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SCL, Encoding a Chloroplast Signal Recognition Particle Receptor, Affects Chlorophyll Synthesis and Chloroplast Development in Rice

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Abstract
Crop yield is largely determined by the solar energy utilization efficiency of photosynthesis; plants with long stay-green periods have greater total photosynthetic production levels and crop yields. Here, a novel seedling chlorosis and lethality (*scl*) mutant exhibiting a yellow leaf and seedling-lethal phenotype was identified in rice (*Oryza sativa* L.). The mutant had deformed chloroplasts and almost no protein complexes in thylakoid membranes. The expression levels of photosynthesis-associated genes were significantly down-regulated in *scl* compared with the wild-type (WT). Positive transgenic lines generated by *Agrobacterium tumefaciens*-mediated transformation of the *scl* mutant with a complementation vector harboring *SCL* cDNA exhibited the normal green leaf phenotype, whereas the *scl* seedling harboring the empty vector displayed the yellow leaf phenotype, indicating that *SCL* is LOC_Os01g72800. A fusion protein expressing SCL with green fluorescent protein revealed the fluorescence signal localized to chloroplasts. The expression patterns of chloroplast development and chlorophyll biosynthesis and degradation-related genes were disordered in *scl* mutant, possibly resulting in the yellow leaf phenotype. These results indicated that the *SCL* loss of function impaired chloroplast development, chlorophyll biosynthesis, and light-harvesting chlorophyll-binding protein transportation in rice.

Keywords Chlorophyll synthesis · Chloroplast development · LHCPs · Rice

Introduction

In plants, photosynthesis is the vital energy metabolic process in which sunlight energy is transformed into chemical energy (Fleming and Van Grondelle 1994). The yield potentials of crops are largely determined by the utilization efficiencies of solar energy through photosynthesis (Long et al. 2006). The utilization efficiency may be greater when the leaf area, daily photosynthetic duration, or leaf area exposure increase (Richards 2000). Thus, selecting plants with a long stay-green period is an effective method to improve total photosynthetic production and crop yield.

Vascular plants perform photosynthesis in chloroplasts; consequently, normal chloroplast development is a prerequisite for photosynthesis. Major advancements have been made in understanding the genetic mechanisms behind chloroplast development and many related genes have been identified in rice (*Oryza sativa* L.). *OsHAP3A*, *-B*, and *-C* encode the OsHAP3 subunits of a CCAAT—box binding complex and they regulate nuclear-encoded chloroplast-targeted gene expression levels and normal chloroplast development (Miyoshi et al. 2003). The gene *V1*, which encodes the chloroplast-localized protein NUS1, is involved in the regulation of chloroplast RNA metabolism (Kusumi et al. 2011), whereas *V2*, which encodes a mitochondrial guanylate kinase, is essential for chloroplast and plastid development (Sugimoto et al. 2007). *OsClpP5* encodes a chloroplast protease that is essential during particular plant developmental stages and cannot be replaced by other *ClpP* genes (Tsugane et al. 2006). *VYL*, which is a homolog of *ClpP6* in *Arabidopsis thaliana*, encodes an ATP-dependent Clp protease proteolytic subunit and plays a crucial role during the early stages of chloroplast development (Dong et al. 2013; Li et al. 2013). *OsPPR1* encodes a pentatricopeptide repeat protein and is required for chloroplast biogenesis in rice (Gothandam et al. 2005).

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Photosynthesis is divided into dark and light reactions and the latter is carried out on the chloroplast thylakoid, which absorbs light energy using photosynthetic pigments. Photosynthetic pigments and protein complexes involved in light reactions, including cytochrome b_6f complexes, ATP synthase complexes, and light-harvesting chlorophyll-binding proteins (LHCPs), are located on the thylakoid membrane. LHCPs, as the most abundant nuclear-coded thylakoid family members in chloroplasts, are posttranslationally inserted into the thylakoid membrane through the chloroplast signal recognition particle (cpSRP) pathway (Ziehe et al. 2018). This pathway requires the cpSRP, its receptor (cpFtsY), and the integral membrane protein Alb3 (Asakura et al. 2008).

Here, we present a novel *seedling chlorosis and lethality* (*scl*) mutant, which exhibited a yellow leaf and seedling-lethal phenotype at the four-leaf stage. Map-based cloning, sequencing, and complementation analyses indicated that *SCL* encodes a cpSRP receptor. The phenotypic and physiological characterizations and the expression analysis indicated that *SCL* loss of function impaired chloroplast development, chlorophyll biosynthesis, and LHCP transportation in rice.

Materials and Methods

Plant Materials and Growth Conditions

The seedling yellow and lethality (*scl*) mutant was derived from Nipponbare (*Oryza sativa* L. ssp. *japonica*) by natural variation. The mutant and wild-type (WT) were grown in a plant incubator under a 16-h light (300 µmol photonsm^{-2} s^{-1})/8-h dark photocycle at a constant 30 °C. The youngest fully expanded leaves at the three-leaf stage were used for chlorophyll content measurement, transmission electron microscopic analysis, blue native gel electrophoresis, and RNA extraction, unless otherwise noted. Because the *scl* mutant was lethal at the seedling stage, heterozygous plants (*SCL*/*scl*), from which lines with etiolated phenotypes could be isolated, were collected for preservation. F_1 seeds were obtained by crossing heterozygous plants with *indica* variety '9311.' They were harvested and planted independently. The F_2 populations, from which lines with etiolated phenotypes could be isolated, were used as the mapping population.

