results revealed that the number and size of chloroplasts per cell in the mutant were very similar to those of the WT, whereas the grana stacks appeared less dense and a lot of plastoglobules were observed in the chloroplasts of *pgl3* (Figs. 4d–4g). These results indicated that the process of senescence was accelerated in the mutant.

The transcription levels of ROS scavenging-related genes, *OsAPX2* (encoding ascorbate peroxidase 2), *OsCatB* (encoding catalase), and *OsPOD2* (encoding peroxidase precursor 2), were obviously down-regulated, while *OsAPX1* (encoding ascorbate peroxidase 1) and *OsPOD1* (encoding peroxidase precursor 1) showed no apparent change in *pgl3* (Fig. 4h). The transcription level of senescence-inducible gene, *OsAMTR1* (encoding aminotransferase), was obviously up-regulated in *pgl3*. The changes in expression levels of these genes may be responsible for the leaf senescence exacerbated in *pgl3*.

3.5 *pgl3* is sensitive to heat treatment

Continuous heat treatment will cause a reduction in cell-protective abilities and advance the aging process (Li et al., 2015). We could see that WT and *pgl3* exhibited a normal phenotype except for leaf color (Fig. 5a). *pgl3* presented a severely wizened phenotype in the leaf tip under high temperatures for 5 d and the whole plant had become dry and withered under a high-temperature for 9 d, whereas there was no obvious signs of aging in the WT plants (Figs. 5b and 5c). The Chl a, Chl b, and Chl levels of *pgl3* were rapidly decreased compared with WT during heat treatment (Figs. 5d–5f). With the treatment of high temperatures, the electrolyte leakage was barely changed in WT, whereas it was increased sharply in *pgl3* (Fig. 5g). These results indicate that *PGL3* plays important roles in the process of heat tolerance.

4 Discussion

In this study, the *pgl3* plant mutant maintained a pale-green leaf color throughout its life cycle and had a sharp drop in Chl content compared with WT (Fig. 1). Those phenotypes were similar to the other leaf color mutants in rice although the pathways of those genes might be different. The leaf is the main organ for photosynthesis and the major source of carbohydrates. A Chl deficiency usually leads to the decline of supply nutrients for the rice grain at the filling stage, resulting in the reduction of grain yield and quality in *pgl3*. *AtcpSRP43* is a homolog of *PGL3* and its mutant has similar phenotypes to *pgl3* (Klimyuk et al., 1999). By map-based cloning, the *PGL3* gene was finally delimited in an 85-kb region on chromosome 3, including eleven ORFs (Fig. 2a). Sequence analysis showed that LOC_Os03g03990 was predicted to encode a cpSRP43 protein and to act as a chloroplast precursor, had two nucleotide substitutions in the coding region of *pgl3* and led to the changes of the amino acid sequence coded (Fig. 2b). Furthermore, the leaf color and Chl content of *pgl3* could be restored to the WT phenotype by transformation of the WT gene. Therefore, the gene LOC_Os03g03990 was indeed responsible for the *pgl3* phenotype.

Chl metabolism has been extensively studied in various organisms, and almost all of the Chl biosynthetic genes have been identified in higher plants (Nagata et al., 2005). The *Arabidopsis* cpSRP43, together with cpSRP54, is involved in the pathway of light-harvesting chlorophyll-binding proteins (LHCPs) to the thylakoid (Groves et al., 2001). The cpSRP pathway is necessary for the normal growth and development of a plant (Lv et al., 2015). Therefore, the defect in OscpSRP43 seems to affect the transportation

