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Involvement of the Rice *OsSAUR51* Gene in the Auxin-related Field Resistance Mechanism against Bacterial Blight Disease

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Abstract

Pathogenic infection causes auxins to accumulate in plant cells and suppresses plant defense systems. We isolated the *OsSAUR51* (*Oryza sativa* small auxin-up RNA 51) gene as one of the rice field resistance-related genes from a retrotransposon *Tos17*-tagged 'Nipponbare' mutant (XC20) in which field resistance was reduced against several races of bacterial blight pathogens. *OsSAUR51* expression was induced by infection of the pathogen and exogenous auxin treatment, whereas the *OsSAUR51* gene in the XC20 insertion mutant lost its function due to the *Tos17* insertion. We transformed the *OsSAUR51* gene with the 1.0 kb *OsSAUR51* native promoter into XC20 for complementation analysis and also introduced the *OsSAUR51* gene controlled by RexPhi, an enhanced CaMV 35S promoter, into the rice cultivar 'Dontokoi' to make transgenic plants with enhanced expression of the *OsSAUR51* gene. The *OsSAUR51*-transformed XC20 recovered its bacterial blight resistance, and the RexPhi::OsSAUR51-transformed 'Dontokoi' showed enhanced bacterial blight resistance as compared to untransformed 'Dontokoi'. Auxin accumulation after the bacterial blight inoculation of XC20 was comparatively higher than that of 'Nipponbare', but the auxin levels of *OsSAUR51*-transformed XC20 were lower than those of untransformed XC20. Auxin accumulation after bacterial blight infection in the RexPhi::OsSAUR51-transformed 'Dontokoi' plants was repressed as compared to untransformed 'Dontokoi'. These results suggest that the *OsSAUR51* gene induces a field resistance mechanism and represses auxin accumulation after bacterial blight pathogen infection.

Discipline: Plant protection
Additional key words: mutant, SAUR (small auxin-up RNA), Tos17, *Xanthomonas oryzae*

Introduction

The bacterial blight pathogen (*Xanthomonas oryzae* pv. *oryzae*) is one of the most destructive pathogens of rice in southwestern Japan and South East Asia. There are two types of disease resistance: race-specific true resistance and race-nonspecific field resistance. True resistance is characterized by a high degree of disease resistance in which a few or no pathogenic lesions appear, dominant resistance genes respond to specific races of pathogens, and about 38 resistance genes are known in rice (Khan et al. 2014). Plants with true resistance may become susceptible to other pathogenic races. In contrast, field resistance provides broad-spectrum and durable resistance to disease with little possibility of breakdown against a new pathogenic race. The *Pi21* and *Pi35* genes were identified as field resistance genes to blast disease in rice (Fukuoka et al. 2009, 2013). Several studies are being conducted on field resistance to bacterial leaf blight disease; however, the mechanism for field resistance remains to be elucidated. In order to investigate rice field resistance genes against bacterial blight disease, we inoculated the bacterial blight pathogen race IIIA to retrotransposon *Tos17*-tagged mutant lines of 'Nipponbare' (Miyao et al. 2007) and selected XC20, a line with reduced bacterial blight resistance (Aoki et al. 2006). In this study, we searched for the gene whose function was lost by the insertion of *Tos17* in the XC20 insertion mutant and identified an auxin-related *OsSAUR51* gene.

Auxin affects the expression of many genes and exerts various effects on cell expansion, cell division, and differentiation. In addition, recent studies suggest that auxin plays important roles in plant-pathogen interactions (Bari & Jones

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2009, Kazan & Manners 2009). O'Donnell et al. (2003) reported that the level of IAA increases and reaches its maximum at 72 hours after inoculation of *Arabidopsis* with *Xanthomonas campestris* pv. *campestris*. Moreover, auxin suppresses the induction of chitinase and β-glucanase that are typical pathogenesis-related proteins (Shinshi et al. 1987, Jouanneau et al. 1991). These studies indicate that bacterial infection induces local auxin accumulation and an eventual increase in susceptibility to disease.

Early auxin response genes have been grouped into three major classes: the *Aux/IAA* (auxin/indole acetic acid), *GH3* (Gretchen Hagen 3), and *SAUR* (small auxin-up RNA) gene families (Hagen & Guilfoyle 2002). The *SAUR* gene family members are early auxin response genes in an auxin-signaling pathway. The *SAUR* genes were first identified in soybean hypocotyls (McClure & Guilfoyle 1987). Although many homologous genes in the SAUR family have been identified in plants, the biochemical functions of proteins in this family remain largely unknown. The soybean *SAUR* gene was induced within 2-5 min after auxin treatment, indicating that auxin plays an important role in the transcriptional regulation of *SAUR* genes (Franco et al. 1990). Some SAUR family members (*upa1* to *5*) in peppers (*Capsicum annuum*) are induced by AvrBs3, a type III effector protein in *Xanthomonas campestris* pv. *vesicatoria* (Marois et al. 2002). This report suggests that some SAUR proteins are induced by pathogenic infection. Recent studies on the *SAUR* families have indicated that SAUR proteins suppress the synthesis of auxin and polar auxin transport (Kant et al. 2009), promote cell expansion (Spartz et al. 2012) and hypocotyl/stamen filament elongation (Chase et al. 2012), and activate plasma membrane H^+-ATPases by interacting with PP2C-D phosphatases (Spartz et al. 2014). Jain et al. (2006) found 58 *OsSAUR* gene family members in the 'Nipponbare' genome by conducting a reiterative database search with manual reannotation. In this report, we show the effect of the *OsSAUR51* gene on bacterial blight resistance and discuss the mechanism of auxin-related field resistance to bacterial disease. The results of our experiments suggested that the *OsSAUR51* gene was induced by auxin accumulation after bacterial blight infection, and led to bacterial blight resistance and repressed auxin accumulation.

