Suppression of the Rice Fatty-Acid Desaturase Gene *OsSSI2* Enhances Resistance to Blast and Leaf Blight Diseases in Rice

Chang-Jie Jiang,¹ Masaki Shimono,¹ Satoru Maeda,¹ Haruhiko Inoue,¹ Masaki Mori,¹ Morifumi Hasegawa,² Shoji Sugano,¹ and Hiroshi Takatsui¹

¹Plant Disease Resistance Research Unit, Division of Plant Science, National Institute of Agrobiological Sciences, Kannondai 2-1-2, Tsukuba, 305-8602 Japan; ²College of Agriculture, Ibaraki University, Ami 300-0393, Japan

Submitted 3 October 2008. Accepted 9 March 2009.

Fatty acids and their derivatives play important signaling roles in plant defense responses. It has been shown that suppressing a gene for stearyl acyl carrier protein fatty-acid desaturase (SACPD) enhances the resistance of *Arabidopsis* (*SSI2*) and soybean to multiple pathogens. In this study, we present functional analyses of a rice homolog of *SSI2* (*OsSSI2*) in disease resistance of rice plants. A transposon insertion mutation (*OsSSI2-Tos17*) and RNAi-mediated knockdown of *OsSSI2* (*OsSSI2-kd*) reduced the oleic acid (18:1) level and increased that of stearic acid (18:0), indicating that *OsSSI2* is responsible for fatty-acid desaturase activity. These plants displayed spontaneous lesion formation in leaf blades, retarded growth, slight increase in endogenous free salicylic acid (SA) levels, and SA/benzothiadiazole (BTH)-specific inducible genes, including *WRKY45*, a key regulator of SA/BTH-induced resistance, in rice. Moreover, the *OsSSI2-kd* plants showed markedly enhanced resistance to the blast fungus *Magnaporthe grisea* and leaf-blight bacteria *Xanthomonas oryzae* pv. *oryzae*. These results suggest that *OsSSI2* is involved in the negative regulation of defense responses in rice, as are its *Arabidopsis* and soybean counterparts. Microarray analyses identified 406 genes that were differentially expressed (≥ 2 -fold) in *OsSSI2-kd* rice plants compared with wild-type rice and, of these, approximately 39% were BTH responsive. Taken together, our results suggest that induction of SA-responsive genes, including *WRKY45*, is likely responsible for enhanced disease resistance in *OsSSI2-kd* rice plants.

Plants combat pathogen infections by activating various defense pathways in which the plant hormones salicylic acid (SA), jasmonic acid (JA), and ethylene (ET) play major signaling roles (Kachroo and Kachroo 2007). Each hormone mediates distinct but interacting defense pathways. Upon pathogenic infection, endogenous SA levels rapidly increase in many dicots such as *Arabidopsis* and tobacco. This leads to the induction of a battery of pathogenesis-related (PR) genes and the activation of systemic acquired resistance (SAR) (Durrant and Dong 2004; Vallad and Goodman 2004). JA and ET have been

shown to mediate induced systemic resistance (ISR) that is elicited by the colonization of plant roots by certain nonpathogenic rhizobacteria (Bostock 2005; Heil and Bostock 2002; Vallad and Goodman 2004). In many cases, SA- and JA/ET-mediated defense pathways appear to interact antagonistically (Bostock 2005; Felton and Korth 2000; Kunkel and Brooks 2002). It has been shown that a transcriptional coactivator, named nonexpression of PR genes (NPR1), plays a key role in SA signaling and also in the negative crosstalk between SA and JA signaling in *Arabidopsis* (Dong 2004; Pieterse and Van Loon 2004; Spoel et al. 2003).

Defense signaling in rice, the most important cereal crop for human consumption worldwide, appears to differ in some aspects from the signaling in many dicots. One such difference is that the endogenous SA levels in rice are twofold those in dicots, and the SA levels do not increase further in response to pathogen infection (Silverman et al. 1995). This raises questions regarding the role of SA as a defense signaling substance in rice. However, several lines of evidence indicate functional conservation of SA-mediated defense signaling even in rice. A study of 28 modern rice cultivars showed that the SA levels in seedlings significantly correlated with the basal resistance to the blast fungus *Magnaporthe grisea* (Silverman et al. 1995). Depletion of endogenous SA by overexpressing the bacterial *nahG* gene that encodes salicylate hydroxylase increases the susceptibility to avirulent isolates of *M. grisea* (Yang et al. 2004). Exogenous application of benzothiadiazole (BTH), a functional analog of SA, induces defense-related gene expression and enhances resistance to the sheath blight fungus *Rhizoctonia solani* (Rohilla et al. 2002), leaf blight bacteria *Xanthomonas oryzae* pv. *oryzae* (Babu et al. 2003), and *M. grisea* (Schweizer et al. 1999; Shimono et al. 2007). Moreover, overexpression of *Arabidopsis* NPR1 or its rice ortholog *OsNPR1/NH1* in rice plants enhances the resistance to *X. oryzae* pv. *oryzae* (Chern et al. 2001, 2005b; Fitzgerald et al. 2004; Yuan et al. 2007), while RNAi-mediated knockdown of *OsNPR1* compromises the resistance (Yuan et al. 2007). These findings suggest that rice has an SA signaling pathway similar to that in *Arabidopsis*. We have previously identified a rice transcription factor, *WRKY45*, that is highly specifically induced by SA and BTH. Overexpression of this gene dramatically enhanced resistance to *M. grisea* (Shimono et al. 2007) and *X. oryzae* pv. *oryzae* (*unpublished*). Meanwhile, RNAi-mediated knockdown of *WRKY45* almost completely compromised the BTH-induced resistance to these pathogens. Thus, *WRKY45* is a key transcription factor in BTH-induced disease resistance (Shimono et al. 2007). Interestingly, the SA signal-

Corresponding author: Hiroshi Takatsui; Telephone and Fax: +81-29-838-8383; E-mail: takatsuih@affrc.go.jp

Rice Annotation Project (RAP) code Os01g0919900.