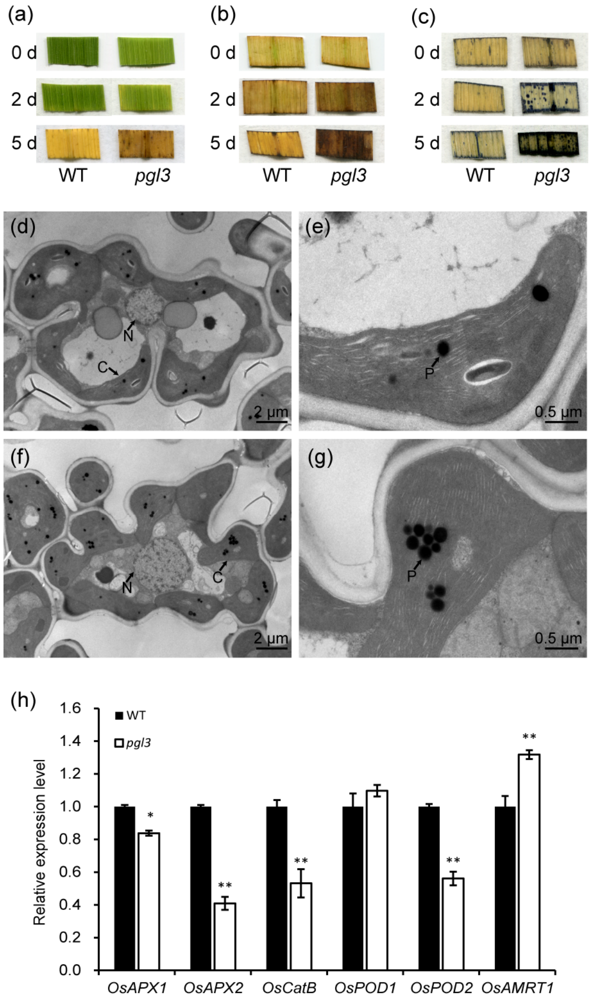

Fig. 4 Senescent phenotype in *pgl3* at the heading stage
(a) WT and *pgl3* leaves were incubated in the dark for 0, 2, and 5 d. (b, c) WT and *pgl3* leaves were stained with DAB (b) and NBT (c) during dark incubation. (d–g) Ultrastructure of the chloroplast in the leaves of WT (d, e) and *pgl3* (f, g). N, nucleus; C, chloroplast; P, plastoglobule. (h) RT-PCR analysis of senescence-associated genes in WT and *pgl3*. The values are presented as the mean±SD from three biological replicates. * $P\leq0.05$, ** $P\leq0.01$ vs. WT

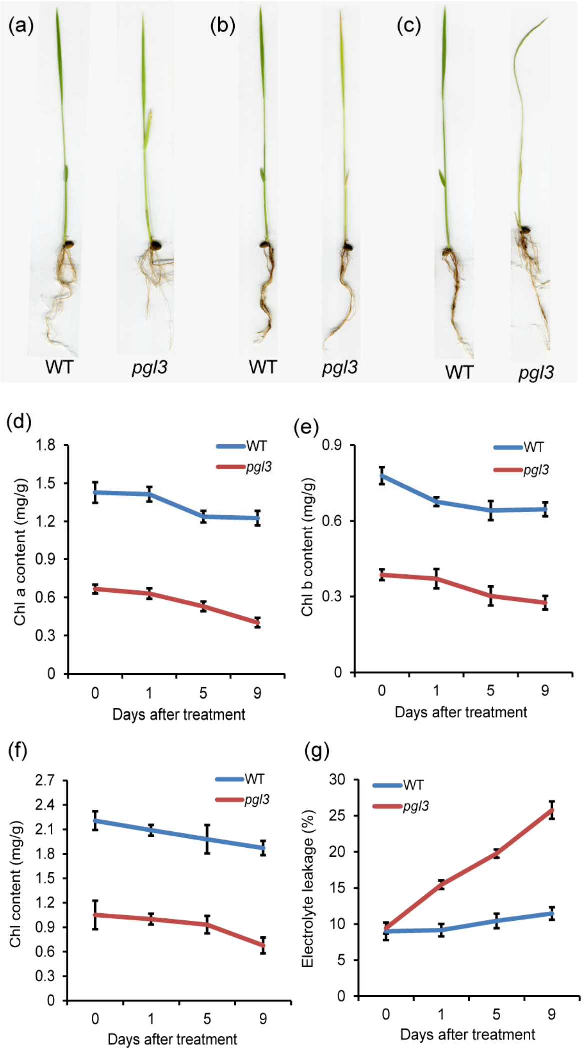

Fig. 5 Effects of high temperature stress on WT and *pgl3*
The plants of WT and *pgl3* were grown under heat stress for 0 d (a), 5 d (b), and 9 d (c). The changes in Chl a (d), Chl b (e), and Chl (f) content under heat stress for 0, 1, 5, and 9 d. (g) Changes of electrolyte leakage under heat stress for 0, 1, 5, and 9 d. The values are presented as the mean±SD from three biological replicates

of LHCPs, leading to impaired Chl biosynthesis as well. Among the Chl synthesis-associated genes, we found that the expressions of three genes, including *DVR*, *PORA*, and *PGL3*, were reduced at different levels at the seedling stage in *pgl3* (Fig. 3a). The chlchlorophyll deficiency in *pgl3* is likely due to the apparent down-regulation of *DVR* and *PORA*. *PORA* expression is repressed by light (Sakuraba et al., 2013), which can explain why the expression level of *PORA* decreased sharply after being exposed to light, indicating that it was not essential for the process of greening. The cpSRP targets proteins in the chloroplast thylakoid membrane, and responds to high light intensity (Hutin et al., 2002). Divinyl reductase converts 8-vinyl groups on various Chl intermediates to ethyl groups, which is indispensable for Chl biosynthesis (Wang et al., 2010). The expression levels of *PGL3* and *DVR* rapidly increased in WT plants after illumination, indicating that *PGL3* and *DVR* may play a major role in Chl synthesis during greening in rice.

