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ORIGINAL PAPER

Map-based cloning of a spotted-leaf mutant gene *OsSL5* in *Japonica* rice

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Abstract A *japonica* rice mutant, spotted-leaf 5 (*sl5*), was identified from YUN32 by EMS mutagenesis. The number of spots in leaves increased from maturity to late maturity in *sl5*, however, the leaves did not dry and withered. The *sl5* mutant exhibited significantly lower height, spike length, primary branch number, second branch number, and 1,000-grain weight than YUN32. Genetic analysis shows that *sl5* is controlled by a single recessive gene. *OsSL5* was mapped into a 40-kb interval flanked by markers MX4 and MX5 on chromosome 7 by map-based cloning. Four ORFs, including one *SPL5* gene, were identified in this region. Sequencing reveals that the G base at site 3,647 of the *OsSL5* coding region was changed to A. The mutant *OsSL5* site was different from that of the *SPL5* mutant, with the background of *indica* rice. *OsSL5* is thus a new *SPL5* allele which encodes a putative splicing factor 3b subunit 3. QPCR shows that *OsSL5* expression in the *sl5* mutant is significantly lower than that in YUN32. The spotted leaf-related genes *RLIN1*, *SPL28*, and *SPL18* expressions were significantly decreased, whereas the *SPL7* and *SL* gene expressions significantly increased. The *OsSL5* gene may be important for rice cell apoptosis regulation.

Keywords Rice · Spotted-leaf mutant · Map-based cloning · Expression analysis

Introduction

Spotted-leaf (*spl*) mutants are ubiquitous in plants and exhibit different phenotypes, colors, and spot sizes. These spots commonly appear to be brown. The *spl* mutants also exhibit other shades, including reddish-brown and white. Spots in this leaf mutant type are generated even without the presence of pathogens, mechanical or pesticide damages, and adverse conditions. Many spots are related to plant cell apoptosis and disease resistance. The *spl* mutants that exhibit natural, chemical, or physical mutagenesis are currently separated from maize (Johal et al. 1995), *Arabidopsis thaliana* (Dietrich et al. 1994), barley (Wolter et al. 1993) and rice (Takahashi et al. 2007).

Caused genes of spotted-leaf have been isolated and cloned in rice. *SPL6* (Matin et al. 2010) and *SPL28* (Qiao et al. 2010), for instance, are found in chromosome 1. The transport of compounds associated with *SPL28*-encoding clathrin is regulated between the vesicle and the cytoplasm during vesicle formation. Other genes, such as *LRD1* (*BL1*) and *SPL2* (Yoshimura et al. 1997; Kojo et al. 2006), are found in chromosome 2. *spl2* mutant plants exhibited accumulated higher amounts of H_2O_2 and increased rates of cell death in response to wounding; *BL4* (Yoshimura et al. 1997) and *RLIN1* (Sun et al. 2011) are detected in chromosomes 3 and 4, respectively. The *rlin1* mutant gene encodes coproporphyrin III oxidase. This gene is also