*The e-Xtra logo stands for “electronic extra” and indicates that a supplemental table is published online.

ing pathway of rice has two branches: the *WRKY45*-dependent and the *OsNPR1*-dependent paths, downstream of SA (Shimono et al. 2007). This feature is markedly distinct from the SA signaling pathway in *Arabidopsis*, which is primarily mediated by NPR1 (Wang et al. 2006). JA and ET have also been implicated in the rice defense pathway (Iwai et al. 2006; Mei et al. 2006).

In addition to plant hormones, several studies have also unveiled important roles for fatty acids (FA) in mediating the signaling in plant defense responses. Using a genetic screen for suppressors of the *npr1* mutant, the *suppressor of salicylate insensitivity of npr1-5* (*ssi2*) mutant (Shah et al. 2001) was identified, which is defective in a gene encoding stearyl acyl carrier protein (ACP) FA desaturase (SACPD) (Kachroo et al. 2001). SACPD catalyzes the conversion of stearic acid (18:0)-ACP to oleic acid (18:1)-ACP during FA biosynthesis. A mutation in this gene decreases the levels of 18:1 and increases those of 18:0 in *ssi2* plants (Kachroo et al. 2001). The *ssi2* plants exhibit spontaneous lesion formation; severely retarded growth; elevated SA levels; constitutive PR gene expression; and enhanced resistance to *Peronospora parasitica*, *Pseudomonas syringae* (Shah et al. 2001), and *Cucumber mosaic virus* (Kachroo et al. 2001, 2003a; Sekine et al. 2004). On the other hand, *ssi2* plants lack the JA-responsive expression of the antifungal defensin gene *PDF1.2* and are more susceptible to *Botrytis cinerea* (Kachroo et al. 2001). The lack of JA response in *ssi2* plants has been attributed to the absence or reduced levels of a yet unknown JA-coactivating signal because no alteration was observed in the biosynthesis or perception of JA in *ssi2* plants (Kachroo et al. 2003a and b). All these *ssi2* plant phenotypes were completely rescued by restoring the 18:1 levels via loss-of-function mutations in the genes encoding glycerol-3-phosphate acyltransferase (ACT1) (Kachroo et al. 2003a) or G3P dehydrogenase (G3Pdh), which catalyzes the acylation of G3P with 18:1 (Kachroo et al. 2004; Nandi et al. 2003). Moreover, the exogenous application of glycerol to wild-type plants resulted in *ssi2*-like phenotypes, which is consistent with the reduction in the 18:1 levels by this treatment (Kachroo et al. 2004, 2005). These findings strongly suggest an important role for 18:1 in the modulation of SA- and JA-mediated signaling in plants. Similar morphological and defense-related phenotypes have recently been observed in soybean plants when *GmSACPD-A/B* genes were silenced (Kachroo et al. 2008). The reduced 18:1 level also induces several resistance (*R*) genes in both *Arabidopsis* and soybean (Chandra-Shekara et al. 2007; Kachroo et al. 2008). In rice, an *SSI2* homolog gene (*OsSSI2*) was downregulated in the early phases of interaction between the rice resistance gene *Pi33* (resistance cv. IR64) and *M. grisea* avirulence gene *ACE1* (avirulent strain PH14:*ACE1*) (Vergne et al. 2007). No such change in *OsSSI2* expression was observed when IR64 was infected by a virulent strain of *M. grisea*, PH14 (Vergne et al. 2007). These results imply a potential role for *OsSSI2* in defense signaling pathways in rice.

In this study, we performed functional analyses of *OsSSI2* (Os01g0919900), with a focus on its role in the defense response of rice. We show that *OsSSI2* is a rice ortholog of *SSI2* and is involved in the negative regulation of defense responses in rice, similar to its counterparts in *Arabidopsis* and soybean. RNAi-mediated *OsSSI2* knockdown (*OsSSI2*-kd) markedly enhanced the resistance of rice to fungal blast and bacterial leaf-blight diseases—the two most devastating rice diseases worldwide. Microarray analyses revealed extensive overlap of *OsSSI2*-regulated genes with BTH-responsive genes including *WRKY45*, suggesting that the enhanced resistance in *OsSSI2*-kd plants is at least partly due to the upregulation of *WRKY45*, most probably through the activation of the SA signaling pathway.

RESULTS

SACPD-like gene family in rice.

