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Regulatory Roles of CORONATINE INSENSITIVE 1b (OsCOI1b) in Rice Leaf Senescence

비의 잎 노화에 관여하는 CORONATINE INSENSITIVE 1b (OsCOI1b)의 조절 기작 규명

BY
SANG-HWA LEE

AUGUST, 2014

MAJOR IN CROP SCIENCE AND BIOTECHNOLOGY
DEPARTMENT OF PLANT SCIENCE
THE GRADUATE SCHOOL OF SEOUL NATIONAL UNIVERSITY
Regulatory Roles of CORONATINE INSENSITIVE 1b (OsCOI1b) in Rice Leaf Senescence

UNDER THE DIRECTION OF DR. NAM-CHON PAEK
SUBMITTED TO THE FACULTY OF THE GRADUATE SCHOOL
OF SEOUL NATIONAL UNIVERSITY

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JULY, 2014

APPROVED AS A QUALIFIED DISSERTATION OF SANG-HWA LEE
FOR THE DEGREE OF MASTER
BY THE COMMITTEE MEMBERS

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ABSTRACT

Regulatory Roles of CORONATINE INSENSITIVE 1b (OsCOI1b) in Rice Leaf Senescence

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Jasmonic acid (JA) is an important phytohormone involved in several developmental processes, including senescence and plant immunity. CORONATINE INSENSITIVE 1 (COI1), encoding a JA receptor, is one of the central factors in JA-responsive signaling pathway in Arabidopsis (Arabidopsis thaliana). Arabidopsis harbors a single COI gene, termed COI1, and its molecular-genetic and physiological functions in JA signaling have been extensively studied using coi1 mutants. However, rice (Oryza sativa) has three COI homologs, termed OsCOI1a, OsCOI1b, and OsCOI2. The phylogenetic analysis suggests that the three OsCOI genes arose from two different duplication events after monocot-dicot divergence. However, it still remains elusive whether each OsCOI has distinct, synergistic, or redundant functions in rice development. Here, we show that using the T-DNA insertion oscoi1b-1 knockout mutant, OsCOI1b is mainly associated with leaf senescence under senescence-promoting conditions. The detached leaves of oscoi1b-1 mutant exhibited a strong stay-green phenotype in dark-induced senescence conditions and delayed leaf senescence in natural field conditions, with
substantial retention of chlorophylls and photosynthetic capacity. Furthermore, several senescence-associated genes, including senescence-inducing hormone-related genes were down-regulated in oscoi1b-1 mutants, including ETHYLENE INSENSITIVE 3 (EIN3) and ORESARA1 (ORE1), one of the important cascade of leaf senescence in Arabidopsis. These results strongly suggest that JA-responsive signaling crosstalk with ethylene-responsive signaling to promote leaf senescence. Among the agronomic traits, seed weight and spikelet fertility were significantly decreased in the stay-green oscoi1b-1 mutant, leading to severe reduction of grain yield, indicating that OsCOI1b function or JA signaling is involved in the spikelet fertility and seed filling. Possible divergent roles of the three OsCOI homologs are discussed.

Keywords: Jasmonic acid, COI1, Leaf senescence, OsCOI1b, Rice, Stay-green

Student number: 2012-23333
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# ABBREVIATION

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>ABA</td>
<td>Abscisic Acid</td>
</tr>
<tr>
<td>COI1</td>
<td>CORONATINE INSENSITIVE 1</td>
</tr>
<tr>
<td>DAH</td>
<td>Days After Heading</td>
</tr>
<tr>
<td>DAS</td>
<td>Days After Sowing</td>
</tr>
<tr>
<td>DDI</td>
<td>Days of Dark Incubation</td>
</tr>
<tr>
<td>DIS</td>
<td>Dark Induced Senescence</td>
</tr>
<tr>
<td>DT</td>
<td>Days after Treatment</td>
</tr>
<tr>
<td>ET</td>
<td>Ethylene</td>
</tr>
<tr>
<td>JA</td>
<td>Jasmonic Acid</td>
</tr>
<tr>
<td>MeJA</td>
<td>Methyl Jasmonate</td>
</tr>
<tr>
<td>MYA</td>
<td>Million Years Ago</td>
</tr>
<tr>
<td>SA</td>
<td>Salicylic Acid</td>
</tr>
<tr>
<td>SAG</td>
<td>Senescence-Associated Gene</td>
</tr>
<tr>
<td>WGD</td>
<td>Whole Genome Duplication</td>
</tr>
</tbody>
</table>
INTRODUCTION

Leaf senescence constitutes the final stage of leaf development, and it accompanies with the expression of senescence-associated genes (SAGs) regulated by senescence-associated transcriptional factors (senTFs) [1], leading to massive degradation of proteins and macromolecules, such as chlorophylls (Chls), carotenoids, and lipids [2]. The onset of leaf senescence is autonomously induced in an age-dependent manner. However, it is often triggered by endogenous factors, such as phytohormones and some metabolites [2, 3], and exogenous factors, including pathogens, ultraviolet light, salt, and drought stresses [4-6]. The in-depth understanding of senescence mechanism is important in cereal crop production because leaf senescence is closely associated with the grain filling and grain weight [7, 8].

Jasmonic acid (JA) and other oxylipin derivatives, generally called jasmonates, control many developmental processes, including leaf yellowing [9], root elongation [10], cell cycle progression [11], and seed fertility [12]. JA is also involved in the plant defense processes against wounding [13] and pathogenesis [14]. JA is synthesized from the precursor compound α-linolenic acid by six consecutive reactions, involving LIPOXYGENASE (LOX), ALLENE OXIDE SYNTHASE (AOS), and 12-OXO-PHYTODIENOATE REDUCTASE 3 (OPR3) enzymes in the JA biosynthesis pathway [10]. Newly synthesized JA is introduced to an F-box protein,
CORONATINE INSENSITIVE 1 (COI1) [12, 15], acting as a JA receptor in the E3 ubiquitin-ligase SKP1-Cullin-F-box complex (SCF^{COI1}), to initiate the JA-responsive pathway [16]. The SCF^{COI1} complex interacts with JASMONATE ZIM DOMAIN (JAZ) family proteins in a JA-dependent manner [17], leading to degradation of JAZ proteins by the 26S proteasome [18]. With a basic helix-loop-helix transcription factor MYC2 [19], JAZ proteins act as the main inhibitors for the transcription of genes in the JA-responsive pathway in Arabidopsis (*Arabidopsis thaliana*). MYC2 widely regulates the transcriptome of JA signaling pathway [20], and several direct target genes of MYC2, including *VEGETATIVE STORAGE PROTEIN2* (VSP2) and *PLANT DEFENSIN1.2* (PDF1.2), have been identified [10, 17].