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involved in spot formation in leaves and stems. Such formations are mainly related to H_2O_2 metabolism and illumination. *SPL7* (Yamanouchi et al. 2002; Kojo et al. 2006) and *SPL8* (Liu et al. 2003) are found in chromosome 5. By contrast, no spots have been identified in the *spl7* mutant during the seeding stage. However, small yellow spots have been found on leaves during the tillering stage to the heading stage. These spots disappear after exposing the leaves to high-temperature illumination or UV irradiation. *SPL7* encodes heat stress transcription factor (HSF). HSF is activated by thermally stimulated factors when UV irradiation or illumination and temperature are decreased. HSF is then combined with a thermally stimulated protein gene promoter site in the nucleus to promote thermally stimulated protein expression and thermally stimulated response. Several genes have also been detected in other chromosomes. The *lms1* mutant started to appear small brown spots on the leaves in elongation stage, and the spots gradually spread to whole leaves and sheaths (Ma et al. 2012). The *lbsl1* displayed light brown spots during the whole growth period, and the *LBSL1* gene was mapped between markers RM586 and RM588 (Feng et al. 2013). *BL2* (Matin et al. 2010), small spots in the seedlings appeared at the four leaves stage and gradually grew into a large round and black area with a gray center on the leaf surface, the gene was not cloned. *BL3* (Yoshimura et al. 1997), for example, are found in chromosome 6. *SPL5* (Chen et al. 2012; 2013) and *SPL9* (Liu et al. 2003) are detected in chromosome 7. The *spl5* mutant plants exhibited small reddish brown spots scattered extensively on leaves, from the tillering stage to heading stage. *SPL5* encodes for a splicing factor 3b subunit (SF3b3) and may regulate cell death by splicing mature RNA. *OsLSD1* (Wang et al. 2005) and *LMI1* (Liu et al. 2003) are found in chromosome 8. *SPL10* (Babu et al. 2011) and *SPL18* (*OsAT1*) (Mori et al. 2007) are located in chromosome 10. The *spl10* showed lesions similar to *spl5* mutant and have blast-resistant functions. Spots are mainly generated on the edge of the *spl18* mutant leaves. The *SPL18* gene encodes the OsAT1 protein, which is highly homologous to acyltransferase in tobacco. The OsAT1 protein is also important in the allergic reaction in tobacco. Studies verified that spot formation is related to the PR protein. *SPL1* (*SL*) (Fujiwara et al. 2010), *SPL11* (Zeng et al. 2004; Kojo et al. 2006) and *SPL29* (*t*) (Li et al. 2010) are found in chromosome 12. *SPL1* (*SL*) encodes a cytochrome P450 monooxygenase, which is important in serotonin formation and disease resistance in plants (Fujiwara et al. 2010). *SPL11* regulates cell death and encodes a U-box-ARM-like protein, which induces E3 ubiquitin ligase catalytic activity. *SPL11* can be potentially applied to breed rice blast fungus–resistant rice because this gene encodes the phenotype manifesting resistance to rice blast fungus (Zeng et al. 2004).

The *spl* gene provides important genetic resources that can be used to investigate plant cell apoptosis. This gene is also important in breeding disease resistant rice. A stable *spl* mutant, designated as *sl5*, was obtained in this study by subjecting YUN32 to ethyl methyl sulfide (EMS)-induced mutagenesis. The agronomic, physiological, and biochemical characteristics of the mutant were analyzed. In the study, *OsSL5* was found in the 40 kb range of chromosome 7 by using the map-based cloning method. The sequencing results show that *OsSL5* from Japonica rice is a new allele of *SPL5*. QPCR (real-time quantitative PCR) was performed to analyze *OsSL5* and multiple cloned spl-related gene expressions in *sl5* and YUN32. The results also show that *OsSL5* is important in apoptosis regulation. These results further provide a new genetic resource that could be used to analyze the rice *spl* formation mechanism.

Materials and methods

Plant materials and growth condition

The *sl5* mutant was identified from the YUN32 mutant library which was genetated by EMS mutagenesis. The *sl5* and YUN32 were planted in paddy field by normal management in Fuyang, Hangzhou, in 2013. Each of *sl5* and YUN32 were prepared for fifteen rows in each plot, with six plants per row. This arrangement was repeated three times. Fifteen plants in the two middle rows were selected to investigate the agronomic characteristics at the mature stage. Plant height, effective panicles, spike length, primary and secondary branch numbers, seed setting rate, 1,000-grain weight, and other characteristics were surveyed. Mean values and significance for agricultural traits were performed through Student's *t* test analysis.

Genetic analysis and allelic test

The *sl5* mutant was used as the female parent to cross with the *indica* cultivar TN1, NJ06, and 93-11. The F_1 plants self-fertilized to produce three F_2 generations. The leaf phenotypes of the F_1 and F_2 plants were investigated. The segregation ratio of the normal and the spot leaf individuals from the F_2 generation at the heading stage was studied. A Chi square test was conducted. For the allelic test, *sl5* was crossed with the *spl5* mutant and the leaf phenotype of F_1 plants was observed.