Photosynthetic Pigment Content Measurements

The samples were gathered and incubated in 10 ml of 80% acetone for 48 h in the dark. The photosynthetic pigments chlorophyll a (Chl a), chlorophyll b (Chl b), and carotenoid (Car) from WT and *scl* mutant seedling were spectrophotometrically measured at 663 nm, 645 nm, and 470 nm, respectively, and then their contents were calculated in accordance with previously published methods (Wellburn 1994; Zhou et al. 2015).

Transmission Electron Microscopy

Fresh leaf samples were collected from WT and *scl* plants for transmission electron microscopy. The samples were fixed in 2.5% glutaraldehyde with phosphate buffer (pH 7.2) at 4 °C for 16 h. The sectioning and staining of the leaf samples were performed as described previously (Li et al. 2011). Then, the leaf materials were viewed under a Tecnai G^2 F20 S-TWIN transmission electron microscope (FEI, USA).

Chlorophyll Fluorescence and Blue Native Gel Electrophoretic Analyses

Chlorophyll fluorescence analyses were performed using an IMAGING-PAM chlorophyll fluorometer after dark adapting the seedlings for 1 h (Mishra et al. 2020). A blue native gel electrophoretic analysis of chloroplast pigment protein complexes was performed in accordance with the method of Lima et al. (2006).

Mapping and Cloning of *SCL*

In total, 45 individuals from the F_2 population having the mutant phenotype were used for preliminary mapping. Polymorphic markers between 'Nipponbare' and '9311' were screened using simple sequence repeat and insertion/deletion markers. Furthermore, another 537 plants having the mutant phenotype were used for fine mapping. In total, nine new insertion/deletion markers were developed based on sequence divergences between 'Nipponbare' and '9311' (Supplementary Table S1). The 43.3-kb target region from the *scl* mutant was amplified and then sequenced by Hangzhou Tsingke Biological Engineering Technology and Service Co. Ltd (Hangzhou, China). The candidate gene was assembled and analyzed using Lasergene 7. For mutant complementation, the construction of a binary vector containing the full-length cDNA sequence of the WT (*pCAMBIA1390-SCL*) was introduced into the protoplasts of heterozygous plants (*SCL*/*scl*) via *Agrobacterium tumefaciens*-mediated transformation and the empty vector served as the control. The PCR screen and sequencing used for transgenic *scl* mutant lines used the primer pair CX-F/R (5′- AGCCTT GGCATCCCTACAAT-3′ and 5′-CGAGACCAAGCAGGA AACAT-3′, respectively).

RNA Extraction and Quantitative Reverse Transcription PCR (qRT-PCR) Analysis

Total RNA was extracted from different seedling organs using a Quick Extract RNA Extraction Kit (Axygen,

Hangzhou, China) following the manufacturer's procedures. The first-strand cDNA was reverse transcribed using a ReverTra Ace quantitative PCR RT Master Mix Kit with gDNA remover (Toyobo, Shanghai, China). qRT-PCR was performed using a Power SYBR Green PCR Master Mix kit (Applied Biosystems, Foster City, CA, USA). The qRT-PCR was performed using an Applied Biosystems® 7500 Real-Time PCR System (Invitrogen, USA). The rice *Actin* gene (LOC_Os03g50885) was used as a normalization control. The relative expression levels of the genes were calculated following the protocol of Wu et al. using the $2^{-\Delta\Delta CT}$ method (Wu et al. 2007). The means from three replications were used for the analysis. The sequences of primer pairs used for the qRT-PCR analysis are listed in Supplementary Table S2.

Subcellular Localization

To investigate the subcellular localization of *SCL* in rice, the full-length *SCL*-coding sequence without the termination codon was introduced into the *pCAMBIA1390* vector. The *pCAMBIA1390*:*SCL*::*GFP* vector was introduced into rice protoplasts and GFP fluorescence was observed in the transformed protoplasts using a Zeiss LSM700 laser scanning confocal microscope (Zhang et al. 2011).

Statistical Analyses

Data were entered into Microsoft Excel 2007 and charts were constructed using Illustrator CS5. Statistical analyses included chi-square tests and a variance analysis was performed using SAS 9.1.3 software (SAS Institute Inc., Carrboro, NC, USA). Histogram columns represent the means of data from three biological replicates and ** indicates highly statistically difference at $p < 0.01$ compared with the control, as determined by Tukey's test.

Results

Phenotype of the *scl* Mutant

A novel rice leaf color mutant, named seedling chlorosis and lethality (*scl*), was identified in a paddy field of the *japonica* rice variety 'Nipponbare' and was the result of natural variation. The *scl* mutant showed a chlorotic phenotype from germination to the four-leaf stage and displayed shorter leaf lengths and seedling heights than those of the wild-type (WT) (Fig. 1a). As expected, Chl a, Chl b, Chl, and Car contents were remarkably reduced in the mutant compared with WT at the four-leaf stage (Fig. 1b). After the four-leaf stage, the fourth leaf of the mutant aged first, then the third leaf withered, and finally, the whole seedling died (Fig. 1c, d).

SCL Affects Chloroplast Development and Photosynthesis

A transmission electron microscopy analysis, revealing chloroplast ultrastructures, was conducted to investigate chloroplast development in the *scl* mutant. The chloroplast ultrastructures in the three-leaf stage WT rice seedlings were tidy, compact, and included well-structured thylakoid grana, whereas the chloroplasts of the mutant *scl* had no obvious grana thylakoids and thin stroma (Fig. 2). Photosynthetic products are stored as starch, and the stored starch usually exists in a granular form, called starch grains. The *scl* mutant had no starch grains, instead having oval-shaped vesicles and deformed chloroplasts. In contrast, the WT had well-developed starch grains and normal chloroplasts. Thus, SCL appears to play vital roles in early chloroplast development.