The diverse physiological functions of *COI1* have been well studied using Arabidopsis *coi1* mutants. Due to JA insensitivity, Arabidopsis *coi1* mutants show pleiotropic defectiveness, including male sterile (*coi1-1*) [15] and apical dominance (*coi1-37*) [21]. The *coi1* mutants also showed a stay-green phenotype under dark-induced senescence (DIS) conditions (*coi1-1*) [22] and by methyl jasmonate (MeJA) treatment (*coi1-2*) [23], indicating that Arabidopsis COI1 function is closely associated with leaf senescence. Most of higher plants have more than one *COI* homologs (Supplementary Fig. 1); Arabidopsis has a single *COI1* (At2g39940) gene while rice has three *COI* homologs, *OsCOI1a* (Os01g0853400), *OsCOI1b* (Os05g0449500), and *OsCOI2* (Os03g0265500) [24]. Amino acid sequences of *OsCOI1a* and *OsCOI1b* are considerably similar and share more than 80% identity, while
OsCOI2 is relatively less similar (63%) to the two OsCOI1 proteins. To date, unlike the Arabidopsis COI1 functions, the physiological roles of rice COI homologs are not intensively studied. Two different groups recently reported the RNA interference (RNAi)-mediated gene silencing mutants of OsCOI1 genes [25, 26]. In addition to the MeJA-insensitive phenotype, [25] reported that the OsCOI1 RNAi lines showed altered plant height, internode length, and grain length, possibly due to uncontrolled cell elongation. Furthermore, another OsCOI1 RNAi lines [26] showed increased susceptibility to chewing insects. Because their OsCOI1 RNAi lines were developed using the conserved sequence of OsCOI1a and OsCOI1b, they were actually double knockdown mutants of the two OsCOI1 homologs [25, 26]. Thus, it is not clear whether the two COI1 homologs acts synergistically, redundantly, or independently in rice. Although ectopic expression of rice COI genes in the Arabidopsis coi1-1 mutants recovered the JA responsiveness [24], the existence of three COI homologs in rice strongly suggests that they may have distinct roles in the JA-responsive signaling during development. Thus, the elucidation of functions of each COI homolog is essential to understand the COI-mediated JA-responsive pathway in rice.

Here we show that in addition to MeJA insensitivity, the T-DNA insertion oscoi1b-1 knockout mutants stayed green during DIS, similar to Arabidopsis coi1 mutants. The oscoi1b-1 mutation also delayed the age-dependent senescence in the field conditions, with high retention of grana thylakoid structure and photosynthetic capacity, indicating that OsCOI1b is largely
associated with the regulation of leaf senescence. During senescence, several SAGs and phytohormone signaling- or synthesis-associated genes were significantly down-regulated in *oscoi1b-1* mutants. Unlike the previously reported *oscoi1* RNAi lines, *oscoi1b-1* mutants were quite normal during vegetative growth. However, the main agronomical traits, such as spikelet fertility and grain weight, decreased significantly. The possible function of *OsCOI1b* and divergent roles of three rice COI homologs are discussed.
MATERIALS AND METHODS

Plant Materials and Growth Conditions

The wild-type *japonica* rice cultivar ‘Dongjin’, and *oscoi1b-1* and *nyc1-1* mutants were grown in the paddy field under natural long days (>14 h light/day) in Suwon, South Korea (37°N latitude). *oscoi1b-1* mutant was obtained from Prof. Gynheung An at Kyung-Hee University, Korea [27, 28]. For DIS, detached leaves from 3-week-old plants or the whole plants were incubated in complete darkness. Arabidopsis wild-type (Col-0) and *coi1-16* plants were grown on the soil at 22-24°C under cool-white fluorescent light (90-100 μmol m⁻² s⁻¹) in long days (16 h light/8h dark) and short days (10 h light/14 h dark). Arabidopsis *coi1-16* mutant seeds (CS67817) were obtained from the Arabidopsis Biological Resource Center (ABRC, USA).

Gene Tree Analysis

Amino acid (aa) sequences of the orthologs to OsCOI1b were obtained from EnsemblPlants Release 21 (http://plants.ensembl.org/) and aligned by Clustal Omega (https://www.ebi.ac.uk/Tools/msa/clustalo/) [29]. Aligned aa sequences were introduced to MEGA 6 [30] using maximum-likelihood method employing the Jones-Taylor-Thornton (JTT) model with 1000-bootstrap test replicates to construct phylogenetic tree.
Synteny Analysis and Ks Value Calculation

In order to retrieve homologous gene pairs with e-value threshold $1e^{-10}$ with the top 5 alignment per gene, BLASTP [31] was used with *Oryza sativa* peptide sequences, and MCScanX [32] was used for obtain the synteny block which had collinearity within the BLASTP results. Ks value of each gene pairs in the MCScanX results was calculated using built-in perl module of MCScanX, `add_ka_and_ks_to_collinearity.pl`. Ks values of OsCOI1a-OsCOI2 and OsCOI1b-OsCOI2 were calculated separately. DNA sequences of these gene pairs were aligned by Prank [33] and parsed into AXT file using perl module, `parseFastaIntoAXT.pl`, provided by KaKs-calculator homepage (https://code.google.com/p/kaks-calculator/). Finally, Ks values were calculated by KaKs-Calculator 1.2 [34] using LWL model described previously [35]. All of the rice DNA and peptide sequences used in these analyses were downloaded from the Rice Genome Annotation Project Release 7 (http://rice.plantbiology.msu.edu).

Chlorophyll (Chl) Quantification

For the measurement of total Chl concentration, pigments were extracted from leaf tissues with 80% ice-cold acetone. The Chl concentrations were determined by the spectrophotometric methods as described previously [36].

SDS-PAGE and Immunoblot Analysis
Protein extracts were prepared from the leaf tissues. To extract total proteins, the leaf tissues from 2-month-old rice plants grown under long-day conditions (14.5 h light/9.5 h dark) were ground in liquid nitrogen and 10 mg aliquots were homogenized with 100 μL of the sample buffer (50 mM Tris, pH 6.8, 2 mM EDTA, 10% glycerol, 2% SDS, and 6% 2-mercaptoethanol). Homogenates were centrifuged at 10,000 × g for 3 min, and supernatants were denatured at 80°C for 5 min. 4 μL of samples were subjected to 12% (w/v) polyacrylamide SDS-PAGE separation. Subsequently, resolved proteins were electroblotted onto a Hybond-P membrane (GE healthcare). Photosystem protein antibodies against Lhca1, Lhcb1, Lhcb2, Lhcb4, D1, and PsaA (Agrisera, Sweden) used for immunoblot analysis. The level of each protein was examined using the ECL system with WESTSAVE (AbFRONTIER, Korea) according to the manufacturers’ protocols.

Measurement of Ion Leakage Rates

Ion leakage was measured as described previously [37] with minor modifications. Membrane leakage was determined by measurement of electrolytes (or ions) leaking from rice leaf disc (1 cm²). Three leaf discs from each treatment were immersed in 6 mL of 0.4 M mannitol at room temperature with gentle shaking for 3 h, and conductivity of the solution measured with a conductivity meter (CON6 METER, LaMOTTE Co., USA). Total conductivity
was determined after sample incubation at 85°C for 20 min. The ion leakage is expressed as the percentage of initial conductivity divided by total conductivity.

