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ORIGINAL PAPER

Characterization and fine mapping of the rice premature senescence mutant *ospse1*

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Abstract Premature senescence can limit crop productivity by limiting the growth phase. In the present study, a spontaneous premature senescence mutant was identified in rice (*Oryza sativa* L.). Genetic analysis revealed that the premature senescence phenotype was controlled by a recessive mutation, which we named *Oryza sativa premature senescence1* (*ospse1*). The *ospse1* mutants showed premature leaf senescence from the booting stage and exhibited more severe symptoms during reproductive and ripening stages. Key yield-related agronomic traits such as 1,000-grain weight and seed-setting rate, but not panicle grain number, were significantly reduced in *ospse1* plants. Chlorophyll content, net photosynthetic rate, and transpiration rate of *ospse1* flag leaves were similar to the wild-type plants in vegetative stages, but these parameters decreased steeply in the mutant after the heading stage. Consistent with this, the senescence-associated genes *OsNYC1* and *OsSgr* were up-regulated in *ospse1* mutant during premature leaf senescence. The *ospse1* locus was mapped to a 38-kb region on chromosome 1 and sequence analysis of this region identified a single-nucleotide deletion in the 3′ region of an open reading frame (ORF) encoding a putative pectate lyase, leading to a frame shift and a longer ORF. Our results suggested that the premature senescence of the *ospse1* may be regulated by a novel mechanism mediated by pectate lyase.

Abbreviations

ABA	Abscisic acid
CC	Chlorophyll content
MeJA	Methyl jasmonate acid
NPR	Net photosynthetic rate
ORF	Open reading frame
SAG	Senescence-associated gene
SPAD	Soil plant analysis development
TR	Transpiration rate
qRT-PCR	Quantitative reverse transcription polymerase chain reaction

Introduction

Senescence is the final stage of leaf development encompassing the period from maturation to death. Leaf senescence is a genetically regulated, highly ordered process controlled by developmental stage. However, leaf senescence is also affected by complex interactions between endogenous and environmental signals (Buchanan-Wollaston 1997; Gan and Amasino 1997; Nam 1997). During the senescence, leaves lose their ability to conduct

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photosynthesis and anabolism, while the catabolism of chlorophylls, lipids, proteins, and nucleic acids becomes dominant. Released leaf nutrients are mobilized and recycled to other tissues or organs, such as seeds, storage organs or developing leaves and flowers (Buchanan-Wollaston 1997; Thomas and Howarth 2000). Therefore, leaf senescence is an altruistic process contributing to the fitness of the whole plant by ensuring optimal production of offspring and better survival of plants in their temporal and environmental conditions (Lim et al. 2007).

Premature senescence is an undesirable agronomic trait, decreasing crop productivity potential by reducing the crop growth phase. In rice (*Oryza sativa* L.), premature senescence appears to be common in a number of high-yielding *indica* cultivars and *indica–japonica* hybrids (Duan et al. 1997); premature leaf senescence during reproductive development and grain filling stages is directly related to reduction of biomass and grain yield (Ray et al. 1983). Similar examples have been shown under abiotic stresses in many crops, such as sorghum (Borrell et al. 2000a, b), maize (Bänziger et al. 1999) and durum wheat (Benbella and Paulsen 1998; Hafsi et al. 2000). In contrast, appropriate timing of leaf senescence maintains high photosynthetic capacity and increases crop yield (Gentinetta et al. 1986; Thomas and Howarth 2000).

A number of senescence-associated genes (SAGs) have been cloned through genetic and genomic approaches from various plant species, including barley, tomato, *Brassica napus, Arabidopsis,* asparagus, maize, rice and radish (Ay et al. 2008; Drake et al. 1996; Gombert et al. 2006; King et al. 1995; Morquecho-Contreras et al. 2010; Ori et al. 1999; Sato et al. 2009; Shimada et al. 1998). SAGs can be grouped into three major categories according to their regulatory functions in senescence: (1) phytohormone responsive elements: most of the phytohormone pathways are involved in leaf senescence; for example, ethylene accelerates senescence, but cytokinin delays senescence. The genes involved in hormone signaling pathways are an important part of the regulation of SAGs. For example, the expression of *isopentenyl transferase* (*IPT*), which is involved in cytokinin biosynthesis, can effectively delay leaf senescence (Gan and Amasino 1995; McCabe et al. 2001; Robson et al. 2004). AHK3, one of the three cytokinin receptors in *Arabidopsis*, plays a major role in controlling cytokinin-mediated leaf longevity (Kim et al. 2006). *OsDOS*, *RPK1*, *ARF2*, and *EDR1*, which are involved in jasmonic acid (JA), abscisic acid (ABA), auxin, and ethylene signaling pathways, also act during leaf senescence (Kong et al. 2006; Lee et al. 2011; Okushima et al. 2005; Tang et al. 2005). (2) Transcription factors: many transcription factors involved in the regulation of leaf senescence have been identified by suppression subtractive hybridization or microarray analysis (Buchanan-Wollaston et al. 2005; Liu et al. 2008; Sperotto et al. 2009). For example, *ORS1*, *AtNAP* and *Gpc-B1*, encoding NAC family transcription factors, play important roles in controlling leaf senescence in *Arabidopsis* and wheat (Balazadeh et al. 2011; Guo and Gan 2006; Uauy et al. 2006). Transcription factors WRKY53 and WRKY6 are involved in regulating leaf senescence in *Arabidopsis* (Hinderhofer and Zentgraf 2001; Miao et al. 2004; Robatzek and Somssich 2001). (3) Degradation-related proteins: for instance, ORE9, an F-Box protein, may play a role in senescence through a proteolysis pathway in *Arabidopsis* (Woo et al. 2001), and NYC1, OsAkαGal and Sgr are involved in the degradation of chlorophyll and/or thylakoid membrane during rice senescence (Kusaba et al. 2007; Lee et al. 2009; Park et al. 2007).