Primary mapping of the *OsSL5* gene

The F_2 individuals with spotted leaves derived from a cross of the *sl5* mutant and TN1 were used to map the *OsSL5* gene. Genomic DNA was extracted using the

cetyltrimethyl ammonium bromide method (Rogers and Bendich 1985). The DNA samples from 21 mutant plants were pooled equally. One hundred and sixty-three pairs of rice SSR primers were evenly distributed on 12 chromosomes and used for polymorphism screening. A total of 50 mutant individuals were used for linkage analysis of the *sl5* gene and flanking markers. The volume of the PCR system was 10 μL and contains 1 μL of template DNA, 1 μL each of forward and reverse primers (10 μmol/L), 0.1 μL of dNTPs (10 μmol/L), 1 μL of 10× PCR buffer solution, and 0.05–10 μL of r-*Taq* enzyme with ddH_2O. PCR was performed for 38 cycles as follows: pre-denaturation at 94 °C for 4 min, denaturation at 94 °C, annealing at 55 °C, and extension at 72 °C for 30 s. Temperature was held constant at 15 °C during PCR. The PCR products were subjected to 4–5 % agarose gel electrophoresis, observed, and photographed using a gel imager after gel-red staining.

Fine mapping of the *OsSL5* gene

The published rice genome sequence (http://rpg.dna.affrc.go.jp/E//toppage.html) and BLAST (http://blast.ncbi.nlm.nih.gov/) program were used to compare the genome sequence of *Oryza sativa* L. ssp. *japonica* var. Nipponbare with that of *O. sativa* ssp. *indica* 93-11. Indel markers were developed using the Software Primer 5 to narrow down the target gene (Table 1).

Sequencing of candidate gene

The full-length genomic sequences of the candidate genes were downloaded from the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov). The *OsSL5* gene was amplified by KOD FX fidelity enzyme (Toyobo) using wild type and *sl5* genomic DNA as template. The amplified products were sequenced in Shanghai Sunny Biotechnology Co., Ltd. The sequencing primers are shown in Table 2. The wild type gene and its mutant site were confirmed after the sequencing results were compared.

Histological staining

The Thordal-Christensen et al. (1997) method was used to detect H_2O_2 reactant accumulation in the mutant leaf. The spotted *sl5* mutant leaves and the wild-type YUN32 leaves of heading stage (approximately 10 cm) were immersed in a 1 mg/mL 3,3′-diaminobenzidine (DAB; pH 3.8) solution and incubated at 25 °C for 8 h. The leaves were boiled in 96 % ethanol for 10 min to induce decolorization. The decolorized leaves were immersed subsequently in fresh ethanol for 4 h at 25 °C to observe and record the resulting color.

QPCR for *OsSL5* analysis and related *spl* gene expression

Real-time fluorogenic quantitative PCR was performed to determine the relative spot formation mechanism in *OsSL5* and to analyze *spl*-related gene expressions in the wild type and the *sl5* mutant. The total RNAs in the wild type and the mutant leaves at the four-leaf stage were extracted to synthesize the first-strand cDNA by reverse transcription. The volume of the PCR system was 10 μL and contained the following: 1 μL of cDNA template; 1 μL each of upstream primer and downstream primer (10 μmol/L); 5 μL of 2× SYBR green PCR master mix (Applied Biosystems); and 2 μL of ddH_2O. Each sample has three replicates. PCR amplification was performed for 40 cycles under the following conditions: enzyme activation at 95 °C for 10 min, denaturation at 95 °C for 15 s, annealing at 60 °C for 5 s, extension at 72 °C for 15 s, and fluorescence detection at 76 °C for 3 s. $2^{-\Delta\Delta Ct}$ was used to calculate relative gene expression. The *spl*-related gene expression

Table 1 Primers designed for *OsSL5* gene mapping

Marker	Forward primer (5′–3′)	Reverse primer (5′–3′)
RM6574	AACCTCGAATTCCTTGGGAG	TTCGACTCCAAGGAGTGCTC
RM542	TGAATCAAGCCCCTCACTAC	CTGCAACGAGTAAGGCAGAG
MX1	GCTTGGAGAGGTTGTTCTCG	TGGAGCATGGATAGTGCAAA
MX2	AGACCTCAGACAGGCTCGAC	ATTTTTCCGACGGCGAA
MX3	TGGCTGTGAAACGGATTATG	CAGAGTTTTCACGAGCACCA
MX4	GCCGCATTTAATGTGATGTG	AGTGGATGAACCGACTCCAG
MX5	ATGTTGTAAAACCGCCGAAG	GGATTTGCAACCCAAAACAC
MX6	TGGCTGCTATGGATGTTTGA	TTGCACAGCAAAGGAAACAG
MX7	GTGCCAAGTAAGCATGAGCA	CAAGCCCCAGGATTTTATGA
MX8	TGAACCATCTGTCGCTCTTG	CATCTCGGTTTGATGGTCCT
MX9	TTGTATGCAGCAGCTGAACC	GACGGTCAATCTGGGAAGAG
MX10	TTGAGGCCCACAAATTAAGG	TCCGGAAGTCTTGCGTATTT