ROS are thought to play a vital role in plant senescence (Mhamdi et al., 2010). This is consistent with a loss in ROS scavenging capacity during the progression of senescence. So, the transcription levels of some ROS scavenging genes were detected, such as *OsAPX1*, *OsAPX2*, *OsCatB*, *OsPOD1*, and *OsPOD2*. For example, overexpression of *OsAPX1* in rice prevents the over-accumulation of H_2O_2 and reduces lipid peroxidation, thereby protecting plant development under stress (Sato et al., 2011). The transcription levels of all ROS scavenging genes, which have a similar function, were obviously down-regulated except for *OsPOD1* in *pgl3* (Fig. 4h). Under normal conditions, redundant ROS were cleared by the ROS scavenging system in the plant. So *pgl3* accumulated considerable ROS, which were detected by DAB and NBT during the aging process (Figs. 4b and 4c). The *PGL3* may function as a regulator, which directly or indirectly regulates the level of ROS accumulation. Accumulation of unnecessary ROS induced the premature senescence of leaves and may even be involved in programmed cell death. This can explain why the characteristics of premature senescence exhibited early in *pgl3*. Furthermore, *OsAMTR1* is a specific expression gene of the aging process, which can be used as a molecular marker during the aging process (Liang et al., 2015). *OsAMTR1*, as a senescence-induced gene, was significantly higher in *pgl3* compared with that in the WT.

In addition, the number of plastoglobules also can be a sign of senescence, and it strongly increases during light stress, senescence, and in mutants blocked during thylakoid formation (Austin et al., 2006; Bréhélin et al., 2007). Although the WT produced some plastoglobules attached to thylakoid membranes, the number of plastoglobules in WT is far less than that in *pgl3* at the heading stage (Fig. 4d). A rise in conjugated ROS led to leaf senescence in *pgl3*. The process of senescence usually shows some important characteristics such as visible color changes, reduction in photosynthesis, and the degeneration of chloroplasts (Huang et al., 2016). Leaf senescence will be accelerated when plants are exposed to environmental stresses, such as drought and heat conditions, which cause more ROS generation (Lee and Park, 2012). During high temperature treatment, the plant of *pgl3* grew extremely slowly and quickly turned withered and even died (Figs. 5a–5c). Through the characterization of Chl content and electrolyte leakage, *pgl3* demonstrated the characteristics of premature senescence (Figs. 5d–5g). We may therefore infer that stable chloroplast structures can hold a steady level of electrolytes, and the aim of delaying leaf senescence has been achieved.

In conclusion, we have conducted a study on a pale-green leaf mutant throughout the development of Xiushui11 rice. This mutation was controlled by a single nuclear gene *PGL3*, which encodes for the cpSRP43 protein. *PGL3* responded positively to the abiotic stress to delay senescence mainly by regulating the expression levels of ROS scavenging-associated genes. These results will not only provide a basis for further research on the *PGL3* gene, but will also accelerate the explanation of the mechanism of OsSRP43 protein involved in Chl synthesis and ROS scavenging to rice senescence.

Compliance with ethics guidelines

Jing YE, Yao-long YANG, Xing-hua WEI, Xiao-jun NIU, Shan WANG, Qun XU, Xiao-ping YUAN, Han-yong YU, Yi-ping WANG, Yue FENG, and Shu WANG declare that they do not have any conflict of interest.

This article does not contain any studies with human or animal subjects performed by any of the authors.

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List of electronic supplementary materials

Table S1 Molecular markers used for mapping of the mutation
Table S2 List of genes used for real-time PCR analysis
Fig. S1 Rice quality traits in WT and *pgl3*

中文概要

题　目：*PGL3* 对水稻叶绿素合成和叶片衰老的影响

目　的：研究 *PGL3* 的遗传机制与生物学功能。

创新点：研究一个水稻叶色突变体的鉴定与基因克隆，并探讨其对叶绿素合成和叶片衰老的影响。

方　法：通过乙基甲磺酸（EMS）诱变，获得了一个淡绿叶突变体 *pgl3*，运用图位克隆法对 *PGL3* 进行定位，并对 *PGL3* 的功能进行研究。

结　论：*PGL3* 通过调节叶绿素合成相关基因的表达水平影响叶绿素合成。*pgl3* 叶片中清除活性氧基因转录水平下调，导致活性氧大量积累，表明 *pgl3* 可以加速衰老。此外，高温可以抑制植物的生长并加速 *pgl3* 衰老。

关键词：淡绿叶；叶绿素合成；活性氧；衰老；水稻