In addition to molecular strategies that have identified senescence-associated genes, genetic strategies have identified many mutants with phenotypes affecting senescence. In rice, several loci conferring premature senescence have been mapped. For example, *Pse(t)*, identified from a T-DNA insertion mutant library, is located on the long arm of chromosome 7 (Li et al. 2005), the recessive mutant *pls1* is located on chromosome 2 (Wang et al. 2006), and the dominant mutant *PSL3* is located on chromosome 7 (Fang et al. 2010). A recessive mutant in *SMS1* on chromosome 8 affects both early senecence and male sterility in rice (Yan et al. 2010). However, no genes from these loci have been isolated and the molecular regulatory network for early leaf senescence in rice remains largely unknown.

Here, we report the characterization of a novel spontaneous premature-senescence mutant, *ospse1*, in rice. In this mutant, premature senescence substantially affects agronomic traits. We use genetic analysis and mapping to reveal that *ospse1* is a single recessive gene on the long arm of chromosome 1. Sequence analysis of the *ospse1* region identified a single-nucleotide deletion in a gene encoding a putative pectate lyase. These results provide the basis for the isolation of this novel premature senescence gene and the elucidation of a senescence mechanism likely mediated by pectate lyase.

Materials and methods

Plant materials

The spontaneous premature senescence mutant *ospse1* was derived from an *indica* rice line CW312. Two F_2 populations derived from crossing *ospse1* to CW312 and Nipponbare (*japonica* cultivar) were used for the genetic analysis and molecular mapping of *ospse1*. All the plants were grown at the experimental station of South China Agricultural University in Guangzhou, China.

Chlorophyll content measurement

The samples for chlorophyll content measurement were taken from the tip of flag leaves. Samples of 100 mg leaf disk tissue were soaked in 20 ml 80 % acetone for 16 h in the dark until the disks became colorless. Chlorophyll concentration was measured as described previously (Yang et al. 1998).

Measurements of photosynthesis and transpiration

Net photosynthetic rate (NPR) and transpiration rate (TR) in the field were measured with a LI-6400 portable photosynthesis system (Li-Cor, USA). Artificial light of 1,200 μmol m^{-2} s^{-1} inside the leaf chamber was employed to ensure consistency in the measurements. All measurements, in the widest part of the leaves, were carried out between 9:00 a.m and 12:00 noon, according to the manufacturer's instructions. Means and standard deviations were obtained from five replicates.

Observation and measurement of chlorophyll in detached leaves with hormone and stress treatments

The youngest fully expanded leaves were detached and incubated in 3 mM MES buffer (pH 5.8) at 25 °C. A concentration of 50 mM ABA or methyl jasmonate (MeJA) was added to the MES solution for phytohormone treatments. The degree of leaf greenness was measured using a Minolta Chlorophyll Meter SPAD-502 (Minolta Camera Co., Japan).

RNA isolation and RT-PCR

The mRNA samples were extracted using TRIzol (Invitrogen, Carlsbad, CA, USA) as described by the manufacturer. The quantification of total mRNA was done spectrophotometrically at 260 nm, and mRNA was treated with DNase I according to the manufacturer's instructions (Invitrogen, USA). The mRNAs (1.5 μg each sample) were used to synthesize first-strand cDNA with an oligo (dT) primer in 20 μl reaction volume using SuperScript II (Invitrogen, USA). PCR was performed in a total volume of 20 μl with 1 μl of the RT products, 0.4 μm gene-specific primers (Table S1), and 1 unit of *ExTaq* (TaKaRa, Japan). The PCR was conducted with 28–34 cycles of 94 °C for 30 s, 56 °C for 30 s and 72 °C for 1 min. The PCR products were resolved by electrophoresis on 1.2 % agarose gels and visualized with Universal Hood II (Bio-Rad, USA).

To test the expression profiles of two SAG*s* in the wild type (WT) and the mutant plants, leaf samples were collected every 5 days from the heading day. The qRT-PCR was conducted with gene-specific primers (Bio-Rad, USA). The PCRs were carried out with 30 cycles of 94 °C for 15 s, 58 °C for 60 s, 72 °C for 30 s.

Genetic analysis and molecular mapping

The segregation ratio of premature senescence phenotype (at the heading stage) in the F_2 plants from the cross of *ospse1*/CW312 was analyzed using Excel software for Windows.

For genetic mapping of the *ospse1* locus, we developed InDel (insertion/deletion) markers based on the sequence difference between the *japonica* variety Nipponbare and the *indica* variety 93–11. The primers used for genetic mapping are listed in Table S1.