Table 2 Primer sequences used in *OsSL5* gene sequencing

Primer	Forward primer	Reverse primer
X1	GCACGAGTGATGTGAGTGC	TTTGTAACAAGGGGCCAGAG
X2	GGAGCAGTCAATGGGTGAAC	AATTCTGACCAGCTGATTCCA
X3	CTTCCGAACCAAACAAGAGC	GGAGGTGGAGGTTGAGGAAT
X4	CCACACCGACAAGTCTACGA	TGGAGCTTCTTTGTCGTTACC
X5	GCTCCCTCGTCGATCTGATA	CATCTCATGCTGGTTCTCCA
X6	AGCTGCTGCCAACACATATC	TTTATCTCCTAAAAGTGGCTTGC
X7	ACCCAGTTCTCAGCACCAAC	CAAGTGCTGCATTATTTTGGAA
X8	TCACTCATTGCAACTGTGTTCTC	GTTGCATGACAGAGCCAATG
X9	TGGATGAATATGCCAAGCTG	GGGTCCTTGCGAGTAATACG
X10	CATGCCAGAGTGCAAACATC	CTTAAGTTTGTGGCGATTACCTG
X11	CCAGCACGAAACATAAGTGC	TGGGAGGCCTGTCTAGTTTG
X12	ATGGAGTCCCCCACCGCCG	TCAGTTGAGTGCGTAATGC

Table 3 Spotted leaf-related gene expression analysis primer sequence

Gene	Forward primer sequence (5′–3′)	Reverse primer sequence (5′–3′)
OsSL5	GAGGGACTCCAAGGAAAGTGTTAT	TTGCAGCAAACTTCATCAGGAC
SPL7	GGTCACGGGAGAAGTCGAGC	GTCTCCGTGGCCGTGGCTGA
SPL11	ACGGATTGATATGCCTGACGAT	GATGCTTGCCTTATTGTCCTCA
SPL28	GTGAAAGCAAGAAGTCAGTTTAAGG	CTAACAAGATGAACAACGAGACAGA
SL(SPL1)	CCTCCGCGTCCGAAATCCCCCGACC	CCACTCGAAGTGGTAGAGCAAGC
SPL18	CCATACGGGCAGAATGGTC	CCTCATGCAGGTGACGCAACTTAA
RLIN1	AGGAAAGCTAACAAACGATTGGA	ATCATACACCTGAAGAGGGAACT
β-Actin	CCATTGGTGCTGAGCGTTT	CGCAGCTTCCATTCCTATGAA

primers are shown in Table 3 (Livak and Schmittgen 2001). Student's *t* test analysis was performed to determine the significance of each gene's expression.

Results

Phenotypic and agronomic characteristics of the mutant

Sparse rust points were observed at the leaf tip when the second leaf of the *sl5* mutant grew. Rust points were dispersed on the leaf surface during leaf growth. The agronomic characteristics of the *sl5* mutant were investigated. The results show that plant height reduced from 85 to 71.7 cm, and spike length decreased from 19 to 14.8 cm. The internode also significantly reduced in mature leaves compared with the wild-type leaves. The secondary branch number of *sl5* decreased from 31 to 5.3 and directly affected the *sl5* grain number (Fig. 1; Table 4).

Relationship between spot formation and reactive oxygen species accumulation

YUN32 (Fig. 2a) and *sl5* mutant (Fig. 2b) were stained with DAB. No H_2O_2 accumulated in YUN32 (Fig. 2c). By contrast, numerous H_2O_2 deposition spots were observed in the mutant (Fig. 2d). This finding indicates that the emergence of spots on the *sl5* leaf was caused by the excessive reactive oxygen species (ROS) in the rice cells.

Genetic analysis of the *OsSL5* gene

The mutant *sl5* was crossed with *indca* cultivar TN1, NJ06, and 93-11 to determine the inheritance pattern of *OsSL5*. All F_1 plants showed normal phenotype without spotted-leaf. This finding indicates that *sl5* is controlled by a recessive gene. In three F_2 populations investigated, the number of normal plants and *spl* plants fitted to a 3:1 ratio well ($\chi^2 = 1.1228,\ 1.0756,\ 1.6260 < \chi^2_{0.05} = 3.84$; Table 5). These results indicated that the spotted-leaf phenotype of *sl5* is controlled by a single recessive gene. The F_1 plants derived from the cross of *sl5* and *spl5* mutants exhibited leaf spots like *spl5*. Thus, *OsSL5* is allelic to *SPL5*.