To explore why the *scl* mutant lacked starch grains, we examined the Chl fluorescent parameters between the mutant and the WT. The maximum photochemical quantum yield of photosystem II (*Fv/Fm*) decreased obviously in the mutant compared with the WT (Fig. 3a). The protein complexes in chloroplast thylakoid membranes have key roles in light energy absorption, transfer, and conversion in seedling. Blue native gel electrophoresis was used to analyze the protein complexes in the chloroplast thylakoid membranes. We observed almost no protein complexes in thylakoid membranes of the *scl* mutant (Fig. 3b). Moreover, the expression levels of the photosynthesis-associated genes, such as *LHCB1*, *LHCB4*, and *CAB1R*, were significantly down-regulated in *scl* compared with the WT (Fig. 3c). These observations indicated that *SCL* affects photosynthesis.

Map-Based Cloning of *SCL*

scl is a seedling-lethal mutant and therefore homozygous plants cannot complete a normal life cycle to produce seeds; consequently, heterozygous plants were used to preserve the mutant gene. F_1 plants were obtained by crossing the mutant heterozygous line with the *indica* rice variety '9311' and they showed the normal green color at the seedling stage. The F_2 population obtained from F_1 selfing had both green and mutant yellow leaf phenotypes at the seedling stage. Among 1,336 F_2 generation plants, 1,031 had normal green leaves and 305 had yellow leaves. The segregation ratio of the population was 3:1 ($\chi^2 = 3.36 < \chi^2_{0.05} = 3.84$), indicating that the *scl* mutant phenotype was controlled by a single recessive nuclear gene.

To isolate the recessive nuclear gene, 45 F_2 mutant individuals were used for initial mapping with 163 genome-wide markers. The *SCL* locus was located roughly on chromosome 1 between markers RM5794 and RM12276 (Fig. 4a). New genetic markers were designed after comparing the sequences between the *japonica* cultivar 'Nipponbare'

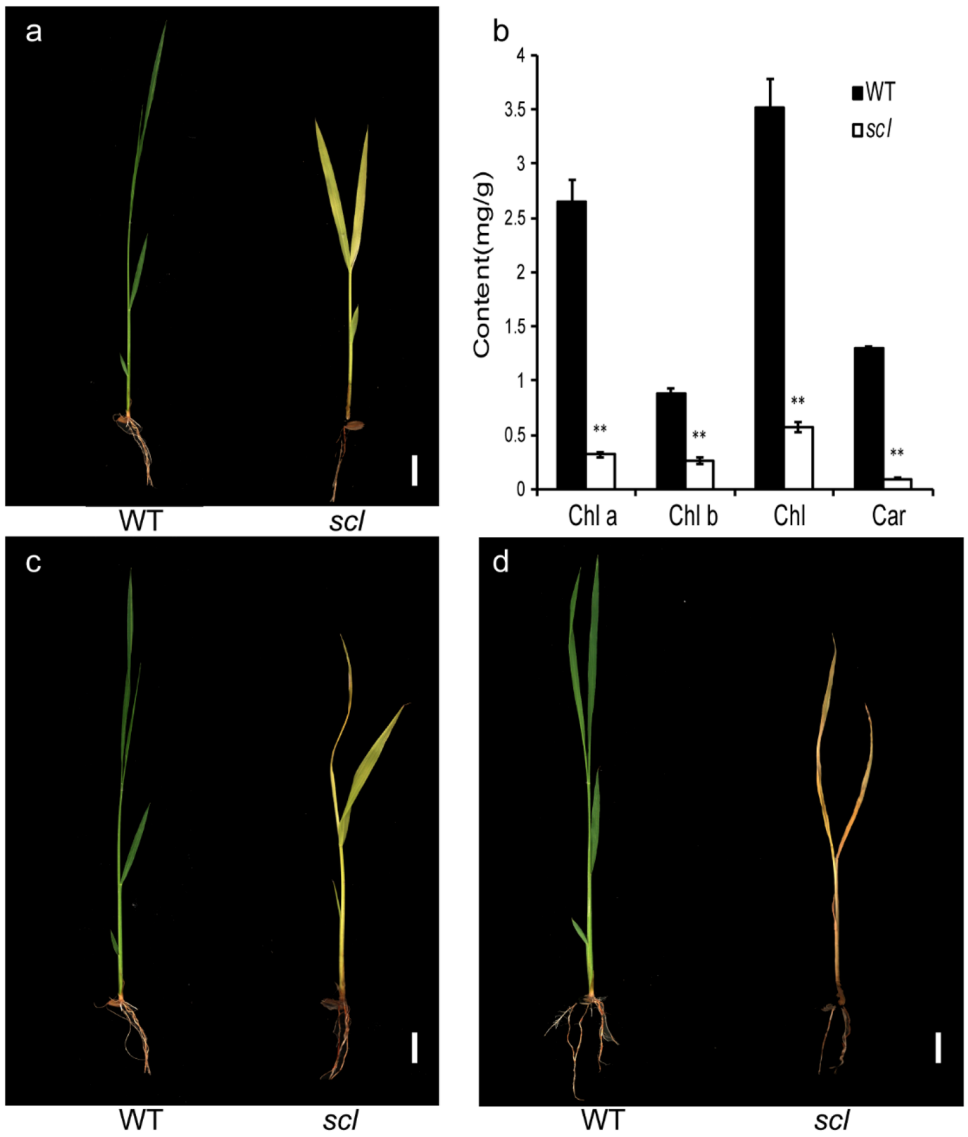

Fig. 1 Characterization of WT and *scl* mutant rice seedlings. Phenotypes of 9- (**a**), 12- (**c**), and 15-day-old (**d**) WT and *scl* seedlings. Bar = 1 cm. **b** Photosynthetic pigments contents. The values are presented as the means ± SDs from three biological replicates. **Highly significant at $p \leq 0.01$ compared with WT