Material and methods

1. Plant materials and bacterial strains

The seeds of rice cultivars 'Nipponbare', XC20 and 'Dontokoi' were harvested from the fields at our research center. The bacterial blight strains T7174 (race IA), T7147 (race II), T7133 (race IIIA), H75373-1 (race IV), and H75304-4 (race V) were used to investigate bacterial blight resistance.

2. Isolation of the *OsSAUR* gene

Previously, we identified a 5.7 kb fragment in *Xba* I-digested genomic DNA of the XC20 insertion mutant that was closely associated with a decrease in bacterial blight resistance (Aoki et al. 2006). A 3.0 kb DNA fragment with *Tos17* and the adjacent DNA was amplified from the 5.7 kb DNA fragment by an inverse PCR method (Izawa 1997) using primers IPCRTos1 (5′-TTGCACTAGAGAAC TGAGTA-3′) and IPCRTos2 (5′-CAAGTCGCTGATTTCT TCACCAAGG-3′). The amplified 3.0 kb DNA fragment was subcloned in the pDrive vector of the PCR Cloning Kit (Qiagen, Hilden, Germany), and the nucleotide sequence adjacent to *Tos17* was determined using a CEQ8000 Sequencer (Beckman Coulter, California, USA).

Dr. Eiichi Minami of the National Institute of Agrobiological Sciences (NIAS) provided a rice genomic library of 'Nipponbare' (Minami et al. 1989). The 1.0 kb *OsSAUR51* DNA fragment including about 0.3 kb of the *OsSAUR51* promoter region, the *OsSAUR51* ORF, and about 0.3 kb of the *OsSAUR51* 3'-untranslated region was amplified by PCR from the genomic DNA of 'Nipponbare' with primers OsSAUR51-5-1 (5′-ACTAGTCCACTGCAG CAGCA-3′) and OsSAUR51-3-1 (5′-ATACCATGTTTT GCAGCTGCCA-3′) (Fig. 2). A genomic clone containing the *OsSAUR51* gene was screened from the rice genomic library using the 1.0 kb *OsSAUR51* DNA fragment as a probe with the AlkPhos Direct Labeling and Detection System Kit (GE Healthcare, Little Chalfont, UK).

3. Transformation of the *OsSAUR51* gene into XC20

The *OsSAUR51* complementation vector was constructed by fusing the 2.2 kb *Sma* I – *Xba* I DNA fragment containing the *OsSAUR51* gene with the 1.0 kb *OsSAUR51* native promoter and a HPT (hygromycin B phosphotransferase) selectable marker in the pPZP202 binary vector (Hajdukiewicz et al. 1994). The *OsSAUR51* complementation vector was used for transforming XC20 with *Agrobacterium tumefaciens* strain EHA 101. Transformed calli were selected with 50 μg/ml hygromycin (Toki 1997). Southern hybridization confirmed the presence of the *OsSAUR51* gene in *OsSAUR51*-transformed XC20 (T_0) by using the 1.0 kb *OsSAUR51* DNA fragment as a probe with the AlkPhos Direct Labeling and Detection System Kit.

4. Transformation of the RexPhi::*OsSAUR51* gene into rice cultivar 'Dontokoi'

The *OsSAUR51* over-expression vector was constructed by inserting the *OsSAUR51* ORF (open reading frame) with the enhanced CaMV 35S promoter RexPhi (Mitsuhara et al. 1996) and NOS terminator into the *Bam* HI/*Kpn* I site in the pPZP202 binary vector. The *OsSAUR51* ORF was amplified by PCR from 'Nipponbare' genomic DNA with primers OsSAUR51-5-Bam

(5′-GCAGGATCCATGATCCATTCCAAGAAGCTC-3′) and OsSAUR51-3-Kpn (5′-CTTCAGGTACCTTTAGCTA CAAACTGCAAA-3′) that were designed to artificially introduce the 5'-*Bam* HI and 3'-*Kpn* I sites flanking the *OsSAUR51* ORF. The *OsSAUR51* over-expression vector was used to transform the rice cultivar 'Dontokoi' with *A. tumefaciens* strain EHA 101. Transformed calli were selected with 50 μg/ml hygromycin (Toki 1997). 'Dontokoi' lacks a true resistance gene, but does have intermediate field resistance against bacterial blight pathogens. The nucleotide sequences of the *OsSAUR51* gene in 'Dontokoi' were identical to those in 'Nipponbare', thereby making it easier to monitor bacterial blight field resistance. Southern hybridization confirmed the transformed *OsSAUR51* gene in the RexPhi::OsSAUR51-transformed 'Dontokoi' (T_0) by using the 1.0 kb *OsSAUR51* DNA fragment as a probe with the AlkPhos Direct Labeling and Detection System Kit.

5. Analysis of *OsSAUR51* mRNA expression

The XC20 insertion mutant and 'Nipponbare' were grown in a greenhouse, and bacterial blight race IIIA was inoculated on their leaves or 10 μM IAA was applied at the five-leaf stage. Leaves were harvested at 0, 3, 5, 7, 9, and 14 days after bacterial blight inoculation or at 0, 0.2, 0.5, 1, 2, 3, 6, 12, 24, and 48 h after IAA treatment. Total RNAs were isolated from these leaves with an RNeasy Plant Mini Kit (Qiagen). Each 1μg of the isolated total RNAs was analyzed by RT-PCR with an mRNA Selective PCR Kit (TaKaRa) using primers OsSAUR51RT-5 (5′-AAGCAGCCGCCATGATCCAT-3′) and OsSAUR-51RT-3 (5′-TCTTCAGAAACCTTTAGCTA-3′). The amplified 459 bp signal was then subcloned into pDrive and sequenced.