A genomic database search revealed that the rice genome encodes seven SACPD-like proteins that have 46 to 86% amino acid sequence identity with *Arabidopsis* SSI2 (Fig. 1). Phylogenetically, these proteins fall into two subgroups. One includes Os01g0919900 and Os04g0379900, which share 86 and 82% amino acid sequence identity, respectively, with SSI2. The other contains the five remaining members (Os08g0200100, Os03g0423300, Os02g0504800, Os08g0199400, and Os01g0880800), which share 46 to 71% amino acid sequence identity with SSI2 and are more closely related to *Arabidopsis* At1g43800 (SACPD6) and soybean *GmSACPD-C*. Prediction of subcellular targeting by using the TargetP and WoLF PSORT prediction programs suggested that Os01g0919900, Os01g0880800, and Os08g0199400 are localized in the chloroplast or mitochondrion, whereas the others are present in chloroplasts. These predictions are consistent with findings that FA synthesis in plants occurs mainly in plastids (Napier 2007). However, cell biological studies are necessary for the conclusive localization of these proteins within living cells. The protein encoded by Os01g0919900 shared the highest sequence identity with *Arabidopsis* SSI2 (86%), referred to as *OsSSI2*, and it was then further characterized with a focus on its role in disease resistance in rice.

Fig. 1. Phylogenetic tree of stearyl acyl carrier protein (ACP) fatty-acid desaturase (SACPD) family proteins in rice, *Arabidopsis*, and soybean. The protein sequences were aligned with Clustal-X software and the tree was constructed using TreeView software. The Rice Annotation Project (RAP) codes and the *Arabidopsis* Genome Initiative (AGI) codes are shown in the figure. The GenBank accession numbers for soybean proteins are as follows: GmSACPD-A, AAX86050; GmSACPD-B, AAX86049; and GmSACPD-C, ABM45911.

Downregulation of *OsSSI2* affects plant growth and FA profiles.

To characterize the loss-of-function effects of *OsSSI2*, we identified and obtained two lines of *Tos17* insertion mutants for *OsSSI2* (*Osssi2-Tos17*: NF7039 and NF8001) from the Rice Genome Resource Center, Japan (Hirochika 2001; Hirochika et al. 2004). The NF7039 and NF8001 lines had *Tos17* insertions in exon 2 and intron 1 of *OsSSI2*, respectively. *OsSSI2* transcripts of normal size were not observed on an RNA blot in homozygous NF7039 plants. Instead, a band for large-sized fusion transcripts that contained *OsSSI2* with *Tos17* sequences was detected, suggesting that this line is a null mutant. On the other hand, nearly normal levels and sizes of *OsSSI2* transcripts were detected in homozygous NF8001 plants (data not shown); therefore, this line was not characterized further.

The NF7039 plants exhibited spontaneous lesion formation in leaves and severely stunted plant growth (Fig. 2). Most mature leaves died of precocious senescence, leaving only one or two youngest expanded leaves alive (Fig. 2). They had very low fertility; consequently, it was difficult to use these plants for functional analyses of *OsSSI2* in rice disease resistance. As an alternative, we generated transgenic rice lines for RNAi-mediated *OsSSI2*-kd. We obtained 14 independent lines of *OsSSI2*-kd rice with barely detectable *OsSSI2* transcripts in the RNA blot analysis (Fig. 3). Most of these lines showed growth and developmental phenotypes very similar to those of NF7039 (data not shown); however, two lines (i.e., *OsSSI2*-kd-1 and *OsSSI2*-kd-2) showed moderate phenotypes with grain sets approximately 60 to 70% of wild type (Fig. 2). Therefore, these *OsSSI2*-kd lines were mainly used for the following experiments. Expression of the two closest *OsSSI2* homologs was unaffected in both the NF7039 and *OsSSI2*-kd lines (Fig. 3), indicating that the phenotypes observed in these lines are specifically due to *OsSSI2* downregulation.

Determination of the FA composition revealed a significant reduction in the 18:1 level accompanied by a large increase in

18:0 accumulation in the *OsSSI2-Tos17* (NF7039) and *OsSSI2*-kd lines compared with the wild-type plants (Table 1). Some changes were observed in the levels of C16 FA, particularly in the NF7039 line, but the changes in the 16:0 levels were rather smaller than those in C18 FA. These results demonstrate that *OsSSI2* is mainly responsible for desaturase activity toward C18 FA.

Suppression of *OsSSI2* upregulates defense-related gene expression and slightly increased free SA levels.

To investigate the potential functions of *OsSSI2* in the defense response of rice, we analyzed the expression of defense-related genes in NF7039 and *OsSSI2*-kd plants. *OsPR1b* is an SA/JA-responsive gene often used as a marker gene for defense responses in rice (Jwa et al. 2006). *OsPR1b* was expressed at high levels in NF7039 and *OsSSI2*-kd plants (Fig. 3), indicating that defense signaling is activated in these plants. The gene for a transcription factor, *WRKY45*, was also upregulated to high levels in the NF7039 and *OsSSI2*-kd plants (Fig. 3). Previously, we showed that *WRKY45* was highly specifically induced by SA and BTH and encodes a transcription factor that plays a key role in SA/BTH-induced resistance to rice blast disease (Shimono et al. 2007). This observation suggests that the SA signaling pathway is activated in these plants.

In *Arabidopsis*, application of 18:1 rescues *ssi2* phenotypes. To examine the effects of 18:1 application on the phenotype of constitutive *WRKY45* and *PR1b* expression in NF7039 and *OsSSI2*-kd plants (Fig. 3), we applied 18:1 to rice plants. We used leaf discs to infiltrate the FA from their cut ends, thereby circumventing its poor penetration through the leaf surface of rice. Gene expression analysis revealed that applying 18:1 significantly reduced the expression of *WRKY45* and *PR1b* in NF7039 and *OsSSI2*-kd plants, while applying 18:0 caused no effects on the expression of both the genes (Fig. 4). These results demonstrate that the lowered level of 18:1 is the causal factor for the activation of defense responses in NF7039 and *OsSSI2*-kd plants.