and the *indica* cultivar '9311.' Using 537 F_2 mutant-type plants, the location of *SCL* was finally narrowed down to a 43.3-kb physical region between the markers B3 and B5, which included six open reading frames (ORFs)(Fig. 4b). Sequence alignment results revealed that the mutant had a 2-bp deletion in ORF4 (LOC_Os01g72800) that resulted in a frameshift mutation (Fig. 4c).

A homolog to *Arabidopsis' cpFtsY*, ORF4 encodes a cpSRP receptor. A *cpFtsY* mutation also causes *Arabidopsis* leaves to turn yellow. Thus, we hypothesized that ORF4 was part of the candidate *SCL* gene. To confirm the candidate gene, the complementation vector *Ubi:SCL::GFP*, harboring the cDNA of *SCL*, was introduced into the *scl* mutant by *Agrobacterium tumefaciens*-mediated transformation. All of the positive transgenic lines (*pCAMBIA1390-SCL/scl*) exhibited the normal green leaf phenotype, whereas the *scl* seedling that were transformed with the empty vector displayed the yellow leaf phenotype, at the seedling stage (Fig. 4d). These results confirmed that *SCL* was LOC_Os01g72800.

Expression and Subcellular Localization of *SCL*

The *scl* mutant died at the four-leaf stage and therefore (qRT-PCR) was performed using RNA samples of various tissues from four-leaf stage WT seedlings to examine the *SCL* expression pattern. The *SCL* gene was mainly expressed in green tissues, such as leaves and leaf sheathes, particularly in leaf IV (Fig. 5a, b). To explore the subcellular localization of the SCL protein, a fusion protein expression system with SCL and green fluorescent protein (GFP) was introduced into rice protoplasts and an empty GFP vector was used as a negative control. Confocal microscopy revealed that the SCL-GFP fluorescence signal localized to chloroplasts, whereas the empty vector's GFP

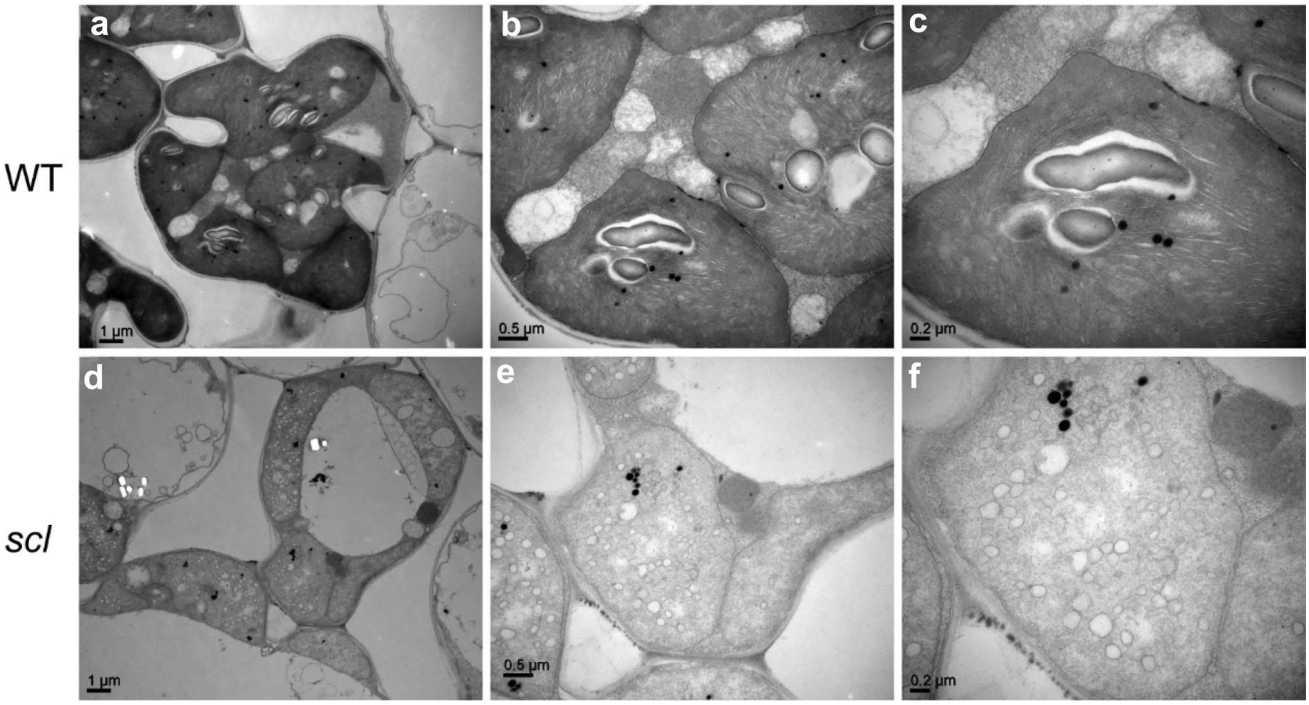

Fig. 2 Chloroplast ultrastructures of WT and *scl* rice seedlings. Three-leaf stages of (**a**–**c**) WT and (**d**–**f**) *scl*