For primary genetic mapping, 135 F_2 individuals with the premature senescence phenotype were selected from the cross of *ospse1*/Nipponbare. Two molecular markers flanking the primary target region were used to screen recombination events from 2,976 F_2 individuals for fine mapping. F_3 progenies of some key F_2 recombinants were further examined to confirm the phenotype and marker genotypes.

Sequence analysis

Gene prediction analysis was performed with the programs rice genome automated annotation system (RiceGAAS) (http://www.ricegaas.dna.affrc.go.jp). The 38-kb region defined by the two flanking markers M20290 and J14-3 was amplified as a set of overlapping fragments from genomic DNAs of WT and *ospse1* plants by PCR and the fragments were subcloned into the TA Vector (Promega, USA) for sequencing.

Results

Phenotypic and physiological characterization of *ospse1* plants

When *ospse1* and WT plants were grown in the field, no clear phenotypic differences were observed at vegetative stages, except that the *ospse1* plants showed weaker growth and fewer tillers as compared to WT plants (Fig. 1a, b). The heading dates of *ospse1* and WT plants are the same. The premature leaf senescence phenotypes of *ospse1,* such as yellowing leaves, were observed in older leaves from the late booting stage onwards (Fig. 1a, b). At the heading and milk stages in *ospse1*, only the flag leaf and the penultimate leaf (2L) remained green and other leaves such as the third (3L) and the fourth (4L) from the top became yellow or dried. In contrast, the 3L and 4L leaves of WT CW312 remained green and healthy at the same stages (Fig. 1c).

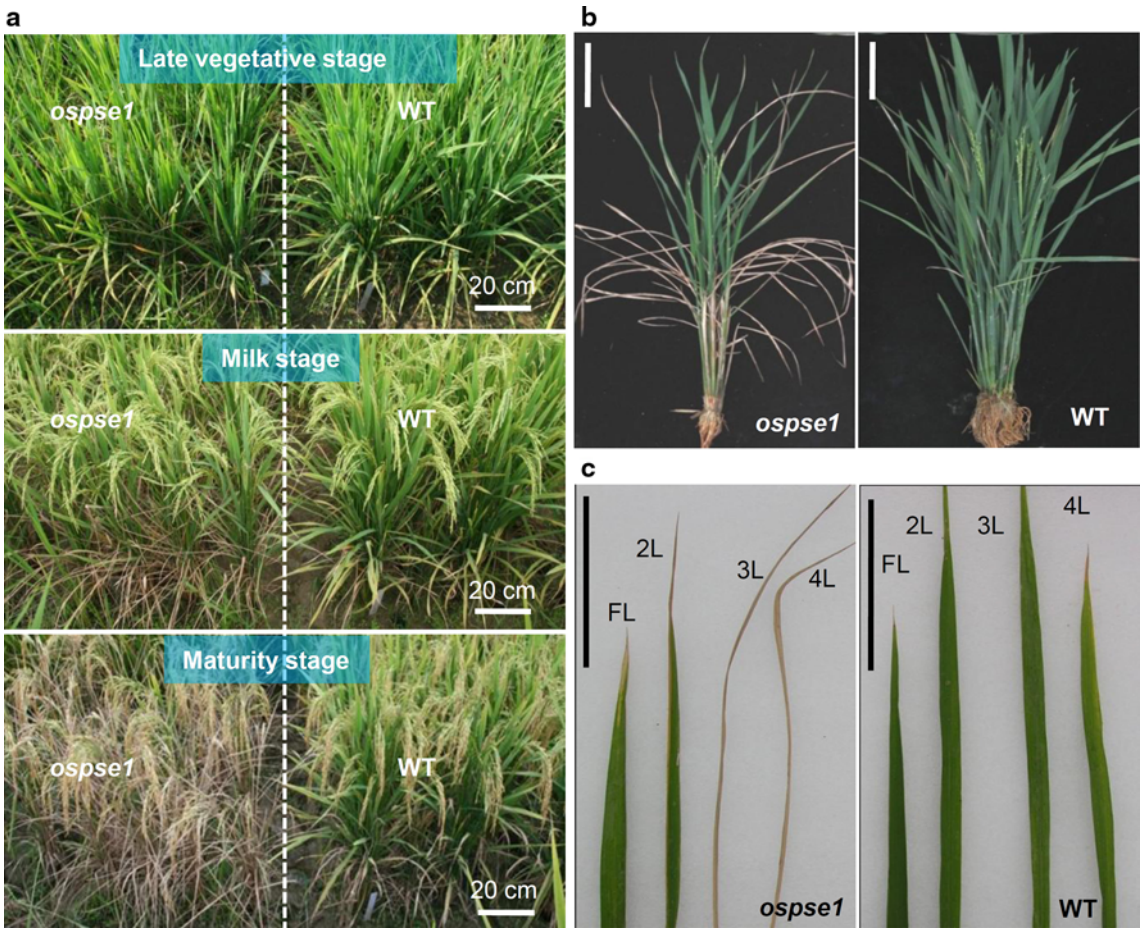

Fig. 1 Phenotypic comparison of wild type (WT) and *ospse1* mutant plants. **a** Phenotype of the WT and *ospse1* mutant plants in the field, at different stages. **b** The *ospse1* mutant plants show premature senescence symptoms as compared to WT plants at heading stage. **c** Morphology of the four leaves from the *top* at heading stage. *FL* flag leaf, *2L* penultimate leaf, *3L* antepenultimate leaf, *4L* the fourth leaf from the *top*. *Bars* 10 cm

We also investigated other yield-related agronomic traits of *ospse1* plants. Pollen fertility was similar in WT and *ospse1* plants (Fig. 2a), but the grain size of *ospse1* plants was smaller and thinner (Fig. 2b). The plant height, the seed-setting rate and the 1,000-grain weight of the *ospse1* plants was reduced significantly compared with WT plants (Fig. 2c–e), but the grain number of each panicle was not affected by *ospse1* (Fig. 2f).