Fig. 2. Growth phenotypes of the wild-type (WT), *Osssi2-Tos17* (NF7039), and *OsSSI2*-knockdown (kd) plants. The plants were grown for 4 months in the greenhouse. The insets show spontaneous lesion formation on the leaf blades of *Osssi2-Tos17* (NF7039) and *OsSSI2*-kd plants.

Determination of the endogenous SA content revealed slight but statistically significant (t test, $P \leq 0.05$) increases in the free SA levels in NF7039 and *OsSSI2*-kd plants compared with the wild-type plants (Table 2). In contrast, no alterations were observed in the SA- β -glucoside (SAG) levels, with the exception of an approximately 80% increase in *OsSSI2*-kd-1 plants (Table 2). These results contrast with the observations made with *Arabidopsis* and soybean, in which mutating *SSI2* or si-

lencing of *GmSACPD-A* or *GmSACPD-B* resulted in the accumulation of several-fold higher levels of SA and SAG (Kachroo et al. 2008; Shah et al. 2001). Smallness of the changes in SA levels has been reported in rice for various treatments, which is probably related to the high basal SA levels in rice plants.

Disease resistance is enhanced in *OsSSI2*-kd rice.

Mutation of the *SSI2* gene in *Arabidopsis* (*ssi2*) enhances the resistance to multiple pathogens, including *Hyaloperonospora parasitica*, *P. syringae* pv. *tomato* DC3000, and *Cucumber mosaic virus* (Kachroo et al. 2001, 2003a; Sekine et al. 2004; Shah et al. 2001). In soybean, silencing of *GmSACPD-A/B* genes enhanced the resistance to *P. syringae* pv. *glycinea* and *Phytophthora sojae* (Kachroo et al. 2008). These results, taken together with the upregulation of defense genes in *OsSSI2*-downregulated rice, prompted us to test whether *OsSSI2* downregulation affects the resistance of rice to *M. grisea* and *X. oryzae* pv. *oryzae*, which are the causal

Fig. 3. RNA blot analysis. Transcripts of *OsSSI2*, two *OsSSI2* homologs, *WRKY45*, and *PR1b* were analyzed in wild-type (WT) and *OsSSI2* mutant plants. *OsSSI2* transcripts were not detected in homozygous *Osssi2-Tos17* (NF7039) and *OsSSI2*-knockdown (kd) plants, whereas the expression of the two closest homologs of *OsSSI2* (i.e., *Os04g0379900* and *Os01g0880800*) was unaffected in these plants. The arrow indicates a band of fusion transcripts encoding *OsSSI2* with the *Tos17* insertion. The salicylic acid/benzothiadiazole-induced gene *WRKY45* and the pathogenesis-related gene *OsPR1b* were constitutively expressed in the NF7039 and *OsSSI2*-kd plants. Leaf blades from the fourth leaves of seedlings were used.

Fig. 4. Restoration of the expression of *WRKY45* and *PR1b* after 18:1 application. Segments of leaf blades from seedlings of wild-type (WT), homozygous *Osssi2-Tos17* (NF7039), and *OsSSI2*-knockdown (kd) plants were incubated in mock, solution containing 18:0, or solution containing 18:1 under light for 12 h at 30°C. Transcripts of *WRKY45* and *PR1b* were analyzed by quantitative reverse-transcription polymerase chain reaction. Averages of three determinations relative to those of Rubq1 are shown with standard deviations (SD).

Table 1. Fatty acid (FA) composition in leaves^a

Genotype	Composition (mol% $\pm$ standard deviation)					
	16:0	16:1	18:0	18:1	18:2	18:3
WT	7.93 $\pm$ 1.47	0.36 $\pm$ 0.41	1.13 $\pm$ 0.18	0.41 $\pm$ 0.03	6.00 $\pm$ 0.61	84.18 $\pm$ 1.48
NF7039	15.36 $\pm$ 0.57	0.12 $\pm$ 0.24	21.00 $\pm$ 4.16	0.18 $\pm$ 0.02	9.33 $\pm$ 1.24	54.02 $\pm$ 2.71
<i>OsSSI2</i> -kd -1	11.01 $\pm$ 0.60	0.48 $\pm$ 0.51	7.86 $\pm$ 3.90	0.29 $\pm$ 0.21	6.50 $\pm$ 1.35	73.85 $\pm$ 3.50
<i>OsSSI2</i> -kd -2	14.09 $\pm$ 1.50	0.37 $\pm$ 0.43	15.99 $\pm$ 1.44	0.20 $\pm$ 0.08	6.31 $\pm$ 0.58	63.05 $\pm$ 2.52

^a FA compositions of the leaf blades from the seedlings of wild-type (WT), homozygous *Osssi2-Tos17* (NF7039), and *OsSSI2*-knockdown (kd) plants are calculated for four samples. The characteristic ion of each FA methyl ester was as follows: methyl pentadecanoate (internal standard), m/z 256; methyl palmitoleate, m/z 270; methyl stearate, m/z 298; methyl oleate, m/z 264; methyl linoleate, m/z 294; methyl linolenate, m/z 292.

pathogens of rice blast and rice leaf-blight diseases, respectively. The number of blast lesions (Fig. 5A) and the length of blight lesions (Fig. 5B) were markedly reduced in *OsSSI2*-kd plants compared with wild-type plants. Thus, *OsSSI2* downregulation enhanced the resistance of rice to these two different pathogens.