Mapping of *OsSL5* gene

To isolate *OsSL5* gene, map-based cloning approach was performed. A total of 163 SSR markers covering the whole rice genome were screened for linkage with *OsSL5*. The results showed that *OsSL5* is located into a region flanked by

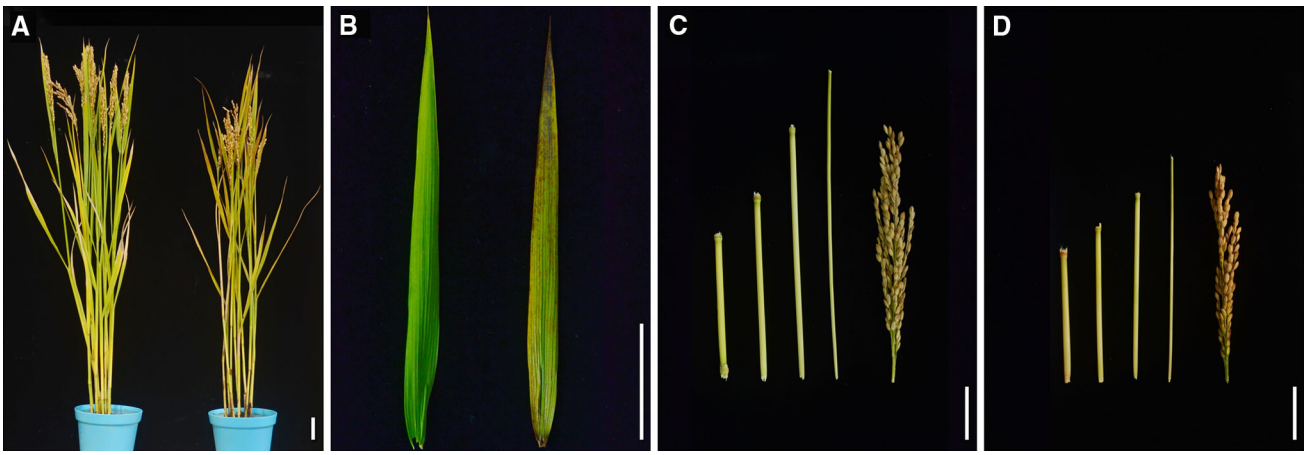

Fig. 1 Phenotypes of wild-type and spotted-leaf mutant *sl5*. **a** Gross phenotypes of wild type and mutant: YUN32 (*left*) and *sl5* (*right*); **b** leaf phenotypes of wild-type and mutant in mature leaves: YUN32 (*left*) and *sl5* (*right*); **c**, **d** refer to the internodes and spikes of YUN32 and *sl5*, respectively. *White bar* 5 cm

Table 4 Major agronomic characteristics of the wild type and *sl5* mutant

Materials	Plant height	Heading date	Panicle length/cm	Primary branch number	Secondary branch number	No. of productive panicles	Seed setting (rate/%)	1,000-grain (weight/g)
YUN32	85 ± 3.35	84 ± 1.02	19 ± 1.06	16.2 ± 1.17	31 ± 3.03	13.1 ± 1.47	82 ± 0.05	29.5 ± 0.14
sl5	71.7 ± 1.63**	83 ± 1.21	14.8 ± 0.43**	12 ± 1.41*	5.3 ± 1.37**	13.5 ± 1.38	78 ± 0.07*	28.5 ± 0.16**

* Difference is significant at the 0.05 level; ** Difference is significant at the 0.01 level

Fig. 2 Partial leaves of YUN32, and *sl5* with different treatments. **a**, **b**, **c**, **d** Refer to YUN32, *sl5*, YUN32, and *sl5* stained with DAB, respectively, during the heading stage