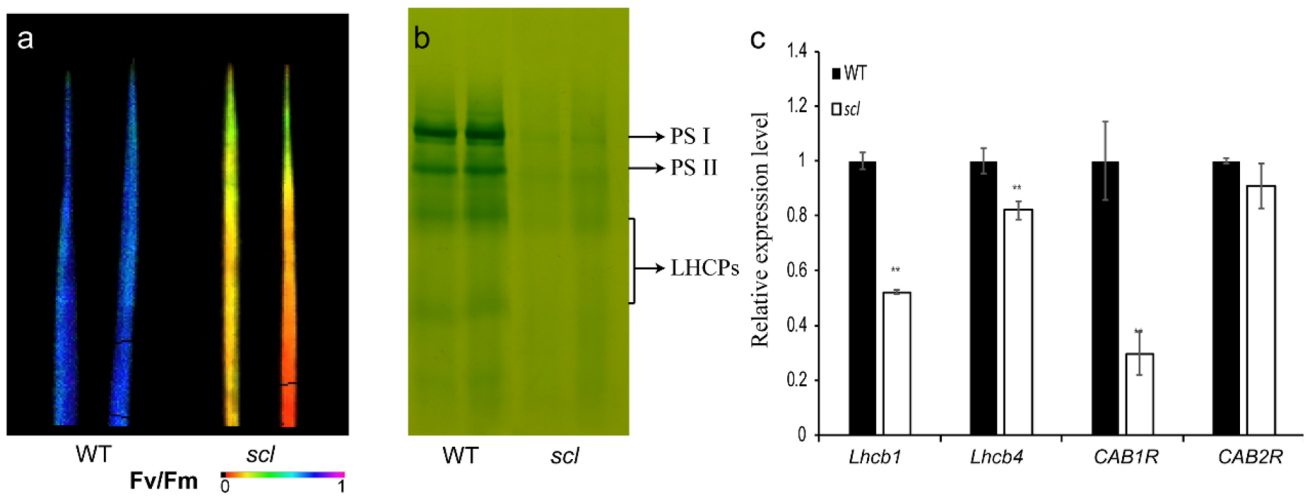

Fig. 3 Photosynthetic characteristics of WT and *scl* seedlings at the three-leaf stage. **a** Maximum photochemical quantum yields of photosystem II (*Fv/Fm*) in WT and *scl* seedlings. The color scale at the bottom of the image depicts *Fv/Fm* values. **b** Gel electrophoresis of thylakoid membrane proteins from WT and *scl* seedlings. PSI, photosystem I; PSII, photosystem II. **c** Photosynthesis-associated gene expression levels in WT and *scl* seedlings at the three-leaf stage. *Lhcb1* (LOC_Os10g41780) and *Lhcb4* (LOC_Os07g37240), light-harvesting chlorophyll a/b-binding proteins of PSII; *CAB1R* (LOC_Os09g17740) and *CAB2R* (LOC_Os01g41710), rice chlorophyll a/b-binding proteins. The values are presented as the means ± SDs from three biological replicates. **Highly significant at $p \leq 0.01$ compared with WT