The loss of chlorophyll is a typical symptom of leaf senescence, and results in leaf yellowing (Oh et al. 1997). Thus, we compared the changes in chlorophyll content (CC) over time between *ospse1* and CW312. The chlorophyll content was similar in the flag leaves of *ospse1* and CW312 on the day of heading, but it rapidly decreased during the grain filling process. After 21 days of heading, CC in the flag leaves of *ospse1* was hardly detectable, whereas that of CW312 was reduced by only about 25 % (Fig. 3a). Consistent with the loss of chlorophyll, the NPR and the transpiration rate (TR) in the flag leaves of *ospse1* plants also decreased rapidly (Fig. 3b, c).

Expression of senescence-associated genes in *ospse1* plants

To test the expression profiles of SAGs in the WT and mutant plants, leaf samples were collected every 5 days starting from the heading day (0 day after heading, DAH). The expression of two SAGs, *OsNYC1* and *OsSgr*, were monitored by qRT-PCR with gene-specific primers (Table S1). The results showed that these two genes were up-regulated in *ospse1* plants during senescence (Fig. 4).

Senescence of detached leaves in response to hormones and stresses

Darkness and ABA are known inducers of leaf senescence (Kusaba et al. 2007; Woo et al. 2001). To test if these factors affect leaf senescence in *ospse1,* detached leaves of *ospse1* and WT plants were treated with continuous light or dark incubation, ABA and MeJA; senescence symptoms were monitored with an SPAD chlorophyll meter. The

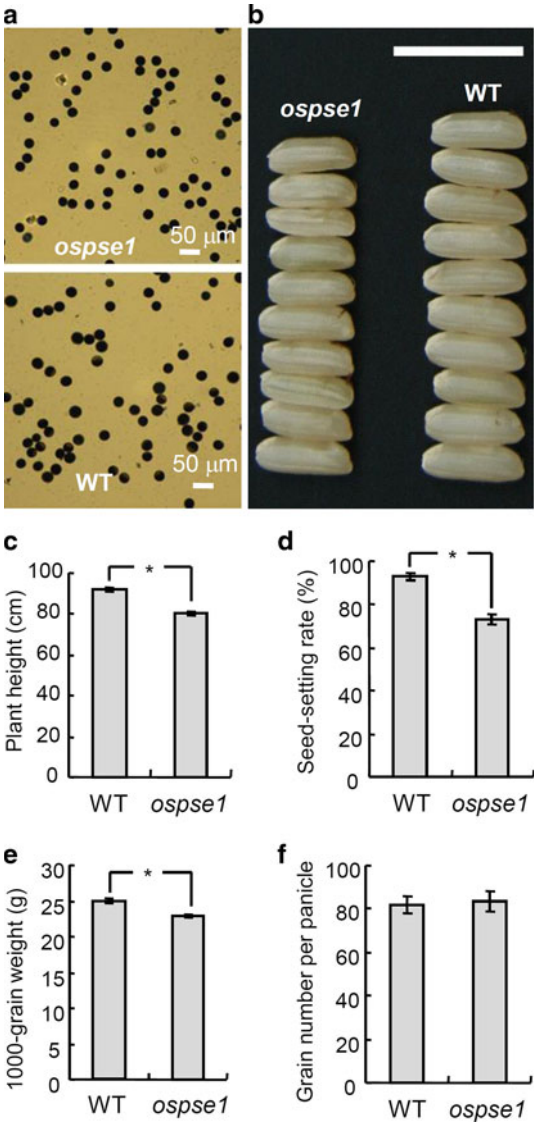

Fig. 2 Comparison of six agronomic traits in WT and *ospse1* plants. **a** Pollen fertility, the pollen grains were stained with 1 % I_2-KI solution, which stains viable pollen *dark purple*. *Bars* 50 μm. **b** Grain size, *Bars* 1 cm. **c** Plant height. **d** Seed setting rate. **e** 1,000-grain weight. **f** Grain number per panicle. *Asterisk* indicates significance at $P < 0.05$ by a *t* test. Data shown are mean ± SE, $n = 8$ (color figure online)

results showed that senescence of detached leaves tended to be faster under dark incubation, ABA and MeJA treatment conditions than that of continuous light incubation (Fig. 5). However, no obvious difference was observed between *ospse1* and WT plants under each condition (Fig. 5).