Table 5 Genetic analysis of the mutant gene *OsSL5*

Cross	F_1		Number of wild type plants	Number of *sl5* type plants	χ^2 (3:1)	*P* value
	Wild type	Mutant				
sl5/TN1	28	0	525	159	1.1228	0.2893
sl5/NJ06	25	0	439	161	1.0756	0.2997
sl5/93-11	30	0	256	72	1.6260	0.2023

two markers, namely, RM6574 and RM542 on chromosome 7. Ten additional indel markers within this region were also developed (Table 1). Among 2,500 F_2 plants, 40 and 60 recombinants were identified by RM6574 and RM542, respectively. Further genetic analysis using 8 indel markers delimited *OsLs5* into a 40-kb interval flanked by MX4 and MX5 (Fig. 3). Only four open reading frames (ORFs) were found in this region (Fig. 3b). Of the four ORFs, two were coded for hypothetically unknown function protein. The other two were coded for proteins with known functions (i.e., S1 RNA-binding domain-containing protein and splicing factor 3B subunit 3; Table 6). This result suggested that the *SPL5* gene is one of the candidate genes.

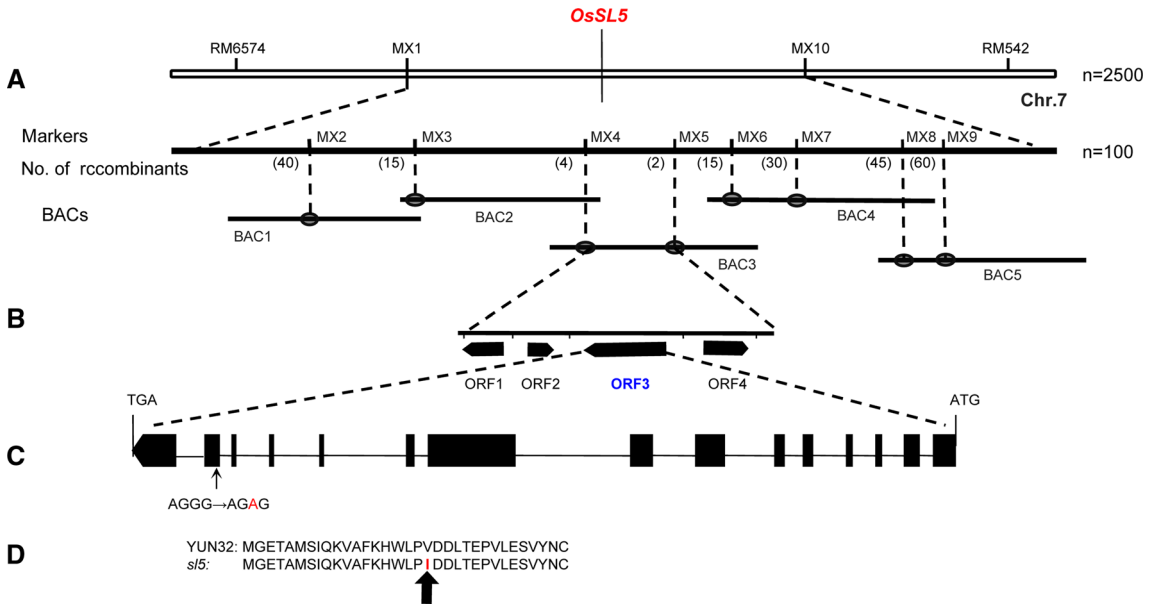

Fig. 3 Mutant *OsSL5* gene positioning, and the confirmation of the selected gene. **a** Fine positioning of the *OsSL5* gene; **b** ORF prediction in the target interval; **c** structure and mutant sites of the *OsSL5* candidate gene; **d** difference between YUN32 and *sl5* amino acids. *Black arrows* represent base mutant site; *thick black arrows* show the difference in amino acids

Table 6 Candidate ORFs identified in the 40 kb delimited interval on the BAC contig AP004273

Candidate ORFs	Gene description
ORF1	S1 RNA binding domain containing protein, expressed
ORF2	Putative uncharacterized protein
ORF3	Putative uncharacterized protein
ORF4	Splicing factor 3B subunit 3

Candidate gene sequencing analysis

The *OsSL5* and *SPL5* genes were sequenced to determine whether the spots on *sl5* leaves are caused by the *SPL5* gene. The *SPL5* gene is 12,900 bp in length, including 15 exons and 14 introns (Fig. 3c). The *sl5* and YUN32 were sequenced for *SPL5* gene using eleven sequencing primers (Table 2) and compared. The CDS of *SPL5* is 4,068 bp in length. The sequencing results showed that the G in the 3,647 position of the *SPL5* coding region in *sl5* was changed to A (Fig. 3c), thereby altering the amino acid sequence (Fig. 3d). Several *japonica* and *indica* rice varieties were sequenced to further ascertain whether the base substitution leads to leaf spots. No mutation sites were found in these rice varieties. The other three candidate genes in this region were further sequenced. The results did not reveal any difference in *SPL5* between the wild-type (YUN32) and the *sl5* mutant. The cDNA sequence of wild type and *sl5* amplified by primer X12 was also compared (Table 2) and showed no difference.