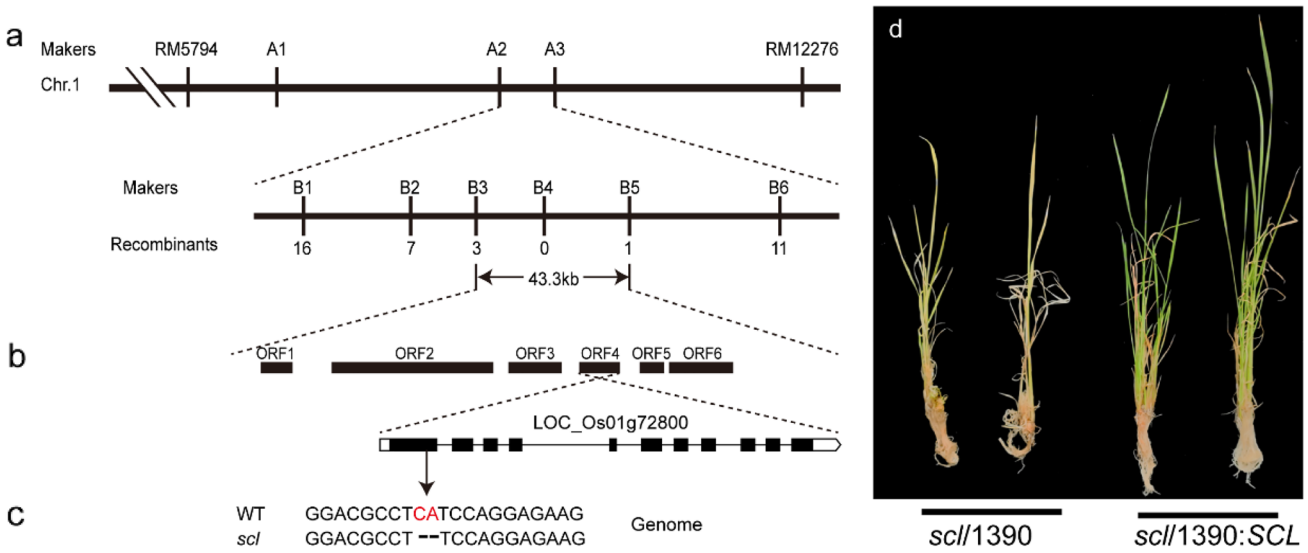

Fig. 4 Map-based cloning and transgenic validation of the *SCL* gene. **a** The *SCL* locus was narrowed to a 43.3-kb genomic DNA region between two markers, B3 and B5. **b** Six open reading frames were predicted in the mapped region and the candidate *SCL* gene was identified as LOC_Os01g72800. **c** Sequence comparison revealing 2-bp deletion in LOC_Os01g72800 of the *scl* mutant. **d** Functional complementation of *SCL*. *scl*/1390, transgenic complementary seedling harboring the empty vector; *scl*/1390:*SCL*, transgenic complementary seedling harboring *pCAMBIA1390-SCL*

fluorescence signal was observed in the cytoplasm and the nucleus (Fig. 5c). These results suggested that SCL plays an important role in early chloroplast development.

Expression Analysis of *SCL* and Chloroplast-Associated Genes

SCL is mainly expressed in leaves and encodes a chloroplast-localized protein and therefore it may be involved in the expression changes of chloroplast-associated genes in the mutant. We thus performed a qRT-PCR analysis of chloroplast development- and pigment metabolism-related genes between *scl* and WT seedlings at the three-leaf stage. The *SCL* transcription level decreased in *scl* compared with in WT. The expression levels of chloroplast development-related genes *SPP* and *SRP43* significantly decreased, those of *V1* and *V2* displayed slight decreases in the *scl* mutant compared with in WT, but the expression level of *PPR1* gene significantly increased in the *scl* mutant (Fig. 6a). Moreover, the transcript levels of the chlorophyll biosynthetic genes, including *HEMA*, *PORA, PORB, YGL1*, *CHLD*, and *CHLI*, were strongly inhibited in *scl* compared with in WT leaves (Fig. 6b). In addition, we also examined the expression levels of genes related to chlorophyll degradation. The transcript levels of *NYC1*, *NOL*, *NYC3, PAO*, and *RCCR1* in the *scl* mutant were higher than in WT leaves. Thus, the loss of *SCL* function results in the massive disordered expression of genes related to chloroplast development and both chlorophyll biosynthesis and degradation and this may be responsible for the yellow leaf phenotype observed in *scl* rice seedlings.

Discussion

When chlorophyll metabolism is disrupted, rice plants usually exhibit a variety of leaf color phenotypes coupled with slow growth, short plant heights, low tiller numbers, and decreased yields (Qiu et al. 2018; Zhang et al. 2013; Huang et al. 2018). Glutamate tRNA reductase, encoded by *HEMA*, and Mg^{2+}-chelatase, encoded by *CHLH*, *CHLD*, and *CHLI*, are the key enzymes in the early stage of chlorophyll synthesis (Jung et al. 2003; Zhang et al. 2006). Protochlorophyllide oxidoreductase (POR), which is an important enzyme in the late stages of chlorophyll synthesis, is encoded by two homologous genes, *PORA* and *PORB*, in rice, and they can catalyze the photoreduction of protochlorophyllide to chlorophyllide during chlorophyll synthesis. This is required for prolamellar body formation in etioplasts (Sakuraba et al. 2013). In rice, the *YGL1* gene encodes chlorophyll synthase, which catalyzes the formation of Chl a in the last step of chlorophyll synthesis (Wu et al. 2007). In this study, we identified a novel rice chlorophyll-deficient mutant, *scl*, which showed short leaf lengths and seedling heights at the seedling stage (Fig. 1). Compared with WT, the expression levels of chloroplast development-related genes, *SRP43*, *V1*, *V2*, and *SPP* were down-regulated, while *PPR1* was up-regulated in the *scl* mutant. The transcription levels of genes