Genetic analysis of *ospse1*

The F_1 plants derived from crosses of *ospse1*/CW312 and *ospse1*/Nipponbare exhibited WT phenotypes, suggesting that the mutant trait is recessive. The genetic segregation in

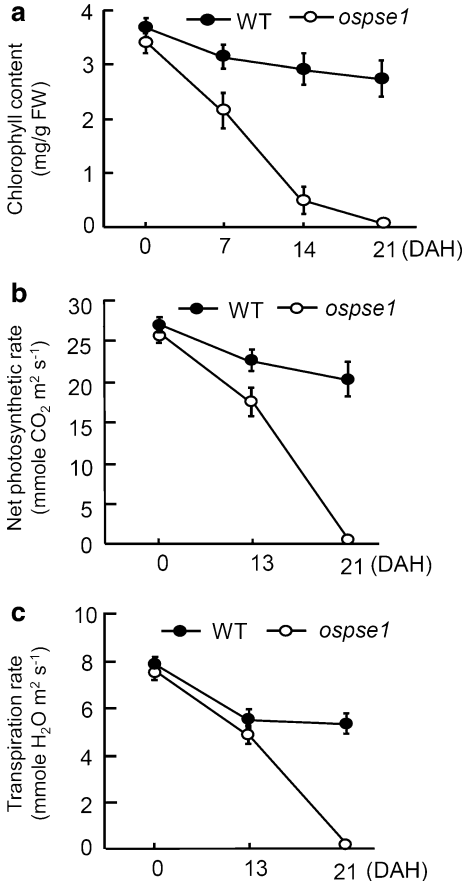

Fig. 3 Physiological characterization of WT and *ospse1* plants after heading. **a** Changes in chlorophyll content in the flag leaf. **b** Net photosynthetic rate in the flag leaf. **c** Transpiration rate in the flag leaf. Data shown are mean ± SD, $n = 5$. DAH day after heading

the F_2 population from the *ospse1*/CW312 cross was 91 *ospse1* to 332 WT plants, which fitted the expected ratio of 3:1 ($\chi_{3:1} = 2.73$), indicating that *ospse1* is controlled by a recessive gene.

Genetic mapping of *ospse1*

To investigate the molecular basis for premature senescence in the mutant, we tried to identify the *ospse1* gene using map-based cloning approaches. First, 33 F_2 plants with the *ospse1* phenotype from the *ospse1*/Nipponbare cross were used to screen markers distributed throughout the rice genome to identify linked markers. Two insertion/deletion (InDel) markers C094-148, C123-3 located on chromosome 1 co-segregated with the *ospse1* phenotype (Table S1). Next, several additional InDel markers in this chromosome region were developed and used to survey 135 F_2 *ospse1* plants. By this primary mapping, the *ospse1* locus was mapped to the interval between two InDel markers, H17-1 and P21-4, with genetic distances of 0.4 and 3 cm to these two markers, respectively (Fig. 6a).

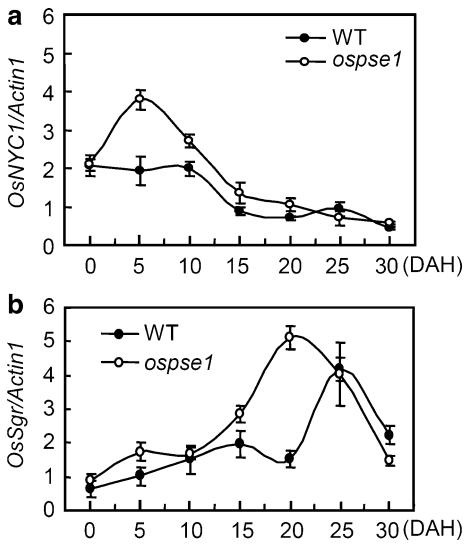

Fig. 4 Expression of senescence-associated genes in *ospse1* plants. Two SAGs, *OsNYC1* (**a**) and *OsSgr* (**b**) were selected for expression profiling in the WT plants and mutants during reproductive and ripening stages. Flag leaf samples were collected every 5 days starting from the heading day. Data are normalized to *Actin1* levels and shown as mean ± SD ($n = 3$). DAH day after heading

To fine-map the *ospse1* gene, a population of 2,976 F_2 plants was used to screen for recombinants with markers H17-1 and P21-4, and 28 recombinants were identified (Fig. 6b). The genotypes of these recombinants were determined by phenotyping of their F_3 progenies. By comparing the PAC/BAC sequences located between H17-1 and P21-4 of *japonica* variety Nipponbare to the sequences of *indica* variety 93-11, we identified seven additional InDel markers in this region. These markers were used to survey the recombinant individuals. Finally, *ospse1* was mapped to a 38-kb region between M20290 and J14-3 (Fig. 6b).

Candidate genes for *ospse1*

Within the mapped region of *ospse1,* which was covered by the sequenced BAC clone OSJNBa0026J14, ten putative open reading frames (ORFs) were predicted by the rice genome automated annotation system (RiceGAAS) (http://www.ricegaas.dna.affrc.go.jp). Out of the ten ORFs, five ORFs (*ORF1*, *ORF4*, *ORF5*, *ORF8* and *ORF10*) are supported by the presence of cDNA sequences in the database, but most of the others are very small, predicted ORFs without cDNA hits (Fig. 6c; Table 1).