Transcript abundance seven days after inoculation with bacterial blight pathogens in the *OsSAUR51*-transformed XC20 mutant was analyzed by RT-PCR with an mRNA Selective PCR Kit (TaKaRa) using primers OsSAUR51RT-5 and OsSAUR51RT-3. Transcript abundance under normal conditions in the RexPhi::OsSAUR51-transformed 'Dontokoi' was analyzed by RT-PCR with an mRNA Selective PCR Kit (TaKaRa) using primers OsSAUR51-5-Bam and OsSAUR51-3-Kpn.

6. Inoculation of bacterial blight pathogens and investigation of bacterial blight resistance

To investigate the field resistance of XC20, we inoculated the leaves of field-grown rice plants at the heading stage with 1×10^7 CFU/ml of bacterial blight strain races IA, II, IIIA, IV, and V by using the clipping method (Kauffman et al. 1973, Horisue 1996). The lengths of lesions of XC20 and 'Nipponbare' were measured four weeks after inoculation. The mean lengths of 30 lesions per genotype were also calculated. To investigate the effect of the *OsSAUR51* gene on bacterial blight resistance, bacterial blight strain race IIIIA was similarly inoculated onto the rice leaves of XC20, 'Nipponbare', 'Dontokoi', the *OsSAUR51*-transformed XC20, and RexPhi::OsSAUR51-transformed 'Dontokoi' in a full-containment greenhouse. The lengths of lesions found on these transgenic lines, and untransformed XC20, 'Nipponbare', and 'Dontokoi' were measured four (T_0) or three weeks (T_1) after inoculation. The mean lengths of ten lesions (T_0) or five lesions (T_1) per genotype were then compared.

7. Measurement of IAA content

Leaves of the *OsSAUR51*-transformed XC20 (T_1), RexPhi::OsSAUR51-transformed 'Dontokoi' (T_1), untransformed XC20, 'Nipponbare', and 'Dontokoi' were harvested three weeks after inoculation with bacterial blight race IIIA. The IAA content in the leaves of each genotype was measured using the method described by Ishimaru et al. (2013). Five leaves were homogenized in liquid N_2 and soaked in 80% acetone in water (4 ml) containing 2.5 mM diethyl dithiocarbamate for 3 h at 4°C. After adding 0.1 nmol of [phenyl-$^{13}C_6$]-IAA as an internal standard, the extraction procedure was repeated. The combined extract was concentrated under reduced pressure, with 1% formic acid in water being added to the residue prior to applying on a Sep-Pak C18 cartridge (Waters). The cartridge was washed with 0.1% formic acid in water, and IAA was eluted with 70% acetonitrile in water. The amount of IAA in the eluate was then quantified by HPLC-ESI-MS/MS as previously described (Ishimaru et al. 2003).

Results

1. Investigation of the field resistance of XC20 against five bacterial blight races

To investigate the race-specific resistance of the insertion mutant, we inoculated five bacterial blight races (race IA, II, IIIA, IV, and V) onto the XC20 insertion mutant and 'Nipponbare' (Fig. 1). XC20 (Fig. 1: XC20) had significantly longer lesions than 'Nipponbare' (Fig. 1: WT) for all five bacterial blight races.

2. Identification and isolation of the *OsSAUR* gene from XC20

We cloned and determined the nucleotide sequences adjacent to the *Tos17* insertion of the 5.7 kb *Xba* I-digested DNA fragment from XC20 by inverse PCR, and used the sequence as a query for a BLAST search of the nr-nt databases at GenomeNet <http://www.genome.jp/>. *Tos17* was inserted at the 30,730th nucleotide of a BAC clone of chromosome 9 (AP005862) (Fig. 2). A start codon for a 432 bp ORF was found 9 bp downstream and a TATA-like box was

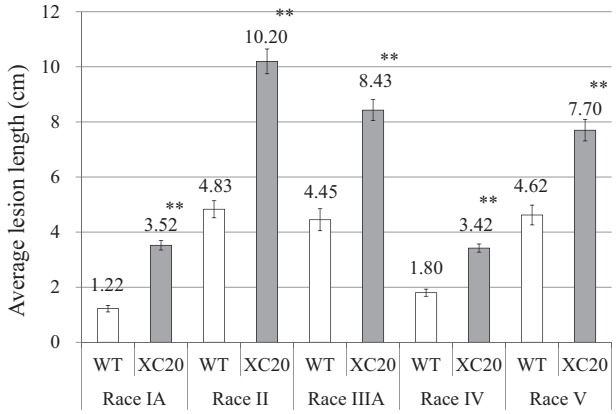

Fig. 1. Comparison of bacterial blight field resistance between 'Nipponbare' (WT) and XC20 (XC20)
The vertical axis is the average length (cm) of 30 lesions four weeks after inoculation with bacterial blight pathogens. Data are the means ± SE. **: Statistical difference from WT at the 1% level.

found 67 bp upstream of the *Tos17* insertion. We identified the *OsSAUR51* gene, a member of the rice *SAUR* gene family (Jain et al. 2006), through a BLAST search of the KEGG GENES database at GenomeNet.