**Measurement of CO₂ Exchange Rates and Fv/Fm Ratios**

CO₂ exchange rate of WT and oscoi1b grown in the paddy field was measured with a LI-6400 (LI-COR Biosciences) by the manufacturer’s instruction. The middle part of flag leaf was placed into the leaf chamber of the instrument and exposed to LED light source at a flow rate of 500 μmol m⁻² s⁻¹. Leaf temperature was maintained at 20°C during measurement. At least three replicates per plant were done for calculation. Fv/Fm was measured using the OS-30p+ instrument (OPTI-SCIENCES, USA). The middle part of flag leaves were adapted in the dark for 5 min to complete oxidation of QA. After dark treatment, Fv/Fm ratio was measured in the paddy field. For both experiments, more than three experimental replicates per plant were done.

**Transmission Electron Microscopy (TEM) Analysis**

To perform TEM analysis, a previous article [38] were used with some modifications. Small leaf pieces were fixed with modified Karnovsky’s fixative, which is consist of 2% paraformaldehyde, 2% glutaraldehyde and
50 mM sodium cacodylate buffer, pH 7.2. After this, samples were washed with 0.05M sodium cacodylate buffer, pH 7.2 three times at 4°C for 10min. Post-fixed the samples at 4°C for 2 h with 1% osmium tetroxide in 50 mM sodium cacodylate buffer, pH 7.2, and wash those twice with distilled water at room temperature. Samples were treated to en bloc staining in 0.5% uranyl acetate at 4°C for overnight and dehydrated in EtOH gradient solution and propylene oxide, finally infiltration with Spurr’s resin. Polymerization step were performed at 70°C for 24 h and sectioning with Ultramicrotome (MT-X). The sections were mounted on copper grids, and were stained with 2% uranyl acetate for 7 min and with Reynolds’ lead citrate for 7 min. Micrographs were made by using LIBRA 120 transmission electron microscope.

**Reverse Transcription (RT) and Quantitative Real-Time PCR (qPCR) Analysis**

For RT reaction, total RNA was extracted from the rice leaf tissues using the RNA Extraction Kit (Macrogen, Seoul, Korea). The first-strand cDNAs were prepared with 2 μg total RNA using M-MLV reverse transcriptase and oligo(dT)$_{15}$ primer (Promega) for total 25 μL, and diluted with 75 μL water. For quantitative real-time PCR (qPCR), a 20 μL mixture was prepared including first-strand cDNAs equivalent to 2 μL total RNA, 10 μL 2× goTaq
master mix (Promega), and 6 μL DW, and gene-specific forward and reverse primers (Table 1). qPCR was analyzed using a Light Cycler 480 (Roche Diagnostics). Rice *Ubiquitin5 (UBQ5)* was used as an internal control. The relative expression of each gene was calculated using the $2^{-\Delta\Delta C_T}$ method, as previously described [39].
### Table 1. Primers used in this study.

A. Primers for verification of OsCOI1b T-DNA insertion

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<th>Forward primer (5′→3′)</th>
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<td>PGA_2715RB</td>
<td>CGTCCGGAATGTTATTAAG</td>
<td>GGACAGCAGTTTCTATCAATC</td>
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<td>SOUTHERN</td>
<td>GTCACGTCTGTAAGAACCCAA</td>
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B. Gene-specific primers used for qRT-PCR

<table>
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<th>Reverse primer (5′→3′)</th>
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<td>AOS2</td>
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<tr>
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<td>AGAGTCGAGCTGAGCTTCTG</td>
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<tr>
<td>UBOQ5</td>
<td>ACCACTTCCAGGCCACTACT</td>
<td>ACGCCCTAAGCTGCTGTT</td>
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RESULTS

Evolutional analysis of the three OsCOI genes in sequence level

Genes have evolved divergently within and among species. Through investigation of whole chloroplast genomes, [40] discovered that monocot and dicot have been split about 140 to 150 million years ago (MYA). Furthermore, rice (*Oryza sativa*) has undergone whole genome duplication (WGD) event about 40 to 50 MYA [41]. After gene duplication, the gene itself suffers from internal and external actions, resulting in non-functionalization, sub-functionalization, and neo-functionalization [42].

The monocot rice differs from the dicot Arabidopsis in the number of COI gene. Arabidopsis has a single copy of COI gene, termed COI1 [12, 15], while rice has three COI homologs, termed OsCOI1a, OsCOI1b, and OsCOI2 [24]. Evidence from tree reconciliation analysis in the EnsemblPlants (http://plants.ensembl.org/), OsCOI genes are identified as co-orthologs, which are duplicated after monocot-dicot speciation. We also found that OsCOI homologs share high similarities with other monocots such as maize and barley rather than the dicot plants including Arabidopsis and soybean (Fig. 1). To determine whether three OsCOI genes were
duplicated or triplicated, nucleotide substitutions of three OsCOI genes were analyzed. The number of synonymous substitutions per synonymous site (Ks) was used as a method to estimate the gene evolution process. Owing to Ks value, OsCOI1 and OsCOI2 might have been split earlier while OsCOI1a and OsCOI1b have undergone duplication more recently (Fig. 2a), suggesting that OsCOI2 may have evolved to differ from two OsCOI1 genes.

It has been reported that rice chromosome 1 (Os01) and Os05 have similar structural blocks, which are the same case as the maize chromosome 6 (Zm06) and Zm08 [43]. We found that OsCOI1a and OsCOI1b are located within the macro-synteny block between Os01 and Os05 (Fig. 2b), indicating that duplication of OsCOI1 was participated in this duplication event. By calculating Ks values of all homologous gene-pairs and macro-synteny block between Os01 and Os05, mean value and standard deviation of them were quite similar (Fig. 2c). Furthermore, Ks value of OsCOI1a-OsCOI1b were located in a similar mean range of Ks of all homologous gene-pairs, however, others were relatively far. These results suggest that the duplication between OsCOI1a and OsCOI1b was possibly accompanied with WGD. As [24] reported that three OsCOI proteins have only several amino acid substitutions in substrate binding site which results in divergent binding activity to OsJAZ proteins, suggesting the possibility of sub-functionalization among OsCOI genes after gene duplication event.
**Fig. 1** COI gene family members in plants. The evolutionary relationship was inferred using the maximum-likelihood method. Numbers at the branch points represent the bootstrap values (> 50 %) of 1000 replicate trees. OsCOI homologs are indicated by red color. The scale bar indicates the number of substitutions per site.
Fig. 2 Homology analysis of three OsCOI genes in sequence level.

a Phylogenetic tree of three OsCOI genes based on Ks values. Ks values of each OsCOI pairs are indicated.

b Collinear structure analysis of the
OsCOI1a and OsCOI1b synteny blocks in Os01 (rice chromosome 1) and Os05 (rice chromosome 5). Os01 and Os05 have the opposite direction. Each red link between Os01 and Os05 means homologous gene-pair at the protein level and a blue link indicates OsCOI1a-OsCOI1b gene pair. 