Table 2. Salicylic acid contents^a

Genotype	SA	SAG
WT	2.25 ± 0.40	8.12 ± 1.85
NF7039	3.50 ± 0.59*	7.83 ± 2.68
<i>OsSSI2</i> -kd -1	4.91 ± 0.48*	14.61 ± 4.79*
<i>OsSSI2</i> -kd -2	2.61 ± 0.22*	7.30 ± 1.34

^a Endogenous salicylic acid (SA) and SA-glucoside (SAG) contents. The SA and SAG contents in the leaf blades from seedlings of wild-type (WT), homozygous *Osssi2-Tos17* (NF7039), and *OsSSI2*-knockdown (kd) plants are shown. Averages of four sets of samples per line are expressed in micrograms per gram fresh weight with standard deviations. The asterisk (*) indicates the statistically significant difference (*P* value ≤ 0.05, Student's *t* test) from the WT.

Fig. 5. Enhanced disease resistance in *OsSSI2*-knockdown (kd) plants. Wild-type (WT) and *OsSSI2*-kd plants were inoculated with **A**, the rice blast fungus *Magnaporthe grisea* and **B**, blight bacteria *Xanthomonas oryzae* pv. *oryzae*. Numbers on the y axes of the graph indicate **A**, the number of fungal blast lesions per 10-cm middle region of leaf blades and **B**, the lesion lengths of bacterial leaf blight. Each value represents the average of 15 to 20 plants. The bars indicate standard deviations (SD).

Glycerol application enhances resistance in rice.

Exogenous application of glycerol lowers the 18:1 levels and enhances disease resistance in *Arabidopsis* (Kachroo et al. 2004, 2005) and in soybean plants (Kachroo et al. 2008). To examine whether glycerol causes similar effects in rice, we spray treated rice plants with 1% glycerol and examined the expression of defense-related genes and blast and leaf-blight resistances. The results showed that glycerol application induced *WRKY45* and *PR1b* expression (Fig. 6A) and significantly enhanced the resistance to both diseases (Fig. 6B).

Fig. 6. Defense response induced by glycerol application. The seedlings of wild-type rice plants were sprayed with mock or glycerol (Gly). **A**, Induction of *WRKY45* and *PR1b* expression by glycerol application. Transcript levels were analyzed by quantitative reverse-transcription polymerase chain reaction. Averages of three determinations relative to those of Rubq1 are shown with standard deviations (SD). **B**, Enhanced resistance to blast fungus *Magnaporthe grisea* (shown on left axis) and blight bacteria *Xanthomonas oryzae* pv. *oryzae* (shown on right axis) after glycerol application. The values on the y axes of the graph indicate **A**, the number of fungal blast lesions per 10-cm middle region of leaf blades and **B**, the lesion lengths of bacterial leaf blight. Each value represents the average of 15 to 20 plants.

DNA-microarray profiling of gene expression in *OsSSI2*-kd plants.

To further characterize the genes influenced by *OsSSI2* down-regulation, we compared the transcript profiles of wild-type and *OsSSI2*-kd plants by using an oligo DNA microarray for 44,000 rice genes. Only genes that are differentially expressed in both *OsSSI2*-kd-1 and *OsSSI2*-kd-2 plants within the criteria of statistical significance (i.e., $P \leq 0.05$ and false discovery rate [FDR] $\leq 5\%$) were selected for data analyses. More stringent filtering (e.g., $P \leq 0.01$ or FDR ≤ 0.01) failed to recover some of the genes whose differential expression was detected in RNA blotting (e.g., *PR1b*). In total, 406 genes were found to be differentially expressed between the wild-type and *OsSSI2*-kd plants by a factor of more than twofold (Table 3; Supplementary Table S1). Among these, 74% (299 genes) and 26% (107 genes) were up- and downregulated, respectively, in *OsSSI2*-kd plants. The differentially expressed genes were classified into different functional groups, using the Kyoto Encyclopedia of Genes and Genomes (KEGG) database. Approximately 42% (169 genes) of the genes were classified as unknown, hypothetical, unclassified proteins, or no hits. *OsSSI2* expression was also repressed by approximately sixfold, while no other SACP-like genes were differentially expressed in *OsSSI2*-kd plants. The genes involved in “metabolism” accounted for 17.5% ($n = 71$) of the genes; among these, 3 genes that encoded phenylalanine ammonia lyase (PAL) were upregulated in *OsSSI2*-kd plants (Table 3). PAL are involved in the SA biosynthesis pathway and implicated in both biotic and abiotic plant responses (Shah 2003). Therefore, the increased free SA levels in these plants (Table 2) may be due to the upregulation of these PAL genes.