3. Analysis of the OsSAUR51 protein

The deduced amino acid sequence of the *OsSAUR51* gene had 144 amino acids with a calculated molecular weight of 15.6 kDa and an isoelectric point (pI) of 6.07. The amino acid sequence of the OsSAUR51 protein was analyzed by the MOTIF search function of the Pfam database at GenomeNet. A motif for "PF02519, auxin responsive protein" was found between the 8th and 103rd amino acids of the deduced sequence of the OsSAUR51 protein. Several SAUR proteins homologous to the OsSAUR51 protein were found via BLAST search at GenomeNet, and the amino acid sequence of OsSAUR51 protein was compared with other homologous OsSAUR proteins by CLUSTALW analysis. The OsSAUR51 protein had little homology with most of the OsSAUR family proteins in 'Nipponbare'. Only two OsSAUR proteins had over 60% similarity to the OsSAUR51 protein. The OsSAUR51 protein had 78% and 66% similarity to the OsSAUR40 and OsSAUR42 proteins, respectively (Fig. 3). A pathogenesis-induced SAUR protein upa5 from *Capsicum annuum* had 36% similarity to the OsSAUR51 protein.

4. Analysis of *OsSAUR51* mRNA expression

We investigated *OsSAUR51* mRNA induction by bacterial blight infection as well as by auxin treatment using RT-PCR (Fig. 4). The amplified *OsSAUR51* mRNA signal was scarcely observed in XC20 (Fig. 4 A: XC20); however, the mRNA signal was observed from 5 to 14 days after inoculation of 'Nipponbare' with bacterial blight pathogens (Fig. 4 A: WT). The signal was also faint in XC20 (Fig. 4 B: XC20), but was observed from 0.2 to 24 h after IAA treatment of 'Nipponbare' (Fig. 4 B: WT). The nucleotide sequence of the amplified DNA fragment was same as the sequence of the *OsSAUR51* gene.

5. Effect of transforming XC20 with the *OsSAUR51* gene on bacterial blight resistance and auxin accumulation

The *OsSAUR51*-transformed XC20 lines (OsSAUR51-1 and OsSAUR51-2) and 'Nipponbare' were

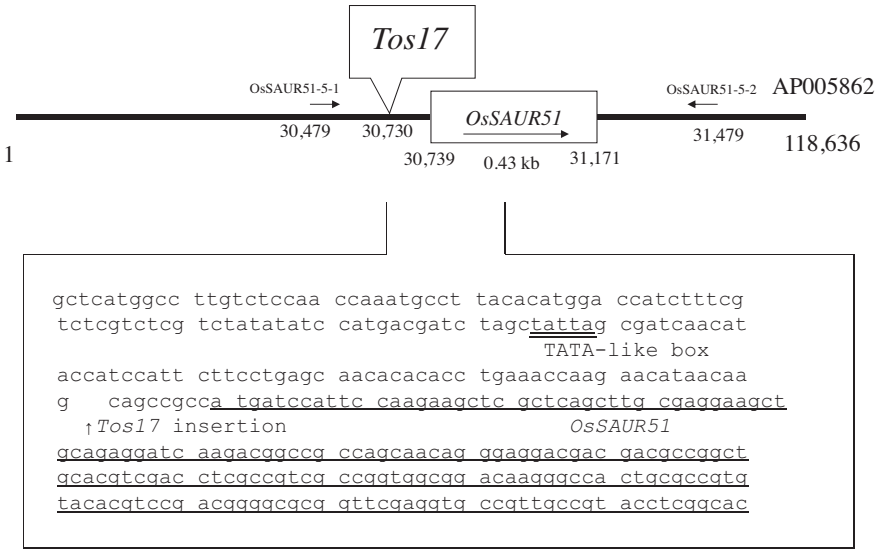

Fig. 2. Analysis of the *Tos17* insertion in XC20
The *Tos17* inserted region is indicated with an upward arrow. The BAC clone is accession number AP005862 in the GenomeNet database. The ORF of the *OsSAUR51* gene is underlined; a TATA-like box is double underlined. Primers OsSAUR51-5-1 and OsSAUR51-3-1 are indicated with arrows.

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OsSAUR51   MIHSKKLAQLARKLQRIKTAASNRE------DDDAGCTSTSPSPVADKGHCAVYTSDGAR
OsSAUR40   .....R......R...V..T.AR--------E...C..T---.......R.TM..A..R.
OsSAUR42   ...A...........QKMVS..AGSGRHTAGTSHDC.ST---ASL.G........A....
upa5       .LSA...IKM..RW.KFAAKQRK.ISFPRNNSNAD..STP.-.SIVE...FV...I.QT.
                  <-------------------------------------------------
                         auxin responsive protein motif (PF02519.6)

OsSAUR51   FEVPLPYLGTTVFVELLRMSQEEFGFAGGDGRITLPCDAAAMEYVMCLLRR-NASEEVER
OsSAUR40   .K...........G..............-...........V..........-....D...
OsSAUR42   ..........A..G...T..H......SE......T..TSV..........-D..K....
upa5       YVF..T..ENE.VMQ..N..E....LPS-G.P......SSF.D.IIS.IKKGV.A.DLHN
           ---------------------------------------------->

OsSAUR51   AFLSSVVTMPCQNSGCTMPPVALHHQFAVCS   Homology
OsSAUR40   .......-.S..D.S.GV......Q..S...S     78%
OsSAUR42   ...C.MA-...H.V.------V.N..L...T      66%
upa5       .I.L.IPSCC.ST.S.HQE--SGNQ.IF..       36%
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Fig. 3. Comparison of the deduced amino acid sequences of the *OsSAUR51* gene with other SAUR proteins
OsSAUR51, OsSAUR40, and OsSAUR42 (all encoded by a single BAC clone AP005862): OsSAUR proteins in rice; upa5: a pepper (*C. annuum*) protein upregulated by a type III effector protein (AvrBs3) of *X. campastris* pv. *Vesicatoria* (AF492629). Dots (.) indicate amino acid residues identical to those of the OsSAUR51 protein; dashes (-) indicate gaps introduced to maximize the alignment. Left right arrow indicates the auxin responsive protein motif (PF02519).