\[ c \] Ks value distribution of rice whole homologous gene pair. Blue, red, and green arrowheads indicate the Ks values of OsCOI1a-OsCOI1b, OsCOI1a-OsCOI2, and OsCOI1b-OsCOI2, respectively. Means and SDs of Ks values of rice whole homologous gene pairs (w) and synteny blocks of Os01 and Os05 (s) described in b are indicated.
The Knockout Mutant of OsCOI1b Stay Green under DIS Conditions

The Arabidopsis coi1-1 mutant is pleiotropic and exhibits a stay-green phenotype under DIS conditions [22], indicating an important role of the JA receptor in the senescence induction of photosynthetically inefficient leaves. To gain insights into the possible functions of the three OsCOI homologs in leaf senescence, we searched the RiceGE database (http://signal.salk.edu/cgi-bin/RiceGE) for the possible knockout mutant lines. While the mutant of OsCOI1a or OsCOI2 is not currently available, we found a single oscoi1b-1 mutant line (PFG_2A.70041.L) in which a single T-DNA fragment is inserted in the 3'-UTR region (Fig. 3a). By RT-PCR analyses, we confirmed that the mutant leaves did not accumulate the OsCOI1b mRNAs (Fig. 3b). In addition, the oscoi1b-1 mutant was insensitive to the methyl jasmonate (MeJA) treatment; while the WT leaves turned yellow completely at 2 days of MeJA treatment, the oscoi1b-1 leaves maintained green color with higher Chl retention (Fig. 4a, b), similar to the stay-green phenotype of Arabidopsis coi1-2 mutant [23], indicating that oscoi1b-1 mutation is a knockout allele.

We next examined the leaf phenotype of oscoi1b-1 mutant under DIS condition. The rice nyc1-1 mutant containing a knockout allele of NYC1, encoding a Chl b reductase [44], was used for the nonfunctional stay-green control. At 4 days of dark incubation (4 DDI), detached leaves of oscoi1b-1 mutant exhibited a stay-green phenotype with higher Chl retention (Fig. 3c,
d), similar to those of nyc1-1 (Fig. 3c, d) and Arabidopsis coi1-16 mutants (Fig. 5a, b). Among the photosystem proteins, oscoi1b-1 mutant retained not only light-harvesting complex II (LHCII) subunits (Lhcb1, Lhcb2, and Lhcb4) but also other photosystem proteins including Lhca1, PsaA, and D1 during dark incubation (Fig. 3e), while nyc1-1 mutant did only LHCII as reported previously [44]. For this reason, Chl a/b ratio in oscoi1b-1 mutant barely changed during dark incubation (Fig. 3f), but Chl a/b ratio in nyc1-1 mutant significantly decreased due to dominant retention of LHCII [44]. Furthermore, we also found that the ion leakage rate, an indicator of membrane disintegration, was significantly lower in oscoi1b-1 mutant than in WT and nyc1-1 mutant (Fig. 3g). These results indicate that mutation of OsCOI1b delays leaf senescence by maintaining the balance of photosystem complexes and the persistence of cell membrane integrity much longer under DIS conditions.
**Fig. 3** *oscoi1b-1* mutants stay green under dark-induced senescence (DIS) conditions.

**a** Gene structure and T-DNA insertion site in the 3’-UTR region of OsCOI1b (PFG_2A.70041.L). Black arrows indicate the forward and reverse primers for RT-PCR. **b** The absence of OsCOI1b transcripts in *oscoi1b-1* mutant was confirmed by RT-PCR. *UBQ5* was used as an internal control. **c-g**
Phenotype (c), changes of total Chl levels (d), the levels of photosystem proteins (e), Chl a/b ratios (f), and ion leakage rates (g) in WT, oscoi1b-1, and nyc1-1 plants during dark incubation. Detached leaves from 1-month-old WT, oscoi1b-1, and nyc1-1 plants were incubated on the 3 mM MES (pH 5.8) buffer with the abaxial side-up at 28°C in darkness. d Antibodies against PSI antenna (Lhca1), PSII antenna (Lhcb1, Lhcb2, and Lhcb4), PSII core (D1), and PSI core (PsaA) were used for immunoblot analysis. d, f, g Black and white bars indicate before (0 DDI) and after 4 days of dark incubation (4 DDI), respectively. Mean and SD values were obtained from three biological replicates. c-g These experiments were repeated more than twice with similar results.
**Fig. 4** *oscoi1b-1* mutant is insensitive to exogenous MeJA treatment.

Color change (a) and total Chl levels (b) of the WT and *oscoi1b-1* leaves before (0 DT) and 2 days of MeJA treatment (2DT). Black and white bars represents WT and *oscoi1b-1* mutants, respectively. The detached leaves from 1-month-old plants were incubated on the MeJA solution (50 μM MeJA, 3 mM MES, pH 5.8) with the abaxial side-up at 28°C in continuous light. **Total Chls were extracted before (0 DDI) and after 4 DDI. Mean and SD values were obtained from more than three biological samples. These results were repeated more than twice with similar results. Student’s t-test was used to calculate statistical significance (**)P **< 0.01). MeJA, methyl jasmonate.**
Fig. 5 Arabidopsis *coi1-16* mutant stay green during DIS conditions.

Color change (a) and total Chl levels (b) of the WT and *coi1-16* leaves before (0 DDI) and after 4 DDI. Black and white bars represents WT and *oscoi1b-1* mutants, respectively. The rosette leaves detached from 3-week-old plants grown in LD (16 h light/8 h dark) conditions were incubated on the 3 mM MES (pH 5.8) buffer with the abaxial side-up at 22°C in darkness. b Total Chls were extracted before (0 DDI) and after 4 DDI. Mean and SD values were obtained from more than three biological samples. These results were repeated more than twice with similar results. Student's t-test was used to calculate statistical significance (**P < 0.01).
**oscoi1b-1 Mutant Exhibits a Functional Stay-Green Phenotype during Natural Senescence**

The stay-green plants are largely classified into two groups, functional and nonfunctional [45, 46]. Functional stay-green plants retain both leaf greenness and photosynthetic capacity much longer during grain filling, while nonfunctional stay-green plants maintain leaf greenness without photosynthetic competence during DIS and natural senescence. Owing to the persistence of photosynthesis capacity, the functional stay-green varieties have long been considered one of the agronomic traits.