The *OsSSI2*-regulated genes also included a large number of transcription factors (7.9%, $n = 32$), protein kinases (6.9%, $n = 28$), and defense-related genes (3.9%, $n = 16$). *WRKY45*, *PR1b*, *PBZ1*, and a *thaumatin-like* gene were upregulated by 5-, >50-, >22-, and >20-fold, respectively, in *OsSSI2*-kd plants (Table 3). Among the defense-related genes, there were five genes for harpin-induced 1 domain-containing proteins and four genes for disease-resistance protein family proteins. In addition, six genes for AAA ATPase, which compose a distinct class of ATPases, were highly upregulated in *OsSSI2*-kd plants (Table 3).

Recently, we used microarray analyses to identify approximately 2,000 BTH-responsive genes (*unpublished data*). In these assays, BTH was applied to the basal cut surface of the shoot instead of being sprayed on the leaves, as reported in our previous study (Shimono et al. 2007). This led to a more efficient BTH response. Comparison of these genes with the list of *OsSSI2*-regulated genes revealed that approximately 39% ($n = 156$) of the *OsSSI2*-regulated genes were common to both lists, and all but 7 genes showed expression changes in the same direction. These results support the notion that *OsSSI2* negatively regulates the SA-signaling pathway in rice. However, a number of *OsSSI2*-regulated genes were BTH unresponsive, suggesting that *OsSSI2* also regulates a signaling pathway or pathways other than the SA signaling pathway.

DISCUSSION

FA and their derivatives are emerging as important signaling molecules in plant defense pathways. In support of this notion, a variety of FA-metabolizing enzymes have been associated with plant defense reactions (Chaturvedi et al. 2008; Kachroo and Kachroo 2007; Shah 2005; Weber 2002; Yara et al. 2007). In this study, we showed that the *OsSSI2* gene for an FA desaturase is involved in the negative regulation of defense responses in rice, similar to its counterparts in *Arabidopsis* (Kachroo et

al. 2001; Shah et al. 2001) and soybean (Kachroo et al. 2008). These data suggest that the function of this particular gene is conserved in defense signaling pathways in both dicots and monocots.

Downregulation of *OsSSI2* resulted in a significant decrease in the 18:1 levels and a large increase in the 18:0 levels (Table 1), indicating that *OsSSI2* is a major contributor to the 18:0-to-18:1 metabolic step. It has been demonstrated that the reduced 18:1 levels are responsible for the phenotypes in the *Arabidopsis ssi2* mutant (Kachroo et al. 2003a, 2004, 2005; Nandi et al. 2003) and those of *GmSACP*-silenced soybean (Kachroo et al. 2008). Similarly, in rice, the 18:1 levels in NF7039 and *OsSSI2*-kd plants appeared to be inversely correlated with the severity of their phenotypes, including spontaneous lesion formation, growth retardation (Fig. 2), and resistance to blight and blast diseases (Fig. 4). Moreover, the application of 18:1 and not 18:0 to leaf discs of NF7039 and *OsSSI2*-kd plants restored the constitutive expression of *WRKY45* and *PR1b* in these plants (Fig. 4). These results demonstrate that 18:1 is a negative regulatory molecule of the defense signaling pathway in rice, as in *Arabidopsis* (Kachroo et al. 2003a, 2004, 2005; Nandi et al. 2003) and soybean (Kachroo et al. 2008). Application of glycerol induced the expression of *WRKY45* and *PR1b* in wild-type rice seedlings and enhanced their resistance to blast and leaf-blight diseases (Fig. 6). This is consistent with *OsSSI2* being an ortholog of the *Arabidopsis* and soybean genes, because the application of glycerol reduces the 18:1 content in wild-type plants and, consequently, mimics the defense phenotypes of the *Arabidopsis ssi2* mutant and *GmSACP*-A/-B-silenced soybean plants. Taken together, these results strongly suggest that the lowered 18:1 levels are responsible for defense phenotypes in *OsSSI2*-kd plants. The levels of stearic acid (18:0) were elevated in the NF7039 and *OsSSI2*-kd lines. Meanwhile, transgenic overexpression of *OsSSI2* in rice decreased the 18:0 levels to approximately 25% of those in the wild-type controls but yielded no appreciable morphological or defense-related phenotypes (data not shown). This result, taken together with the results of exogenous FA application (Fig. 4), suggests that the elevated 18:0 levels in NF7039 and *OsSSI2*-kd lines are not correlated with their phenotypes.

We found that a large number of BTH-responsive genes, including *WRKY45*, *PR1b*, and *PBZ*, overlapped with the *OsSSI2*-regulated genes that we had identified. Many of these genes were also induced by SA treatment, as shown previously

Table 3. Summary of microarray data for representative genes upregulated in *OsSSI2*-kd plants^a

Gene	Putative function	<i>OsSSI2</i> -kd/WT	
		No. 1	No. 2
<i>Os01g0919900</i>	<i>OsSSI2</i>	6.5	6.3
<i>Os05g0322900</i>	<i>WRKY45</i>	5.6	5.0
<i>Os01g0382000</i>	<i>PR1b</i>	69.0	50.7
<i>Os03g0300400</i>	<i>PBZ1</i>	29.9	22.0
<i>Os12g0628600</i>	<i>Thaumatococcus-like</i>	50.2	20.0
<i>Os04g0518400</i>	Phenylalanine ammonia lyase	6.8	15.7
<i>Os05g0427400</i>	Phenylalanine ammonia lyase	7.7	4.8
<i>Os02g0627100</i>	Phenylalanine ammonia lyase	15.0	3.5
<i>Os01g0297200</i>	AAA ATPase	18.0	92.2
<i>Os03g0802500</i>	AAA ATPase	14.4	65.6
<i>Os06g0697600</i>	AAA ATPase	5.6	26.1
<i>Os07g0517600</i>	AAA ATPase	26.0	12.3
<i>Os02g0697600</i>	AAA ATPase	2.9	7.3
<i>Os02g0706500</i>	AAA ATPase	2.3	3.7