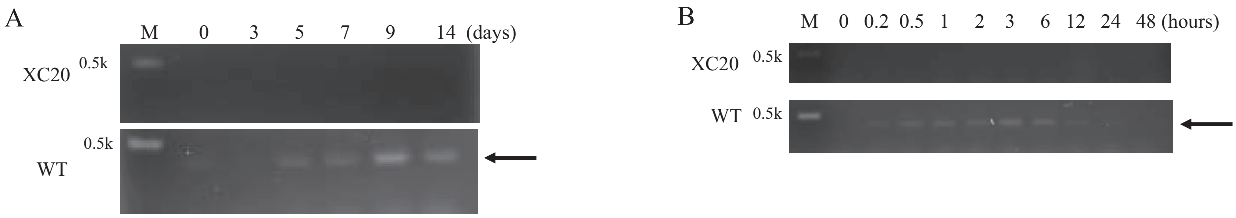

Fig. 4. Effects of bacterial blight infection (A) and auxin treatment (B) on *OsSAUR51* mRNA expression measured by RT-PCR
WT: 'Nipponbare', XC20: XC20 insertion mutant. Lane numbers: Days after bacterial blight infection (A) and hours after 10 µM IAA treatment (B). Lane M: 0.5 kb signal of a 1 kb DNA ladder marker. Expressed *OsSAUR51* mRNA transcripts are indicated with arrows.

inoculated with bacterial blight race IIIA. *OsSAUR51* transcripts were suppressed in XC20; however, *OsSAUR51* transcripts accumulated seven days after inoculation with bacterial blight pathogen race IIIA in 'Nipponbare' and OsSAUR51-1 and OsSAUR51-2 (Fig. 5 A). Although the lesions in XC20 (12.90 cm) were longer than those in 'Nipponbare' (7.40 cm), lesion lengths in OsSAUR51-1 and OsSAUR51-2 (9.30 and 8.80 cm, respectively) were equivalent to those in 'Nipponbare' (Fig. 5 B and C). We also investigated bacterial blight resistance and auxin accumulation in the next generation (T_1) of transgenic lines. A recovery of bacterial blight resistance compared to XC20 (average lesion length = 7.10 cm) was also observed in the OsSAUR51-1 and OsSAUR51-2 lines (average lesion length = 2.42 and 3.76 cm, respectively) (Fig. 5 D). The IAA content increased about 2.7-fold three weeks after inoculation with bacterial blight pathogens (0.78 vs. 2.07 nmol/gFW) in 'Nipponbare' (Fig. 5 E). The IAA content three weeks after inoculating XC20 with bacterial blight pathogens (3.23 nmol/gFW) was higher than that in 'Nipponbare', but the IAA content in the OsSAUR51-1 and OsSAUR51-2 lines (2.02 and 2.20 nmol/gFW, respectively) was lower than that in XC20 and comparable to that in 'Nipponbare'. These results indicate that the introduced *OsSAUR51* gene complemented the XC20 mutation.

6. Effects of OsSAUR51 overexpression on bacterial blight resistance and auxin accumulation

We introduced *OsSAUR51* ORF with the RexPhi enhanced CaMV 35S promoter into the rice cultivar 'Dontokoi', and then obtained three RexPhi::OsSAUR51-transformed 'Dontokoi' lines (RexPhi::OsSAUR51-1, RexPhi::OsSAUR51-2, and RexPhi::OsSAUR51-3). *OsSAUR51* mRNA expression in the uninfected leaves of 'Dontokoi' and the RexPhi::OsSAUR51-1, RexPhi::OsSAUR51-2, and RexPhi::OsSAUR51-3 lines (T_0) was analyzed by RT-PCR (Fig. 6 A). High levels of *OsSAUR51* transcripts were present in all three transgenic lines. 'Dontokoi' and the RexPhi::OsSAUR51-1, RexPhi::OsSAUR51-2, and RexPhi::OsSAUR51-3 lines were inoculated with bacterial blight pathogen race IIIA (Fig. 6 B and C). Lesion lengths in the RexPhi::OsSAUR51-1,