To examine whether the stay-green phenotype of oscoi1b-1 mutant is functional or nonfunctional during natural senescence, oscoi1b-1 mutant plants were grown in the paddy field under natural long day conditions (>14 h light/day at 37° N latitude, Suwon, Korea) with regular rice cultivation practice and thereby the agronomic traits of the mutant were evaluated by comparing with its parental WT japonica cultivar, ‘Dongjin’. While the heading date of oscoi1b-1 mutant was not altered (Fig. 6), the mutant showed delayed leaf senescence during grain filling (Fig. 7a). Consistently, total Chl levels in oscoi1b-1 mutant were significantly higher than those in WT at 40 days after heading (DAH) (Fig. 7b). To check the photosynthetic capacity, two different parameters, $Fv/Fm$ ratio (efficiency of photosystem II) and CO$_2$ exchange rate (photosynthesis capacity) were measured after heading. The $Fv/Fm$ ratio was decreased in WT after 30 DAH while it was...
not much altered in *oscoi1b-1* mutant throughout grain filling (Fig. 7c). The CO₂ exchange rate, an indicator of photosynthetic capacity, decreased drastically in WT but very slowly in *oscoi1b-1* mutant (Fig. 7d).

Next, we compared chloroplast structures of the WT and *oscoi1b-1* leaves at 30 days after seeding (DAS) during vegetative growth and at the completion of grain filling (40 DAH). Using transmission electron microscopy (TEM) analysis revealed that at 30 DAS, chloroplast structures of the WT and *oscoi1b-1* leaves are considerably similar (Fig. 8a, c). At 40 DAH (or 140 DAS), chloroplasts highly retained grana thylakoid structure in the *oscoi1b-1* leaves (Fig. 8d) which was hardly detected in the WT leaves (Fig. 8b). These results strongly suggest that *oscoi1b-1* is the functional stay-green type mutant.

Interestingly, the functional stay-green phenotype of *oscoi1b-1* mutant is quite different from the *coi1-16* mutant during natural senescence, because Arabidopsis *coi1-16* mutant showed a precocious leaf yellowing under both LD (16-h light/8-h dark) and SD (8-h light/16-h dark) conditions (Fig. 9), which is opposite to the stay-green phenotype of *coi1-16* mutant under DIS conditions (Fig. 5a, b). It strongly suggests that the regulatory function of *OsCOI1b* is somewhat different from that of Arabidopsis *COI1* during natural senescence process.
Fig. 6 Heading date of *oscoi1b-1* mutant is not significantly different from that of WT. Heading date was measured in the paddy field, Suwon, Korea (37°N). Mean and SD values were obtained from 10 biological samples. DAS, days after sowing.
Fig. 7 Stay green phenotype of *oscoi1b-1* during natural senescence.

a Phenotype of WT (a parental *japonica* cultivar ‘Dongjin’) and *oscoi1b-1* mutant at 40 days after heading (DAH). b Total Chl levels of WT and *oscoi1b-1* mutant at 0 (black bars) and 40 DAH (white bars). c-d Temporal changes of Fv/Fm ratio (c) and CO₂ exchange rate (d) in WT and *oscoi1b-1* plants after heading. Filled and opened circles indicate WT and *oscoi1b-1* mutants, respectively. Mean and SD values were obtained from three biological replicates. These experiments were repeated more than twice with similar results.
**Fig. 8** Transmission Electron Microscopy (TEM) analysis of the WT and *oscoi1b-1* leaves during natural senescence in the paddy field.

**a-b** Chloroplasts in the mesophyll cells of developing leaves in WT (a) and *oscoi1b-1* mutant (b) at 30 days after sowing (DAS) and **c-d** Chloroplasts in the mesophyll cells of senescing leaves in WT (c) and *oscoi1b-1* mutants (d) at 140 days after sowing (DAS). G, grana thylakoid; PG, plastoglobule; S, starch. Scale bars = 1 μm.
Fig. 9 Arabidopsis *coi1-16* mutant exhibits early leaf senescence.

**a** Photos of LD-grown 4-week-old plants (upper panel) and SD-grown 6-week-old plants. LD, 16 h light/8 h dark; SD, 10 h light/14 h dark. **b** For total Chl quantification, the 2nd cycle of rosette leaves were used. Mean and SD values were obtained from more than three biological samples. These results were repeated more than twice with similar results. Student’s t-test was used to calculate statistical significance (*P < 0.05; **P < 0.01*).
**Altered Gene Expression in oscoi1b-1 Mutant under Dark-Induced Senescence Conditions**

Arabidopsis COI1 JA receptor regulates its downstream genes in the JA signaling pathway at the transcriptional level [23, 47, 48]. To examine whether the stay-green phenotype of oscoi1b-1 mutant is caused by altered expression of its downstream genes, we examined the expression levels of the JA synthesis-associated genes LOX [49] and AOS2 [50], and the JA-responsive genes PDF1.2 [51] (Fig. 10) and VSP2 [52] (Fig. 11) under DIS conditions. Because LOX and AOS2 act upstream of COI1 in Arabidopsis [53], the expression levels of their rice homologs were not altered in oscoi1b-1 mutant (Fig. 12a, b). On the other hand, the expression levels of the rice homologs of Arabidopsis PDF1.2 and VSP2 were significantly down-regulated in oscoi1b-1 mutant both at 0 DDI and 4 DDI (Fig. 12c, d), indicating that oscoi1b-1 mutation affects the expression of JA-responsive genes downstream of OsCOI1b function.

In addition to the JA synthesis- and JA-responsive genes, we further examined the expression levels of a few important SAGs, such as the rice homologs of Arabidopsis ORESARA1 (ORE1) [54] (Fig. 13), ORE9 [55], and SAG12 [56] (Fig. 14), as well as the Chl catabolism-associated genes including NON-YELLOW COLORING1 (NYC1) [44] and PHEOPHORBIDE A OXYGENASE (PAO) [57]. Before dark incubation (0 DDI), the expression levels of these SAGs in oscoi1b-1 mutant were almost the same as those in
WT. However, these gene expression was significantly down-regulated in oscoi1b-1 mutant at 4 DDI (Fig. 12e-i) as well as during natural senescence (Fig. 15). These results indicate that oscoi1b-1 mutation delays leaf senescence at the transcriptional level during both DIS and natural senescence.