^a Shown are the gene expression levels in *OsSSI2*-knockdown (kd) plants relative to that in the wild-type (WT) control. Microarray data are derived from four biologically independent experiments.

by both microarray and RNA blotting analyses (Shimono et al. 2007), indicating that BTH triggers defense responses similar to those triggered by SA in rice plants. Importantly, the expression of *WRKY45* responds to SA and BTH highly specifically among various signaling molecules tested, and *WRKY45* encodes a transcription factor essential for BTH-induced disease resistance (Shimono et al. 2007). Overexpression of *WRKY45* dramatically enhances blast (Shimono et al. 2007) and leaf-blight resistance (*unpublished*); therefore, the enhanced disease resistance of *OsSSI2*-kd plants can be largely ascribed to *WRKY45* upregulation and, perhaps, to its downstream genes.

The endogenous levels of free SA were slightly elevated in *OsSSI2*-knockout and *OsSSI2*-kd plants compared with the wild type (Table 2). However, the total SA contents did not correlate with *OsSSI2* expression in these plants. In addition, the free SA levels in the *OsSSI2*-kd lines did not correlate well with the disease-resistance phenotype (Fig. 4). Thus, the extent to which the SA levels contribute to disease resistance in *OsSSI2*-kd plants remains unknown. Rice has high basal levels of SA, and the SA levels showed little or no response to pathogen infection, which is characteristic of this plant species. On the other hand, rice has a signaling pathway similar to the dicot SA pathway consisting of the counterparts of *Arabidopsis* NPR1 (Chern et al. 2001, 2005b; Fitzgerald et al. 2004; Yuan et al. 2007), NRR (Chern et al. 2005a), and TGA (Fitzgerald et al. 2005). Moreover, *WRKY45*, whose counterpart has not been found in *Arabidopsis*, is also a component of the SA signaling pathway in rice (Shimono et al. 2007). The upregulation of SA/BTH-inducible genes, including *WRKY45*, and the enhanced disease resistance in *OsSSI2*-kd rice, despite the only minor increase in SA levels, may suggest that rice transmits the SA signal in a manner different from those of dicots; for example, through the changes in intracellular localization of SA. Alternatively, suppression of *OsSSI2* somehow resulted in a heightened sensitivity in the perception or transduction of SA signals

in these plants. Similar observations have been made with potato plants, which also contain high basal levels of endogenous SA that does not increase further when infected by *P. infestans* (Yu et al. 1997). However, we cannot completely exclude the possibility that a mechanism independent of SA upregulated the SA/BTH-inducible genes in *OsSSI2*-kd plants. Simultaneous suppression of *OsSSI2* and the genes involved in SA biosynthesis or SA signaling (e.g., *OsNPR1* and *WRKY45*) may aid in addressing this issue.

Regarding *Arabidopsis*, it has been shown that the JA signaling pathway is suppressed in *Arabidopsis ssi2* mutants (Kachroo et al. 2003a and b). However, our microarray data results revealed that the expression of JA-responsive genes (e.g., *Jamyb*) (Lee et al. 2001) was unaltered in *OsSSI2*-kd plants. This may indicate that the JA content or JA signaling are not significantly altered in these plants. However, further biological studies are required to examine the involvement of JA in disease resistance in *OsSSI2*-kd plants.

FA have been implicated in gene regulation through their involvement in the binding and modulation of the protein activities of many transcription factors and enzymes. These compounds have also been shown to affect the nuclear contents of transcription factors in vertebrate cells (Jump 2004). Oleic acid (18:1) has been shown to activate phospholipase D (PLD) in several mammalian cells and also in *Arabidopsis*. In *Arabidopsis*, oleic acid activates PLD δ (Wang and Wang 2001) and thereby decreases H₂O₂-induced cell death (Zhang et al. 2003). If this also occurs in rice, the reduced oleic acid levels may decrease PLD δ activity, leading to failure to suppress H₂O₂-induced cell death. This may account for the spontaneous lesions that appear in NF7039 and *OsSSI2*-kd plants (Fig. 2); these probably arise due to H₂O₂-induced cell death. Thus, oleic-acid-dependent PLD δ might be partly responsible for the differential expression of genes, including BTH-unresponsive genes, and could possibly be responsible for disease resistance in *OsSSI2*-kd plants. It is also known that PLD δ is activated by various defense-related signaling molecules, such as reactive oxygen species, ABA (Zhang et al. 2005), and methyl jasmonate (Profotova et al. 2006), although the involvement of 18:1 in such regulation has not been reported. The involvement of PLD or other oleic-acid-regulated protein factors in defense signaling pathways in *OsSSI2*-kd plants will be an appropriate topic for further investigation.

Six genes for AAA ATPase were highly upregulated in *OsSSI2*-kd plants (Table 3). Notably, these genes are independent of SA. The AAA ATPases form a large family associated with a variety of cellular activities, including proteolysis, protein folding, membrane trafficking, cytoskeletal regulation, organelle biogenesis, DNA replication, and intracellular motility (Vale 2000). Thus, the six AAA ATPases may be involved in some important cellular functions in an SA-independent pathway in *OsSSI2*-kd plants.