Expression analysis of *ospse1* candidate genes

To investigate the expression of the five candidate *ORFs* (*ORF1*, *ORF4*, *ORF5*, *ORF8* and *ORF10*) in different organs, we performed RT-PCR assays to examine gene expression in root, stem and leaf in the WT and mutant plants. The results showed that *ORF1* and *ORF4* were mainly expressed in the leaf and the stem, *ORF8* and *ORF10* were mainly expressed in the leaf, and *ORF5* was expressed in the root, the stem and the leaf. There was no obvious expression change in the leaves between WT and mutant plants (Fig. 7a).

Sequencing analysis of *ospse1* candidate genes

To identify the mutation causing *ospse1*, the 38-kb region containing the ten *ORFs* was amplified from genomic DNAs of WT and *ospse1* plants by PCR and sequenced. Only one change, a nucleotide (C) deletion in *ORF5*, was identified in the *ospse1* mutant (Fig. 7b). This single-nucleotide deletion is present in the 3′ region of *ORF5* encoding a putative pectate lyase, leading to a frame shift and a longer ORF (Fig. 7b). Wild-type *ORF5* encodes a protein of 364 amino acids (AA), while the mutant consists of 396 aa (Figs. S1a).

Expression of *ORF5* during senescence in *ospse1* plants

To understand the possible role of *ORF5* in premature senescence, we examined the transcriptional level of *ORF5* during the senescence in the wild type and *ospse1* plants. The results showed that the *ORF5* slightly responded to the senescence, and there is no remarkable difference between wild type and *ospse1* mutant plants in this process (Fig. 7d).

Discussion

The premature leaf senescence of *ospse1* mutant may start from the early booting stage and accelerate significantly from the late booting stage onwards (Fig. 1). Genetic analysis revealed that a single recessive nuclear gene controls this mutation. By molecular mapping, the mutant locus was localized to a 38-kb region of chromosome 1; no known SAG has been reported in this region. The results suggest that *ospse1* is a newly identified gene associated with leaf senescence in rice.

Before the appearance of premature senescence symptoms in the flag leaves (at the initial heading stage), the major physiological characteristics of *ospse1*, such as CC, NPR, and TR are similar to WT plants. However, accompanying the onset of senescence, CC, NPR, and TR rapidly decreased in *ospse1* plants (Fig. 3). These observations demonstrated that the function of *ospse1* leaves is normal in the vegetative development stages, but that premature senescence leads to a rapid loss of function in *ospse1* leaves.