Analysis of spl-related gene expression

The *sl5* mutant seedling leaf showed a spot-like characteristic with a dry tip. To determine the relationship between the *sl5* mutant gene and other spl-related genes, we conducted real-time fluorescence QPCR and compared the expression levels of *spl*-related genes in the *sl5* mutant and the wild-type. The following genes were analyzed: *SPL7* (heat stress transcription factor), *RLIN1* (coproporphyrin original III oxidase), *SPL28* (clathrin-associated protein compound), *SPL18* (acyltransferase), *SPL11* (E3 ubiquitin ligase), and *SL* (cytochrome P450 monooxygenase). The result showed that the *OsSL5* expression in the mutant was significantly lower than that in the wild-type. The *SPL7* and *SL* gene expressions were significantly increased. By contrast, the *SPL28*, *RLIN1* and *SPL18* gene expressions significantly decreased, but the *SPL11* expression remained unchanged (Fig. 4). The change in the expression of *OsSL5* in the mutant may affect the expression of other related genes. A significant difference was observed between *OsSL5* and other *spl*-related gene expressions. This difference may affect *spl* related gene regulation in rice.

Discussion

Spl mutants are important in plant apoptosis and disease resistance. This study shows that the death of the *spl* mutant is related to ROS, as observed in various mutants, including

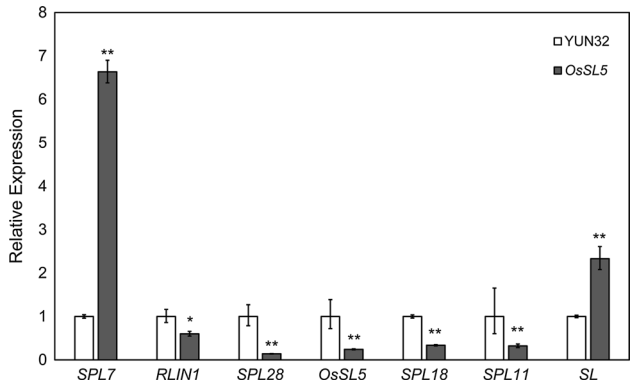

Fig. 4 QPCR analysis of the spotted leaf-related gene expression in wild-type YUN32 and *sl5* mutant. *Difference is significant at the 0.05 level. **Difference is significant at the 0.01 level

spl2 and *spl7* in rice (Yoshimura et al. 1997; Yamanouchi et al. 2002); *lsd1* (Wang et al. 2005) and *acd2* (Mach et al. 2001) in *Arabidopsis*; and *les22* (Hu et al. 1998) and *lls1* (Gray et al. 1997) in maize. Plants develop a radical scavenging system through evolution. This system maintains ROS levels under relatively stable conditions. Metabolic disorders occur if this balance is disrupted. High-content ROS highly oxidizes the cell membrane, thereby affecting cell permeability. Spot-like characteristic was observed on the leaves. Extremely high-content ROS eventually causes cell death. Nevertheless, the disease resistance of *spl* plants is enhanced to a certain degree; for example, *spl11* enables rice to exhibit significant resistance to rice blast and bacterial blight (Yin et al. 2000). *Sl* (*spl1*) and *spl28* affect rice blast and bacterial blight in rice (Mizobuchi et al. 2002). Wu et al. (2008) mutated rice IR64 by using butane, γ-ray, and fast neutrons to obtain 11 new *spl* mutants with different phenotypes. Spots appeared on the leaf and sheath in the two-leaf stage of the *spl1-2* mutant. These spots increased in size afterward. Spots were also observed on the glume and decreased the 1,000-grain weight. Dark brown spots appeared on the tip of the leaf of *Spl3-2* 3 weeks after seeding, and yellow rust spots were found on other parts of the leaf. Yellow strip spots were detected on the leaf vein of *spl6-2* 6 weeks after seeding. After 2 months, the spots became longer with distinct phenotype. Small and discontinuous white spots appeared on the *spl20* leaf 2 weeks after seeding until the tillering stage. Eleven mutants were inoculated with filamentous fungi. Among these mutants, five exhibited resistance. The *spl17*, *spl26*, *spl20*, and G862 mutants are resistant to rice blast fungus, particularly Ca89 and P06-6. The *spl17* and *spl26* mutants are also resistant to bacterial blight, which affects rice disease-resistant. Therefore, the *spl* gene is important in the theoretical research and disease resistance breeding.