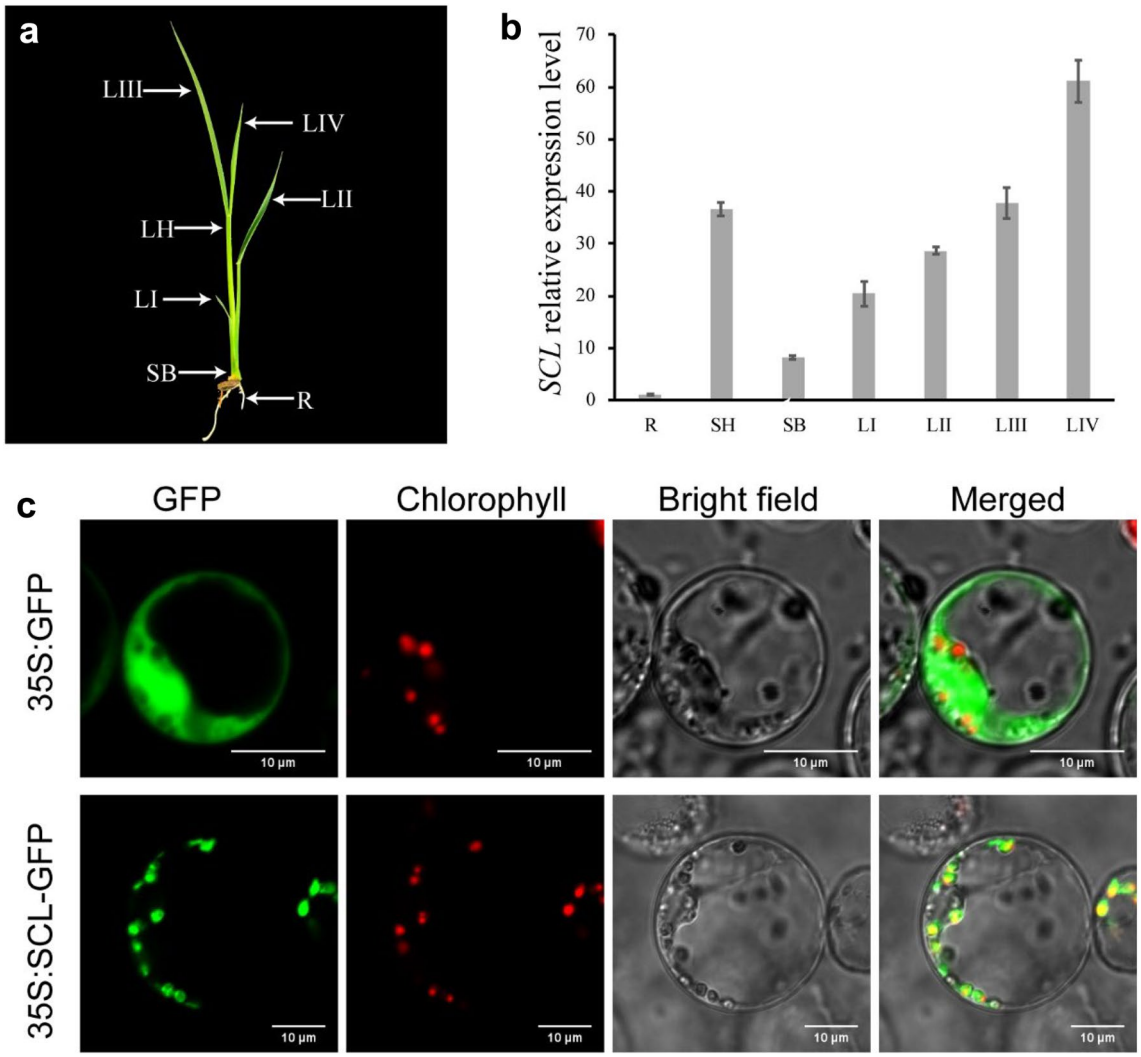

Fig. 5 SCL expression and protein subcellular localization. **a** Schematic representation of young seedling tissues at the four-leaf stage. *R* root; *SB* stem base; *LH* leaf sheath; *LI–LIV* first-to-fourth leaves. **b** Relative expression of *SCL* in different organs of the WT at the four-leaf stage. **c** Transient expression of the empty GFP vector and SCL-GFP fusion protein in rice protoplasts. The values are presented as the means ± SDs from three biological replicates

involved in the chlorophyll biosynthetic were significantly decreased and the expression levels of chlorophyll degradation genes were significantly increased in the *scl* mutant (Fig. 6b). These results indicated that SCL mainly attenuated the chlorophyll synthesis and enhanced the chlorophyll degradation. It may result in feedback regulation of gene expression in chloroplast development. The *scl* mutant exhibited yellow leaves having low levels of chlorophyll, which likely resulted from the low transcription levels of the six chlorophyll biosynthetic genes. Furthermore, the *SCL* protein was localized to chloroplasts, and a qRT-PCR analysis revealed that the *SCL* gene was highly expressed in green tissues (leaf and sheath) (Fig. 5). These results indicate that *SCL* is required for chlorophyll synthesis.

Some chlorophyll-deficient mutants show characteristic chloroplast developmental defects that lead to seedling death (He et al. 2018; Liu et al. 2016). Transmission electron microscopy revealed that chloroplast development in *scl* was abnormal and lacked obvious grana thylakoids (Fig. 2). Consistent with the phenotype, the transcription levels of the chloroplast developmental genes *V1*, *V2*, *SPP*, and *SRP43* were down-regulated (Fig. 6a). Moreover, the expression levels of genes associated with photosynthesis (*LHCB1*, *LHCB4*, and *CAB1R*) were also down-regulated in the *scl* mutant compared with the WT and the maximum photochemical efficiency values in *scl* were also notably decreased compared with those of WT (Fig. 3). As the main organelle of plant photosynthesis, chloroplast impairment may lead to the declines in photosynthetic capacity