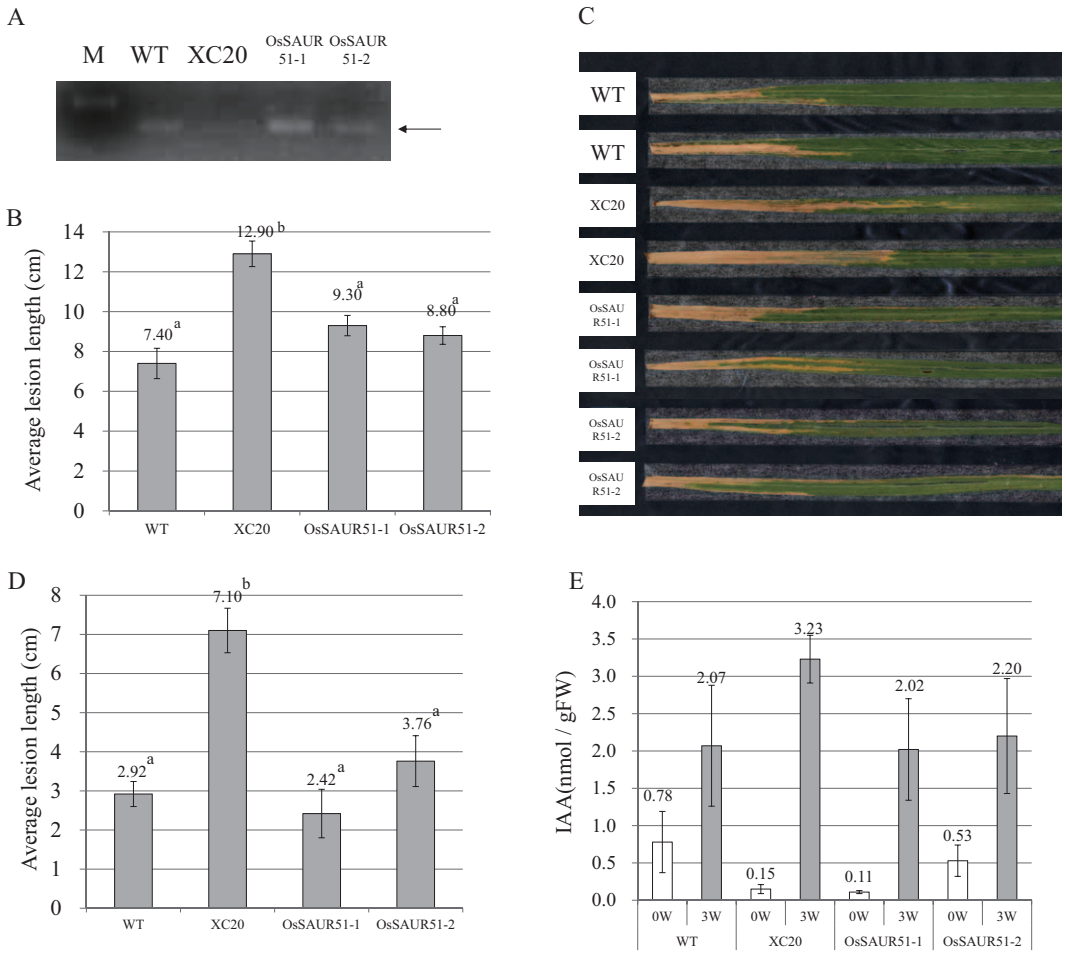

Fig. 5. Field resistance recovery against bacterial blight race IIIA and auxin accumulation of the *OsSAUR51*-transformed XC20 lines
WT: 'Nipponbare'; XC20: XC20 insertion mutant; OsSAUR51-1 and OsSAUR51-2: *OsSAUR51*-transformed XC20 lines. (A) *OsSAUR51* mRNA expression seven days after inoculation with bacterial blight pathogens measured by RT-PCR. Lane M: 0.5 kb signal of a 1 kb DNA ladder marker. (B) Average length (cm) of ten lesions four weeks after inoculation with bacterial blight race IIIA in WT, XC20, OsSAUR51-1, and OsSAUR51-2 (T_0). Data are the means ± SE, and statistically and intentionally different between species a and b at the 1% level. (C) Lesions on the leaves of WT, XC20, OsSAUR51-1, and OsSAUR51-2 (T_0) four weeks after inoculation with bacterial blight race IIIA. (D) Average length (cm) of five lesions three weeks after inoculation with bacterial blight race IIIA in WT, XC20, OsSAUR51-1, and OsSAUR51-2 lines (T_1). Data are the means ± SE, and statistically and intentionally different between species a and b at the 1% level. (E) Average IAA content of the leaves at weeks 0 and 3 after inoculation with bacterial blight race IIIA in WT, XC20, OsSAUR51-1, and OsSAUR51-2 lines (T_1). Data are the means ± SE.

RexPhi::OsSAUR51-2, and RexPhi::OsSAUR51-3 lines (7.25, 5.30, and 6.10 cm, respectively) were shorter than those in 'Dontokoi' (8.90 cm). We also investigated bacterial blight resistance and auxin accumulation in the next generation (T_1) of transgenic lines. Based on the lesion length, enhanced bacterial blight resistance was observed in the RexPhi::OsSAUR51-1, RexPhi::OsSAUR51-2, and RexPhi::OsSAUR51-3 lines (T_1) (3.04, 3.64, and 4.30 cm, respectively) as compared to 8.62 cm for 'Dontokoi' (Fig. 6 D). The IAA content in 'Dontokoi' three weeks after inoculation with bacterial blight pathogen was about 1.8-fold higher than the non-inoculated control (1.73 vs. 3.10 nmol/gFW), but the IAA content in the RexPhi::OsSAUR51-1, RexPhi::OsSAUR51-2, and RexPhi::OsSAUR51-3 lines (1.27, 1.57, and 2.55 nmol/gFW, respectively) was lower than in 'Dontokoi' at three weeks after inoculation (3.10 nmol/gFW) (Fig. 6 E).