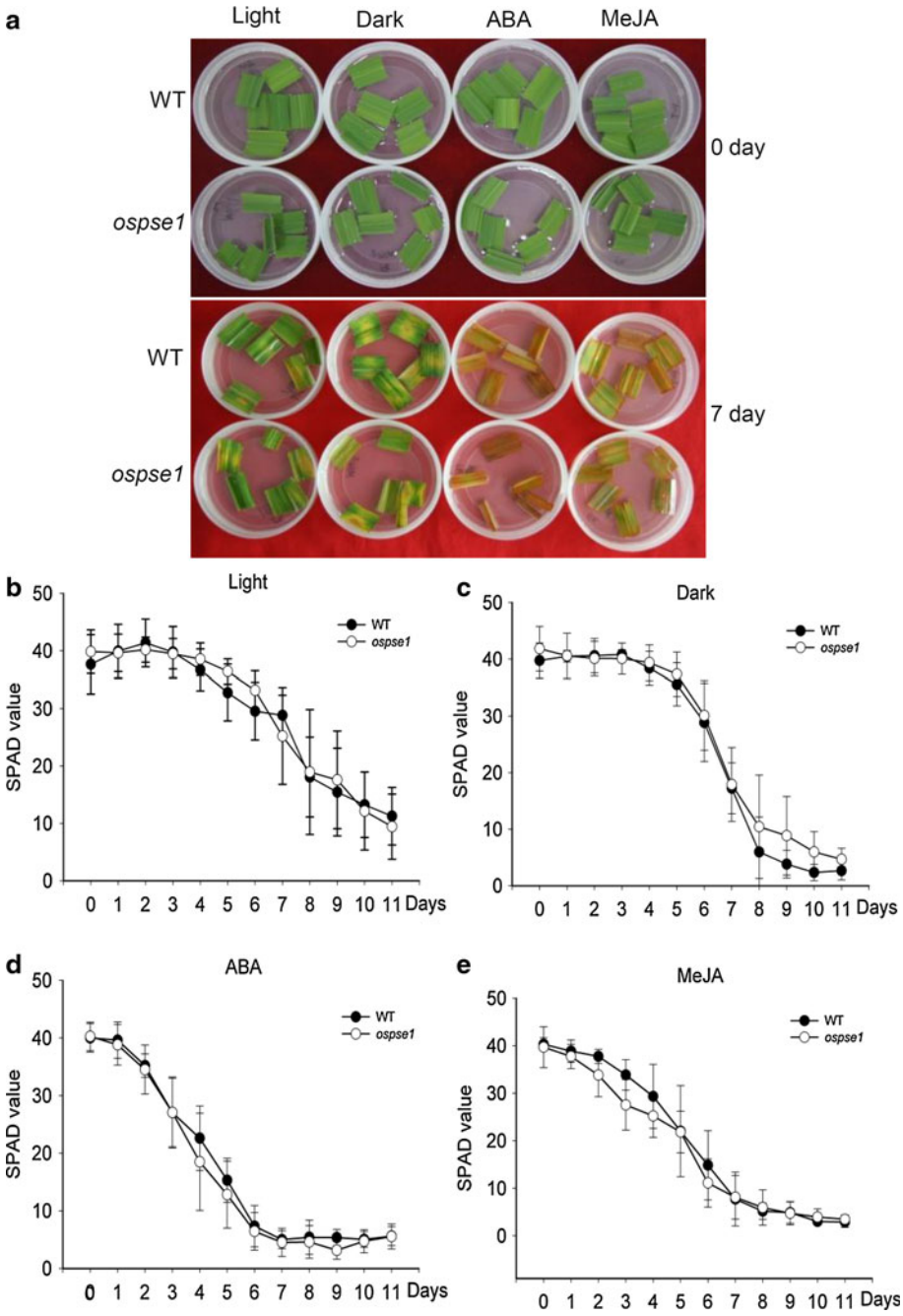

Fig. 5 Senescence of detached leaves in response to hormones and stresses. **a** Phenotype of detached leaves before and after 7-day hormone or stress treatments. **b–e** Chlorophyll content of detached leaves of WT and *ospse1* was measured by the soil plant analysis development (SPAD) value after treatment with continuous light, or dark incubation, ABA and MeJA, respectively. Data shown are mean ± SD, $n = 5$

Bioinformatic analysis indicated that there are ten putative ORFs within the mapped region. However, *ORFs 2*, *3*, *6*, *7*, *9* have no cDNA hits in the database and most of them are very short, suggesting that these five ORFs are not expressed. Consistent with this prediction, no expression was detected for any of these short ORFs in leaf tissue according to a public microarray database (http://www.crep.ncpgr.cn) (Ray et al. 1983; Wang et al. 2010), supporting that these short ORFs could be excluded as candidates for *ospse1*.

In contrast, *ORF1*, *4*, *5*, *8*, and *10* have cDNA hits in the database and were investigated as candidate genes for *ospse1*. We confirmed that all five of the *ospse1* candidate ORFs are expressed in leaf tissue (Fig. 7a). It is well known that the expression of many SAGs is induced by senescence in plants. For example, *SAG101* and *AtNAP*, which encoded an acyl hydrolase and an NAC family transcription factor, respectively, were highly induced during leaf senescence in *Arabidopsis* (Guo and Gan 2006; He and Gan 2002). In our experiments, SAGs such as

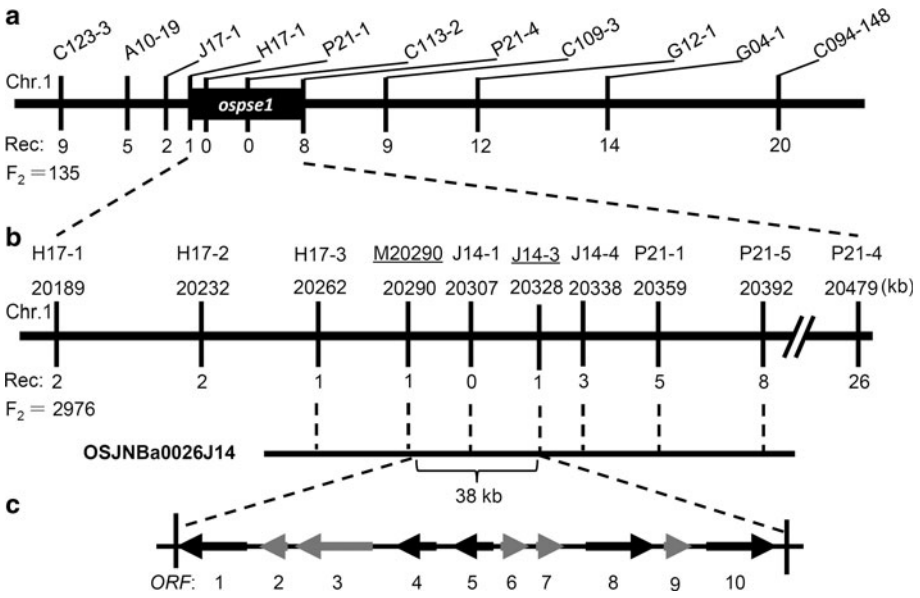

Fig. 6 Mapping of the *ospse1* locus on chromosome 1. **a** Primary genetic mapping using 135 F_2*ospse1* plants. Names starting with *letters* are InDel markers. *Rec* number of recombinants. **b** Fine physical mapping of *ospse1* using 28 recombinants identified from 2,976 F_2 plants by the markers H17-1 and P21-4. *Numbers* (kb) below InDel markers indicate the physical position of the markers on chromosome 1. **c** Predicted ORFs in the mapped *ospse1* region. *Arrows* indicate the order and orientation of ten ORFs within the BAC clone OsJNBa0026J14. ORFs in *black* have corresponding cDNA(s) in the database, ORFs in *grey* show no hits for cDNA or protein

Table 1 Predicted genes in the mapped *ospse1* region (38 kb)