In this study, the *OsSL5* gene encoding for SF3b3 belongs to the SF3b3 splicing family. However, homologous genes in plants have yet to be determined. Other homologous genes have been found in fungi and animals. For example, the homologous gene *prp12-1* in yeast and animals is involved in splicing and cell differentiation (Habara et al. 2001). Yamasaki et al. (2008) found that SF3b3 can form an acceptor by combining with a C-type lectin taken from macrophages to induce non-steady apoptosis. Inflammatory cells, such as neutrophils, are then generated and enter damaged tissues to mediate cell death. Menon et al. (2008) reported that SF3b3 is related to cullin-RING E3 ubiquitin ligase and stabilizes genomes during the cell cycle. Kerins et al. (2010) isolated the *SF3b3* homologous gene *TEG-4* in nematodes, which positively regulates the GLP-1 signal transmission downstream pathway and controls embryonic stem cell proliferation and meiosis. The *SF3b3* gene in mice significantly down-regulates the gene that may induce apoptosis of early sac cells in the embryonic stem cell sac (Jincho et al. 2008). In this study, the *sl5* mutant initially generated spots on the leaf tip in the seeding stage. These spots spread to the whole leaf during growth. The spots further increased in size and number at the late stage of growth. This finding indicates that the *OsSL5* gene could control cell apoptosis. The plant height, spike length, primary branch number, and secondary branch number of the *sl5* mutant were significantly lower than those of the wild type. The most significant observation in this study is the decrease in the secondary branch number. Spotted leaf formation and changes in several parameters were complicated because of various factors. These factors include the interactions and relationships between genes and the single or multiple pathways involved in signal transduction. The *OsSL5* gene is an allelic *SPL5* gene (Chen et al. 2012). However, the severity of the spots in the *sl5* mutant was less than that in *spl5*. This condition may be attributed to the A-to-G single base substitution in the *OsSL5* gene encoding region, which slightly affected the amino acid sequence. The *SPL5* encoding region lacks the base G, and this insufficiency terminates protein translation at early stages. Therefore, *SPL5* may participate in cell apoptosis regulation. QPCR results show that the *PR1* gene (Yin et al. 2000) is highly expressed in *spl5* and *spl1* (*sl*) mutants. The *PR1* gene induces and activates PBZ1 protein expression in the nucleus after rice is infected with rice blast. This report indicates that the *OsSL5* gene induces rice blast resistance. Studies have also shown that *SPL5* regulates precursor mRNA splicing, amino acid metabolism, photosynthesis, glycolysis, ROS metabolism, and stress resistance (Chen et al. 2013). However, the specific regulatory mechanism of *SPL5* splicing remains unclear. The *sl5* is a new weak allelic mutant in the background *Japonica* rice of the *SPL5* gene from *indica* rice (Chen et al. 2012). Further studies on mutants in different subspecies backgrounds will elucidate the molecular mechanism of rice *spl* formation.

Conclusion

A spotted leaf mutant *sl5*, which exhibits more spots at maturity, was successfully isolated in this study. Genetic analysis shows that the mutant is controlled by a single recessive gene, and the allelic test shows that the *OsSL5* gene is allelic to *SPL5*. The *OsSL5* gene was determined to be anchored on chromosome 7 through map-based cloning, and the mapping interval was narrowed to 40 kb by using the most recently designed markers. The candidate genes were amplified, and the sequences were aligned with that of the wild type. The *OsSL5* gene was finally cloned at a mutation site different from that of the *SPL5* mutant gene. The QPCR analysis of the related spotted-leaf genes in the *OsSL5* mutant plant reveals complex connection between these genes.

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