Discussion

There are two types of rice bacterial blight disease resistance: race specific true resistance and race nonspecific field resistance. The Japanese rice cultivar 'Nipponbare' lacks a true resistance gene, but shows relatively high field resistance against bacterial blight pathogens. Lesions from five bacterial blight pathogen races inoculated on retrotrans-

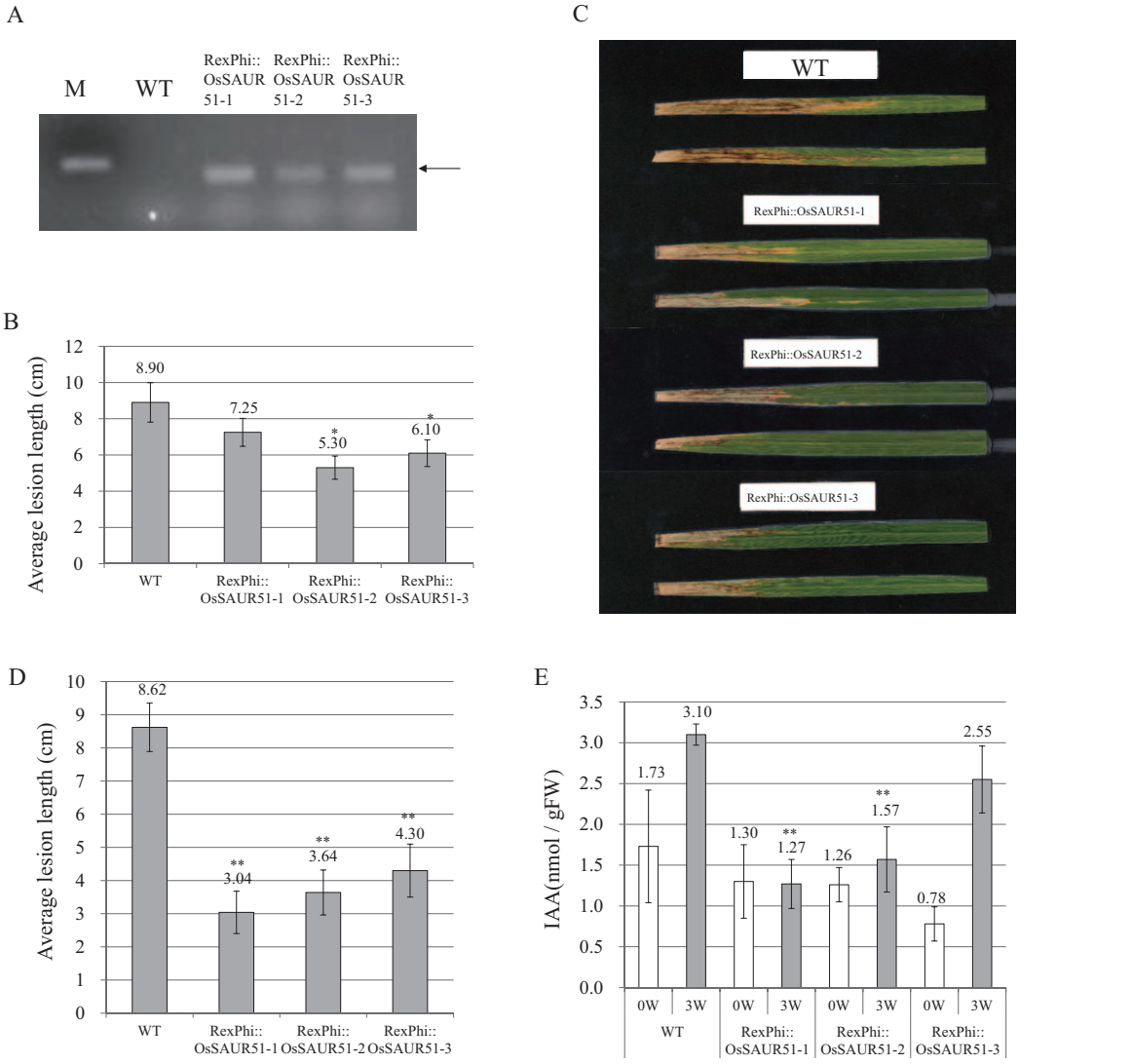

Fig. 6. Field resistance enhancement against bacterial blight race IIIA and auxin accumulation of the RexPhi::OsSAUR51-transformed 'Dontokoi'. WT: 'Dontokoi'; RexPhi::OsSAUR51-1, RexPhi::OsSAUR51-2, and RexPhi::OsSAUR51-3 lines: *OsSAUR51*-transformed 'Dontokoi'

(A) *OsSAUR51* mRNA expression under normal conditions measured by RT-PCR. Lane M: 0.5 kb signal of a 1 kb DNA ladder marker. (B) Average length (cm) of ten lesions four weeks after inoculation with bacterial blight race IIIA in WT, RexPhi::OsSAUR51-1, RexPhi::OsSAUR51-2, and RexPhi::OsSAUR51-3 lines (T_0). Data are the means ± SE. *: Statistical differences from WT at the 1% level. (C) Lesions on the leaves of WT, RexPhi::OsSAUR51-1, RexPhi::OsSAUR51-2, and RexPhi::OsSAUR51-3 lines (T_0) four weeks after inoculation with bacterial blight race IIIA. (D) Average length (cm) of five lesions three weeks after inoculation with bacterial blight race IIIA in WT, RexPhi::OsSAUR51-1, RexPhi::OsSAUR51-2, and RexPhi::OsSAUR51-3 lines (T_1). Data are the means ± SE. **: Statistical differences from WT at the 1% level. (E) Average IAA content of the leaves at weeks 0 and 3 after inoculation with bacterial blight race IIIA in WT, RexPhi::OsSAUR51-1, RexPhi::OsSAUR51-2, and RexPhi::OsSAUR51-3 lines (T_1). Data are the means ± SE. **: Statistical differences from WT at the 1% level.

poson *Tos17*-tagged XC20 were longer than those on 'Nipponbare', and no race specificity in bacterial blight resistance was observed (Fig. 1). Our observations suggested that the *Tos17* insertion caused a loss-of-function mutation in a field resistance gene in the XC20 insertion mutant. The *OsSAUR51* gene was found to exist close to the *Tos17* insertion site in XC20 (Fig. 2). A complementation test conducted by using the clipping method (Horisue 1996) revealed that the *OsSAUR51* gene was involved with bacterial blight field resistance in rice.