ORF	Gene name	Size (bp)	cDNA	Predicted function	Sequencing
ORF1	Predgene11 (*Os01g0546400*)	1,035	AK058673	Putative nodulin MtN21 family protein	Identical
ORF2	Predgene12	180	ND	Hypothetical protein	Identical
ORF3	Predgene13	1,110	ND	Putative nodulin MtN21 family protein	Identical
ORF4	Predgene14 (*Os01g0546500*)	1,029	AK069340	Putative pentatrico peptide repeat-containing protein	Identical
ORF5	Predgene15 (*Os01g0546800*)	1,095	AK066636	Putative pectate lyase family protein	Deletion
ORF6	Predgene16	438	ND	Hypothetical protein	Identical
ORF6	Predgene17	465	ND	Hypothetical protein	Identical
ORF8	Predgene18 (*Os01g0546900*)	1,188	AK073801	Putative transcription factor jumonji (jmjC) domain-containing protein	Identical
ORF9	Predgene19	384	ND	Hypothetical protein	Identical
ORF10	Predgene20 (*Os01g0547000*)	2,718	AK121218	Putative LRR14	Identical

Sequence analysis was conducted with amplified *ORFs* from the gDNA of the wild type and mutant leaves

ND no data available in the database

OsNYC1 and *OsSgr* were up-regulated in *ospse1* during senescence (Fig. 4). However, the transcriptional levels of five *ospse1* candidate genes were not obviously changed between the WT and mutant plants (Fig. 7a), suggesting that the mutation of *ospse1* may not affect its promoter activity or RNA stability.

Sequence analysis of the 38-kb *ospse1* region containing 10 candidate genes identified a single nucleotide deletion in *ORF5* (Table 1). *ORF5* encodes a member of the pectate lyase family. Our results showed that the mutation in *ospse1* does not alter the response to the dark and the tested phytohormones ABA and JA (Fig. 5). Unlike tested SAGs

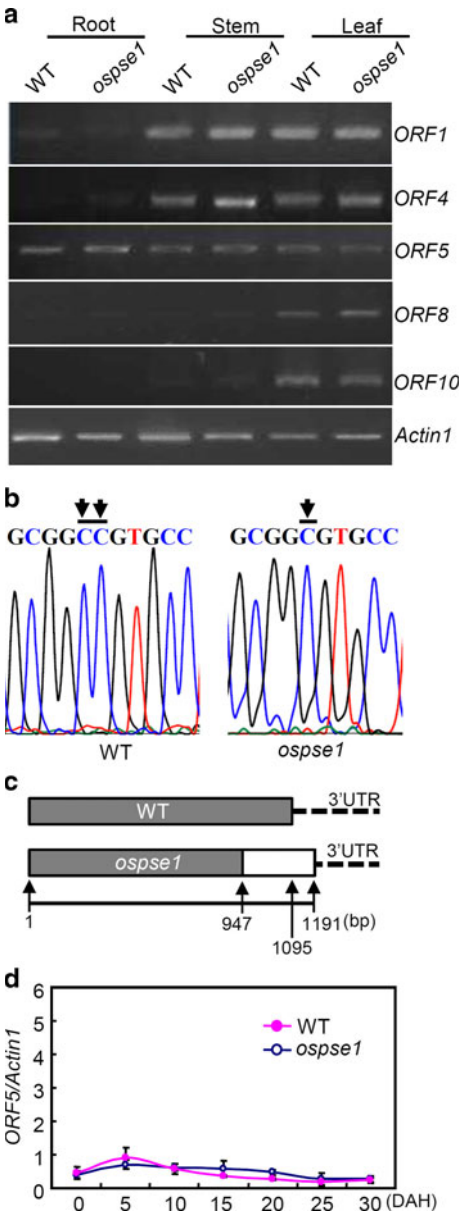

Fig. 7 Analysis of candidate genes for the *ospse1*. **a** Expression profiling of five candidate genes for *ospse1* in different organs. Expression of the ORFs was analyzed by RT-PCR. *Actin1* serves as an internal control for the expression assay. **b** Sequence analysis showed a nucleotide deletion (*arrowed*) in *ORF5* of the *ospse1* mutant. **c** Schematic diagram of cDNA structure of *ORF5* in the wild type or *ospse1* mutant. Wild type *ORF5* consists of 1,095 base pair (bp). A deletion occurs in the position of 947 bp, which causes frame shift and creates a novel stop codon in the 3′ UTR, leading to a longer ORF (1,191 bp). **d** The expression of *ORF5* during senescence in the wild type (WT) and the *ospse1* mutant. Data are normalized to *Actin1* levels and shown as mean ± SD ($n = 3$). DAH day after heading

(Fig. 4), the transcriptional level of *ORF5* did not remarkably change in *ospse1* mutant or in the process of senescence (Fig. 7a, d). Because that pectate lyase is involved in the cell wall degradation (Marin-Rodriguez et al. 2002; Xie et al. 2012), *ORF5* may be a novel degradation-related protein conferring the premature senescence phenotype, which remains an interesting topic for future investigation.

Taken together, our results indicate that *ospse1* may be a novel type of senescence-associated gene that affects senescence pathways via pectate lyase activity. Further functional analysis of *ospse1* will help us to understand the molecular mechanism of this premature leaf senescence, and eventually to manipulate leaf senescence via genetic engineering approaches.

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Conflict of interest The authors declare that they have no conflicts of interests.

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