Abscisic acid (ABA), ethylene, and salicylic acid (SA) are senescence-promoting phytohormones [58], and these hormone signaling pathways crosstalk with JA signaling pathway [10]. Thus, we subsequently checked the expression levels of ABA signaling-associated genes ABA INSENSITIVE5 (ABI5) [59] and ENHANCED EM LEVEL (EEL) [60], ethylene signaling-associated genes ETHYLENE INSENSITIVE3 (EIN3/OsEIL1) [61] and ETHYLENE RESPONSE ELEMENT BINDING FACTOR3 (ERF3) [62], and SA signaling-associated genes SALICYLIC ACID3 HYDROXYLASE (S3H) [63] (Fig. 16) and ISOCHORISMATE SYNTHASE1 (ICS1) [64], revealed that the expression levels of the two hormone signaling-associated genes were significantly down-regulated in oscoi1b-1 mutant under both DIS (Fig. 17) and natural senescence conditions (Fig. 18). Moreover, their gene expression was also down-regulated even during vegetative stage (Fig. 18), suggesting that the oscoi1b mutation also negatively affects other senescence-promoting signaling pathways involving ABA, ethylene, and SA throughout development.
Fig. 10 Amino acid alignment of Arabidopsis PDF1.2 (AT5G44420) to rice PDF1.2 (Os02g0212100) with 97.50% query coverage and 34.62% identity. Amino acid sequences were obtained from the NCBI database and aligned using the Clustal Omega (http://www.ebi.ac.uk/Tools/msa/clustalo/) and BoxShade 3.21 (http://embnet.vital-it.ch/software/BOX_form.html). At, Arabidopsis thaliana; Os, Oryza sativa.
**Fig. 11** Amino acid alignment of Arabidopsis VSP2 (AT5G24770) to rice VSP2 (Os05g0190500) with 95.85% query coverage and 40.23% identity. Amino acid sequences were obtained from the NCBI database and aligned using the Clustal Omega (http://www.ebi.ac.uk/Tools/msa/clustalo/) and BoxShade 3.21 (http://embnet.vital-it.ch/software/BOX_form.html). At, *Arabidopsis thaliana*; Os, *Oryza sativa*.
Fig. 12 Altered expression of MeJA-related genes and SAGs in oscoi1b-1 mutant under DIS conditions.

Relative expression levels of AOS2 (a), LOX (b), PDF1.2 (c), VSP2 (d), ORE1.2 (e), ORE9 (f), SAG12 (g), NYC1 (h), PAO (i) were obtained by normalizing to the transcript levels of UBQ5. The detached leaves from 1-
month-old WT and oscoi1b-1 mutant were used for dark incubation and total RNA was extracted before (0 DDI) and after 4 DDI. Mean and SD values were obtained from more than three biological samples. These results were repeated more than twice with similar results. Student’s t-test was used to calculate statistical significance (*P < 0.05; **P < 0.01).
Fig. 13 Amino acid alignment of Arabidopsis ORE1 (AT5G39610) to rice ORE1.1 (Os02g0579000) and ORE1.2 (Os04g0460600) with 73.68% and 67.72% query coverage and 57.21% and 69.05% identity, respectively. Amino acid sequences were obtained from the NCBI database and aligned using the Clustal Omega (http://www.ebi.ac.uk/Tools/msa/clustalo/) and BoxShade 3.21 (http://embnet.vital-it.ch/software/BOX_form.html). At, *Arabidopsis thaliana*; Os, *Oryza sativa*.
**Fig. 14** Amino acid alignment of Arabidopsis SAG12 (AT5G45890) to rice SAG12 (Os04g0208200) with 99.71% query coverage and 54.42% identity. Amino acid sequences were obtained from the NCBI database and aligned using the Clustal Omega (http://www.ebi.ac.uk/Tools/msa/clustalo/) and BoxShade 3.21 (http://embnet.vital-it.ch/software/BOX_form.html). At, Arabidopsis thaliana; Os, Oryza sativa.
Fig. 15 Expression patterns of the MeJA-related genes and SAGs in developing and senescing leaves of oscoi1b-1 mutant.

**a-i** Total RNA was extracted from the developing leaves (30 DAS) and senescing leaves (130 DAS) of WT and oscoi1b-1 mutant. Relative expression levels of AOS2 (**a**), LOX (**b**), PDF1.2 (**c**), VSP2 (**d**), ORE1.2 (**e**), ORE9 (**f**), SAG12 (**g**), NYC1 (**h**), and PAO (**i**) were obtained by normalizing to the transcript levels of UBQ5. Mean and SD values were obtained from more than three biological replicates. These results were repeated more than three times with similar results. Student’s t-test was used to calculate statistical significance (*P < 0.05; **P < 0.01).
**Fig. 16** Amino acid alignment of Arabidopsis S3H (AT4G10500) to rice S3H (Os04g0581100) with 97.71% query coverage and 60.06% identity. Amino acid sequences were obtained from the NCBI database and aligned using the Clustal Omega (http://www.ebi.ac.uk/Tools/msa/clustalo/) and BoxShade 3.21 (http://embnet.vital-it.ch/software/BOX_form.html). At, *Arabidopsis thaliana*; Os, *Oryza sativa*. 

{alignments}

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AtS3H 1 TVQDLASACGTYGEFLKNHGPEENTITYQVAREFFCHCPESE KNYSDGTYTTRLS
OsS3H 1 TVEAIGSACGTGFEFLKNHGPEQYVEGLRVAREFFHCPESE KNYSDGTYTTRLS
AtS3H 61 TSFNVGAQKVZNWRDFLRLHCPEEEDPILEPSSEFSTPRPET A TWSTRAALYRLLEAI
OsS3H 61 TSFNVRTEKVZNWRDFLRLHCPEEEDIPILEPSSEFSTPRPET A TWSTRAALYRLLEAI
AtS3H 121 SESLGELEDHIGNIIGSAQHMAFNYYPFCPCFELTYGLPGKHDITTVIT LLDQOYSGLG
OsS3H 121 SESLGELETVSAQSAQHMAFNYYPFCPCFELTYGLPGKHDENAIT NLDQOYSGLG
AtS3H 181 QDFDDHVVSGTEKTFTNVIGDOYOISNDSYKSVLHBAVKISIEROLSPFPPSTP
OsS3H 181 QGNGAVVAVNEDALTVNIGDQGALSNDSYKSVLHBYVANISEROLSPFPPSTP
AtS3H 241 AVFPAHELQNEQDSLTVTVEWEFPHNKRSLVASCLESASAPT --
OsS3H 241 AVFPAAGLQGALHPLAYRQAEFPHNMCQGASCLGRSPNDQAV
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Fig. 17 Altered gene expression of the phytohormone-related genes in oscoi1b-1 mutant under DIS conditions.

Relative expression levels of ABI5 (a), EEL (b), EIN3 (c), ERF4 (d), S3H (e), ICS1 (f) were obtained by normalizing to the transcript levels of UBQ5. Materials and methods were exactly the same as those in Fig. 5. These results were repeated more than twice with similar results. Student’s t-test was used to calculate statistical significance (*P < 0.05; **P < 0.01).
Fig. 18 Different expression levels of phytohormone-related genes in developing and senescing leaves of *oscoi1b-1* mutant.