Jain et al. (2006) divided the OsSAUR proteins into two major groups (A and B). Although belonging to group A, the OsSAUR51 protein had little homology with most other members of OsSAUR family protein group A. Only two proteins in group A—OsSAUR40 and OsSAUR42—had over 60% homology with the OsSAUR51 protein (Fig.

3). And most of the OsSAUR proteins are basic (pI > 7.0), and nine OsSAUR proteins (OsSAUR 2, 7, 13, 33, 34, 35, 38, 51, and 58) including OsSAUR51 are acidic (pI < 7.0) out of a total of 58 OsSAUR family members. The pepper upa5 protein was also induced by pathogenic infection; however, the pepper protein has only 36% homology with the OsSAUR51 protein and the two deduced proteins have no unique homologous domains. These results indicate that SAUR proteins whose expression is induced by pathogenic infection have no special domains.

The *OsSAUR51* mRNA in 'Nipponbare' was induced five days after inoculation with bacterial blight pathogens and 0.2 h after IAA treatment, but the *OsSAUR51* mRNA signal was not observed in the XC20 insertion mutant even after inoculation with bacterial blight pathogens or after IAA treatment (Fig. 4). The retrotransposon *Tos17* was inserted between the start codon and a TATA-like box of *OsSAUR51* in XC20, indicating that *OsSAUR51* expression is probably prevented by the *Tos17* insertion (Fig. 2). These results suggest that the suppression of *OsSAUR51* mRNA accumulation resulted in lower bacterial blight resistance. O'Donnell et al. (2003) reported that IAA accumulated after the inoculation of *Arabidopsis* with *X. campestris* pv. *campestris*. In our analysis, the *OsSAUR51* mRNA was induced more rapidly after auxin treatment than after bacterial blight infection. This result leads to a testable hypothesis that *OsSAUR51* mRNA may have been induced by auxin accumulation after bacterial blight infection.

Bacterial blight resistance was recovered in transgenic XC20 (Fig. 5 B, C, and D), indicating that the suppression of *OsSAUR51* mRNA expression lowered the bacterial blight resistance in XC20. The IAA content three weeks after bacterial blight inoculation in XC20 was about 1.6-fold higher than in 'Nipponbare' (2.07 vs. 3.23 nmol/gFW), and the IAA accumulation phenotype of XC20 was complemented in the transformants OsSAUR51-1 and -2 (2.02 and 2.20 nmol/gFW, respectively) (Fig.5 E). These results may suggest that the *OsSAUR51* gene confers field resistance by suppressing auxin accumulation after bacterial blight infection.

To further investigate the effect of the OsSAUR protein's enhanced function, we obtained *OsSAUR51* overexpression transformants in the rice cultivar 'Dontokoi' (RexPhi::OsSAUR51-1, RexPhi::OsSAUR51-2, and RexPhi::OsSAUR51-3). The formation of bacterial blight lesions was repressed in correlation with overexpression of the OsSAUR51 protein in the RexPhi::OsSAUR51-1, RexPhi::OsSAUR51-2, and RexPhi::OsSAUR51-3 lines, thus confirming that the *OsSAUR51* gene functions in field resistance (Fig. 6 B, C, and D.). Recently, several researchers have overexpressed the early auxin response genes in rice to investigate the relationship between auxin synthesis and pathogenesis resistance. Transgenic rice plants overexpressing the rice *SAUR39* gene had a lower auxin level, reduced polar auxin transport, and downregulated expression of some putative auxin biosynthesis genes and transporter genes, suggesting that the SAUR39 protein functions as a negative regulator for auxin synthesis and transport (Kant et al. 2009). Transgenic rice plants constitutively expressing the rice early auxin response genes GH3-8 and OsGH3.1 had increased resistance against rice blast and bacterial blight diseases (Ding et al. 2008, Domingo et al. 2009). These results suggest that some early auxin-induced proteins such as Aux/IAA, GH3, and SAUR may protect plants by reducing auxin accumulation to suppress the expansion of pathogenesis. The morphology of the RexPhi::OsSAUR51-1, RexPhi::OsSAUR51-2, and RexPhi::OsSAUR51-3 lines was indistinguishable from that of 'Dontokoi', and only a very slight difference in IAA content was observed between 'Dontokoi' (1.73 nmol/gFW) and the RexPhi::OsSAUR51-1, RexPhi::OsSAUR51-2, and RexPhi::OsSAUR51-3 lines (1.30, 1.26, and 0.78 nmol/gFW, respectively) before bacterial blight pathogen inoculation (Fig. 6 E). Although the IAA content rapidly increased after inoculation with bacterial blight pathogens in 'Dontokoi' (from 1.73 to 3.10 nmol/gFW, respectively), IAA accumulation remained unchanged even after infection in the RexPhi::OsSAUR51-1, RexPhi::OsSAUR51-2, and RexPhi::OsSAUR51-3 lines (from 1.30, 1.26, and 0.78 to 1.27, 1.57, and 2.55 nmol/gFW, respectively). These results suggest that the OsSAUR51 protein specifically suppresses auxin accumulation after bacterial infection.

In summary, our results confirmed that the *OsSAUR51* gene functions as a field resistance-related gene that is induced by auxin accumulation upon bacterial blight infection, thereby enhancing bacterial blight resistance and suppressing auxin accumulation.

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