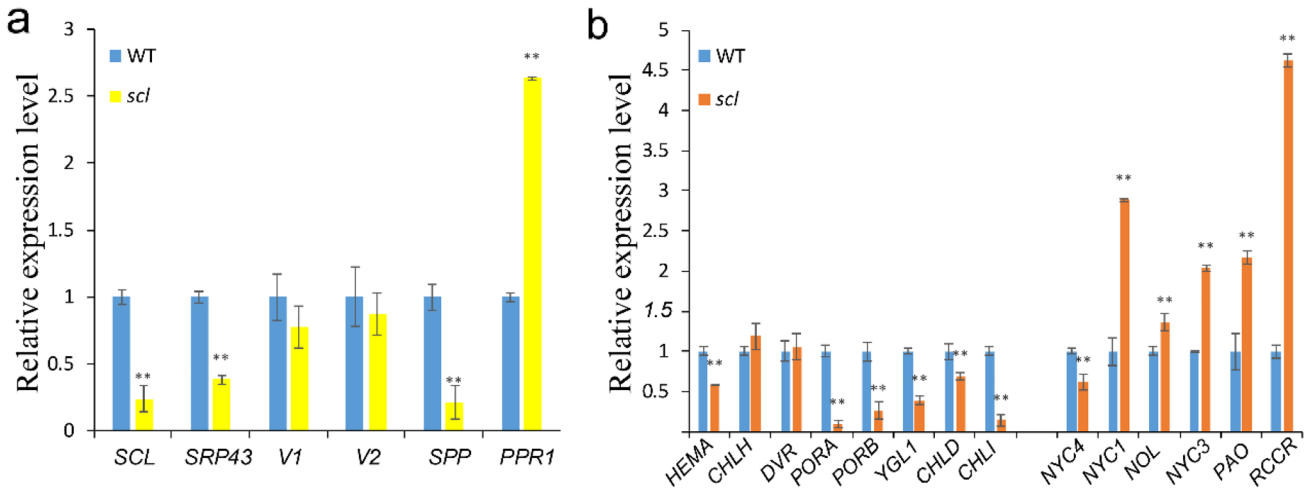

Fig. 6 Quantitative real-time PCR analysis of *SCL* and chloroplast-associated genes in WT and *scl* seedlings. **a** Expression levels of genes associated with chloroplast development. *SRP43*, Chloroplast Signal Recognition Particle 43; *V1* (LOC_Os03g4540), plastid protein; *V2* (LOC_Os03g20460), plastid and mitochondrial guanylate kinase; *SPP* (LOC_Os06g41990), rice stromal processing peptidase; *PPR1* (LOC_Os09g24680), pentatricopeptide repeat protein. **b** Expression levels of genes associated with chlorophyll (Chl) biosynthesis and degradation. *HEMA* (LOC_Os10g35840), glutamyl-tRNA reductase; *CHLH* (LOC_Os03g20700), Mg-chelatase H subunit; *DVR* (LOC_Os03g22780), 3,8-divinyl chlorophyllide, a 8-vinyl reductase; *PORA* (LOC_Os04g58200) and *PORB* (LOC_Os10g35370), two protochlorophyllide oxidoreductases; *YGL1* (LOC_Os05g28200), Chl synthetase; *CHLD* (LOC_Os03g59640), Mg-chelatase D subunit; *CHLI* (LOC_Os03g36540), Mg-chelatase I subunit; *NYC4* (LOC_Os07g37250), THYLKOID FORMATION1, chloroplast precursor; *NYC1* (LOC_Os01g12710), Chl b reductase; *NOL* (LOC_Os03g45194), NYC1-LIKE; *NYC3* (LOC_Os06g24730), α/β hydrolase-fold family protein; *PAO* (LOC_Os03g05310), pheophorbide a oxygenase; *RCCR1* (LOC_Os10g25030), red Chl catabolite reductase. The values are presented as the means ± SDs from three biological replicates. **Highly significant at $p \leq 0.01$ compared with WT

and efficiency in *scl* seedlings. When rice seedlings reach the three-leaf stage, all the nutrients in the seeds are exhausted and the nutrients must then be absorbed or manufactured. The absence of starch grains may indicate that there is no accumulation of photosynthetic products in the *scl* mutant. Thus, an insufficient energy supply may be responsible for the death of the *scl* seedlings.

Using map-based cloning and a functional complementation assay, we identified *SCL*, a homolog of *Arabidopsis cpFtsY*, which encodes a cpSRP receptor. The *scl* mutant had a 2-bp deletion that resulted in a frameshift mutation. The *Arabidopsis cpFtsY* gene plays an important role in LHCPs transportation into thylakoid membranes through the post-translational cpSRP pathway, which requires the stromal components cpSRP54 and cpSRP43, the membrane-bound SRP receptor cpFtsY, and the integral membrane protein Alb3 (Asakura et al. 2008). The *cpSRP54* and *cpSRP43* mutants exhibit pale yellow leaves at the rosette stage, but they are viable in *Arabidopsis* (Hutin et al. 2002). Similar to *scl*, the cpftsY and alb3 mutants in *Arabidopsis* also exhibit severe chlorotic and seedling-lethal phenotypes, resulting from a massive loss of LHCPs (Durrett et al. 2006). cpSRP43 has developed a pathway that functions independently of cpSRP54/cpFtsY in targeting LHCPs to thylakoid membranes (Tzvetkova-Chevolleau et al. 2007). However, we observed almost no LHCPs in the thylakoids membranes of the *scl* mutant, indicating that the cpSRP43-dependent pathway is not sufficient in rice (Fig. 3b). Therefore, we hypothesized that *SCL* is necessary for the transportation of adequate numbers of LHCPs to thylakoid membranes through the cpSRP pathway. This is required to maintain viable rice seedlings.

In conclusion, the *scl* mutant identified here had a 2-bp deletion that resulted in a frameshift mutation and *SCL* encodes a cpSRP receptor, which is homologous to *cpFtsY* in *Arabidopsis*. The experimental results suggest that *SCL* is involved in LHCP transportation to thylakoid membranes and plays important roles in chlorophyll synthesis and chloroplast development.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00344-021-10563-4.

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Author Contributions X.Z. planned the research. S.Y. and J.Y. performed all important experiments. G.Z. performed phenotype evaluation. R.Z., M.W., and F.Y. performed field experiments. J.Y. analyzed the data and wrote the paper.

Declarations

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

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