**a-f** Total RNA was extracted from the developing leaves (30 DAS) and senescing leaves (130 DAS) of WT and *oscoi1b-1* mutant. Relative expression levels of *ABI5* (a), *EEL* (b), *EIN3* (c), *ERF4* (d), *S3H* (e), *ICS1* (f) were obtained by normalizing to the transcript levels of *UBQ5*. For qRT-PCR, Mean and SD values were obtained from more than three biological replicates. These results were repeated more than three times with similar results. Student’s t-test was used to calculate statistical significance (*P < 0.05; **P < 0.01).
Loss of OsCOI1b Function Negatively Affects Spikelet Fertility and Grain Filling Rate

Functional stay-green trait in crop plants is potentially an important yield-increasing trait, due to the prolonged photosynthesis competence during grain filling [65]. Indeed, a few yield-increasing stay-green cultivars have been identified [7]. To examine the relationship between COI1b-dependent functional stay-green phenotype and rice yield, we examined several agronomical traits of oscoi1b-1 mutant, including plant height, number of panicles per plant, number of spikelet per plant, spikelet fertility, and 1000 grain weight. oscoi1b-1 mutant exhibited similar height and internode length compared to WT (Fig. 19a). Besides, the values of other agronomic traits were decreased (Fig. 19b-g). Especially, spikelet fertility and 1000 grain weight of oscoi1b-1 mutant was significantly lower than those of WT (Fig. 19e-g). These results indicate that the functional stay-green trait acquired by the loss of OsCOI1b-mediated JA signaling is rather harmful to grain production in rice, possibly the severe negative effects on other developmental processes including seed fertility and grain filling rate.
Fig. 19 Analysis of agronomic traits in oscoi1b-1 mutant.

a-h Five agronomic traits were examined, such as plant height (a), length of 2nd internode (b), number of panicles per plant (c), number of spikelets per plant (d), spikelet fertility (e), 1000 grain weight (f), panicle phenotype (g), and fertile and sterile spikelets (h) in WT and oscoi1b-1 mutant. Mean and SD values were obtained from more than three biological replicates. a-f Student’s t-test was used to calculate statistical significance (*P < 0.05).
**Transcriptional Relationship of the Three OsCOI Genes**

As mentioned, Arabidopsis contains a single COI gene while rice has three COI homologs (Fig. 1) [24]. Although we showed the critical role of OsCOI1b in promoting leaf senescence, it is highly possible that OsCOI1a and OsCOI2 are also involved in leaf senescence. Thus, we checked the expression of three OsCOI genes under both DIS and natural senescence conditions. Their expression levels increased in senescent leaves compared with presenescent leaves (Fig. 20), especially under DIS conditions (Fig. 20b, d, f), indicating that OsCOI1a and OsCOI2 function are possibly important for leaf senescence stage.

To examine the loss of OsCOI1b function on the transcription of the other OsCOI homologs, we subsequently checked the expression levels of OsCOI1a and OsCOI2 in oscoi1b-1 mutant. During senescence (130 DAS and 4 DDI), the expression levels of OsCOI1a and OsCOI2 in oscoi1b-1 mutant were almost the same as those of WT (Fig. 20c-f), suggesting that the two OsCOI-mediated JA signaling is unlikely related to leaf senescence. However, we found that the expression of the two COI homologs was significantly down-regulated in the presenescent leaves of oscoi1b-1 mutant (30 DAS and 0 DDI) (Fig. 20c-f), suggesting that oscoi1b mutation negatively affects the expression of OsCOI1a and OsCOI2 in developing leaves, not in senescing leaves, by as-yet unknown feedback regulatory mechanism.
Fig. 20 Expression patterns of the three OsCOI genes under natural senescence and DIS conditions.

a-f By qRT-PCR analysis, relative expression levels of OsCOI1b (a, b), OsCOI1a (c, d), and OsCOI2 (e, f) during natural senescence (a, c, e) and DIS (b, d, f) conditions were obtained from normalizing to the transcript levels of UBQ5. OsCOI1b transcripts were not detected in oscoi1b-1 mutant.
Black and white bars indicate WT and *oscoi1b-1* mutant, respectively. In DIS conditions, total RNA was extracted from the leaves from 1-month-old WT and *oscoi1b-1* plants before (0 DDI) and after 4 DDI. In natural senescence conditions, developing leaves (30 DAS) and senescing leaves (130 DAS) of WT and *oscoi1b-1* were used. Mean and SD values were obtained from more than three biological replicates. These results were repeated more than twice with similar results. Student’s t-test was used to calculate statistical significance (*P < 0.05; **P < 0.01*).
DISCUSSION

JA is known as one of the senescence-promoting phytohormones [66]. Involvement of JA signaling in the promotion of leaf senescence has been studied extensively in Arabidopsis [47]. In addition to coi1 mutants in Arabidopsis, the knockout mutant of KAT2 (for 3-ketoacyl-CoA thiolase 2; one of the JA biosynthesis enzymes) and the RNAi-mediated knockdown mutant of the JA-responsive HDA6 (for histone deacetylase 6) retained leaf green color much longer under DIS conditions [22, 67]. However, the JA-deficient mutants of AOS (for allene oxide synthase) and OPR3 (for 12-oxo-phytodienoate reductase 3) did not exhibit the stay-green phenotype [68]. Thus, the relationship between leaf senescence and JA-responsive signaling pathway remains unclear.

Here we show that oscoi1b-1 mutant stayed green during dark incubation (Fig. 3), similar to Arabidopsis coi1-1 [22] and coi1-16 mutants (Fig. 5). Furthermore, the oscoi1b-1 leaves also exhibited a functional stay-green phenotype with high retention of photosynthetic capacity during grain filling period (Fig. 7), possibly due to a fact that several SAGs were significantly down-regulated in oscoi1b-1 mutant under both DIS and natural senescence conditions (Figs. 12, 15, 17 and 18). These results indicates that OsCOI1b signaling pathways is mainly involved in leaf senescence. Especially, the down-regulation of ethylene-responsive EIN3 and ORE1.2 for
leaf senescence appears to be closely associated with delayed senescence OsCOI1b signaling pathway; Arabidopsis \textit{ORE1}, one of the most well-studied SAGs, is a NAC TF that promotes senescence by regulating the expression of hundreds of SAGs involved in the breakdown of nucleic acids and proteins, and the transport of sugar [69, 70]. \textit{EIN3} is a core component of ethylene-responsive pathway [71], and \textit{EIN3} indirectly up-regulates \textit{ORE1} expression through the down-regulation of \textit{miR164} transcription, which cleaves the \textit{ORE1} mRNA [72]. This \textit{EIN3} regulation for \textit{ORE1} expression are considered as a typical age-dependent mechanism in leaf senescence. Indeed, Arabidopsis \textit{ore1} and \textit{ein3} mutants exhibited a strong functional stay-green phenotype during both DIS and natural senescence [54, 72, 73]. Because JAZ and a corepressor HDA6 down-regulates the transcription of \textit{EIN3} [74], COI1-mediated degradation of JAZ results in the increase of \textit{EIN3} expression. Consistently, we found here that in rice, \textit{EIN3} expression were significantly down-regulated in \textit{oscoi1b-1} mutant (Fig. 17), suggesting the direct OsCOI1b-JAZ-\textit{EIN3} signaling cascade (Fig. 21).

Another possibility also exists for the COI1-dependent leaf senescence in Arabidopsis, in that MYC2 directly or indirectly controls the expression of senTFs and other SAGs. COI1 indirectly enhances the transcription levels of the genes in the MYC2-dependent JA signaling pathway by directly degrading JAZ, a negative regulator of \textit{MYC2} expression [17]. Indeed, OsCOI1b showed significant binding capacity with several OsJAZ proteins [24]. Furthermore, it has been recently reported that OsCOI1b activates
OsMYC2 during spikelet development by interacting with OsJAZ1 [75]. Thus, OsCOI1b seems to have important role in the activation of MYC2 dependent transcriptional cascade. MYC2 binds to the G-box motif (CACGTG) on the promoters of various target genes, and down-regulates tryptophan metabolism and pathogen defense responses, while up-regulates other JA responses such as root growth [20, 76]. However, MYC2-target genes related with the promotion of leaf senescence have not been reported yet in Arabidopsis or other plants.

ABA and SA are senescence-promoting phytohormones [77]. The down-regulation of ABA signaling-associated genes (ABI5 and EEL) and SA synthesis-associated genes (S3H and ICS1) in oscoi1b-1 mutant (Figs. 17 and 18) further supports the stay-green phenotype. The two hormone signals crosstalk with JA-responsive signaling pathway [78, 79]. In this scenario, OsCOI1b seems to act as a key component for crosstalking with ABA- and SA-responsive signaling pathways. Taken together, our results suggest that at least in part, the OsCOI1b-mediated promotion of leaf senescence in rice is closely associated with the activation of both JA-responsive MYC2-PDF1.2 and ethylene-responsive EIN3-ORE1 genes at the transcriptional level (Fig. 21).

The number of homolog in a certain functional gene is often different from each higher plant. For example, Arabidopsis has three homologs of protochlorophyllide oxidoreductase (POR) encoding one of the Chl biosynthesis enzymes, while rice has only two POR homologs [80].
Alternatively, rice PORB has overlapping functions corresponding to both Arabidopsis PORB and PORC [81]. As the case of POR homologs, the number of COI homolog is different between Arabidopsis and rice. In contrast with a single copy of COI1 in Arabidopsis, rice has three COI homologs (OsCOI1a, OsCOI1b, and OsCOI2) which may arise from two different duplication events (Figs. 1 and 2). Although oscoi1-RNAi lines have previously been reported, the expression not only OsCOI1a but also OsCOI1b expression became knockdown because their nucleotide sequences are considerably similar [25, 26]. Thus, the exact functions of each OsCOI gene has remained elusive.

Here we first showed the single knockout mutant of OsCOI1b. [25] previously reported that the RNAi-derived oscoi1a oscoi1b double knockdown lines showed increased plant height with longer internode, and these phenotypes are caused by cell elongation instead of cell division, which is one of the typical GA signaling responses. However, plant height and internode length of oscoi1b-1 mutant were almost the same as WT (Fig. 19). Thus, it can be considered that OsCOI1a has major role in the regulation of plant height and internode length, or less possibly, the functions of OsCOI1a and OsCOI1b are partially redundant in plant development.

oscoi1b-1 mutant also showed the functional stay-green phenotype under both DIS and natural senescence conditions (Figs. 3 and 7). Noticeably, the natural senescence phenotype of oscoi1b-1 mutant is quite different from that of Arabidopsis coi1-16 mutant, because coi1-16 mutant
exhibited early leaf yellowing (Fig. 9). These results clearly indicate that the OsCOI1b function during natural senescence is opposite to Arabidopsis COI1 function via an unknown physiological mechanism. It is likely that OsCOIs have been duplicated and sub-functionalized, for example, OsCOI binding activity to OsJAZs [24] during evolution after speciation. Thus it is possible that similar to Arabidopsis COI1, OsCOI1a may have a senescence-promoting function during natural senescence. To date, the reason why rice has three COI homologs are not clear. Isolation of both oscoi1a and oscoi2 single mutants are necessary for revealing the significance of OsCOI evolution in rice.
Fig. 21 Tentative model of COI1b-involved JA signaling pathway leading to leaf senescence.

COI1b is involved in leaf senescence through the up-regulation of EIN3 and MYC2 expression by degrading JAZ in a JA-dependent manner. Red and black lines indicate the post-translational and transcriptional regulations, respectively. COI1b-JAZ signaling crosstalks with SA- and ABA-responsive signaling, directly or indirectly by as-yet unknown mechanisms. SA, salicylic acid; ET, ethylene; JA, jasmonic acid; ABA, abscisic acid.
Gene information  Sequence data from this article can be found in the National Center for Biotechnology Information (NCBI): OsCOI1a, Os01g0853400; OsCOI1b, Os05g0449500; OsCOI2, Os03g0265500; LOX, Os08g0508800; AOS2, Os03g0225900; PDF1.2, Os02g0212100; VSP2, Os05g0190500; ORE1.2, Os04g0460600; ORE9, Os06g0154200; SAG12, Os04g0208200; NYC1, Os01g0227100; PAO, Os03g0146400; ABI5, Os01g0859300; EEL, Os07g0686100; EIN3, Os03g0324200; ERF3, Os01g0797600; ICS1, Os09g0361500; S3H, Os04g0581100; UBQ5, Os01g0328400
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초 록

자스몬산은 노화와 식물 면역을 포함한 여러 발달단계에 연관되어있는 중요한 식물 호르몬이다. 자스몬산 수용체를 번역하는 CORONATINE INSENSITIVE 1 (COI1)은 얘기장대에서 자스몬산-신호 전달 회로의 중요인자이다.

 얘기장대에서는 한 개의 COI 유전자가 존재하는 반면 (COII), 벼에는 3 개의 COII homolog 가 존재한다 (OsCOIIa, OsCOIIb, OsCOII). 벼의 발달에 있어서 각각의 OsCOI들의 기능에 대한 분석이 미비하다. 우리는 유연관계 분석을 통해서 OsCOI가 단자엽-쌍자엽 분화 이후에 2 번의 복제가 일어났을 가능성이 있음을 확인하였다. 또한 T-DNA 삽입 oscoi1b-1 돌연변이체를 통해서 OsCOIIb 가 노화 촉진 조건에서 잎의 노화에 주요하게 관련있을 것임을 밝혀냈다. oscoi1b-1 돌연변이체는 암처리와 자연 노화상태 모두 강한 stay-green 표현형을 보였는데, 이는 엽록소와 광합성 능력의 지속적 유지를 통해서 알 수 있었다. 또한 호르몬 관련 및 노화 관련 유전자들이 oscoi1b-1 돌연변이체에서 적게 발현되었는데, 얘기장대에서 잎 노화에 중요한 기작으로 알려진 ETHYLENE INSENSITIVE 3 (EIN3)와 ORESARA 1 (ORE1)을 포함하고 있다.

이러한 결과들은 JA-신호신호경로는 에틸렌-신호 전달 회로와 상호작용을 통해서 잎의 노화를 촉진한다는 것을 보여준다. 종자의 무게와 이삭의 등숙률과 같은 농업형질은 oscoi1b-1 돌연변이체에서 극심하게 감소되어, 결과적으로 생산성을 감소시켰다. 따라서 OSCOI1b 혹은 JA-신호전달이 이삭의 등숙과 종자 발달에 관련이 있다는 것을 알려준다.

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