In summary, we have shown that *OsSSI2* encodes an FA desaturase and negatively regulates the defense responses in rice partly through suppressing SA-responsive genes. Transcript profiling suggested that *OsSSI2* also regulates SA-independent signaling pathways, which may also play a role in the defense mechanism (Fig. 7). Taken together with the results obtained from *Arabidopsis* and soybean, our results suggest that SSI2 is functionally well conserved in defense signaling pathways across plant species.

MATERIALS AND METHODS

Plasmid DNA construction and plant transformation.

The cDNA clone for *OsSSI2* (accession number: AK058979) was provided by the Rice Genome Resource Center, Japan. To

Fig. 7. Proposed model for the functioning of *OsSSI2* in the rice defense pathway. *OsSSI2* negatively regulates the defense responses in rice partly through suppressing salicylic acid (SA)-responsive genes. *OsSSI2* is also likely to regulate an SA-independent defense signaling pathway mediated by an unknown factor (X).

construct a plasmid for *OsSSI2* RNAi (*OsSSI2*-kd), a part of the 3' untranslated region (nucleotides 1,321 to 1,564) of *OsSSI2* cDNA was amplified by polymerase chain reaction (PCR) and cloned into the pANDA vector (Miki and Shimamoto 2004; Miki et al. 2005), as described previously (Shimono et al. 2007). Rice (*Oryza sativa* cv. Nipponbare) was transformed by an *Agrobacterium tumefaciens* (strain EHA105)-mediated technique, as described earlier (Toki et al. 2006).

Plant materials and growth conditions.

Two insertion mutant lines for *OsSSI2* (*Osssi2-Tos17*: NF7039 and NF8001) that were produced with the endogenous retrotransposon *Tos17* (Hirochika 2001; Hirochika et al. 2004) were obtained from the RGRC. The rice plants were grown in a greenhouse in soil (Bonsol No. 2; Sumitomo Chemical Corp., Tokyo) at 28°C in the day and 23°C at night. The relative humidity in the greenhouse was approximately 70%.

Pathogen culture and inoculations.

The blast fungus *M. grisea* (race 007.0) was grown on an oatmeal agar medium (oatmeal at 30 g/liter, sucrose at 5 g/liter, and agar at 16 g/liter) at 26°C for 10 to 12 days. After removing the aerial hyphae by washing with distilled water and a brush, conidia formation was induced by irradiation under continuous black-blue light (FL15BLB; Toshiba, Osaka, Japan) for 3 days at 24°C. The conidia were suspended in 0.05% Tween 20 at a density of 10^5 conidia/ml and sprayed onto rice plants at the four-leaf stage. After incubation in a dew chamber at 24°C for 24 h, the rice plants were moved back to the greenhouse and cultured there. Disease development was assessed by the number of blast lesions per 10-cm middle region of each fourth leaf at 6 to 7 days after inoculation. The blight bacteria *X. oryzae* pv. *oryzae* (strain T7174) was grown on peptone-sucrose agar (1% proteose peptone, 1% sucrose, 0.1% L-glutamic acid monosodium salt, and 1.6% agar) at 28°C for 2 to 3 days. The bacteria were scraped off the plates, suspended in distilled water (optical density at 600 nm = 0.03), and used to inoculate the fully expanded sixth (the youngest) leaves of rice by using a scissors-dip method (Kauffman et al. 1973). The lesion lengths were measured at 10 to 14 days after inoculation.

RNA analyses.

Total RNA was isolated from the leaf blades of the fourth leaf of rice seedlings using the Trizol reagent (Invitrogen, Carlsbad, CA, U.S.A.). For RNA gel blot analysis, 10 µg of total RNA was separated on a 1.2% agarose gel containing 0.74% formaldehyde and then blotted onto a nylon membrane (Roche Diagnostics, Indianapolis, IN, U.S.A.). In vitro RNA synthesis and digoxigenin (DIG) labeling were performed using a DIG RNA labeling kit (Roche Diagnostics). The blotted membrane was hybridized with DIG-labeled RNA probes, and the signals were detected by a chemiluminescence reaction using CDP-star as the substrate (Roche Diagnostics). Quantitative reverse-transcription PCR was run on a Thermal Cycler Dice TP800 system (Takara Bio, Shiga, Japan) using SYBR premix Ex Taq mixture (Takara Bio) as described previously (Shimono et al. 2007).

Microarray analyses.

The leaf blades of the fourth leaves were collected from 2-week-old rice seedlings of Nipponbare *OsSSI2*-kd-1 and *OsSSI2*-kd-2. Total RNA was isolated using an RNeasy plant kit (Qiagen, Hilden, Germany), according to the manufacturer's instruction. The transcriptomes of Nipponbare and either *OsSSI2*-kd-1 or *OsSSI2*-kd-2 were compared using two-color microarray experiments. RNA labeling and hybridization were carried out as described previously (Shimono et al. 2007), using

an Agilent rice oligo microarray (44K, custom-made; Agilent Technologies, Santa Clara, CA, U.S.A.). Four biological replicates were independently hybridized for each transcriptomic comparison. The Cy3 and Cy5 labels were swapped for technical replicates. Microarray slides were scanned with the Agilent Microarray Laser Scanner (model G2505B) and the raw images were processed using Feature Extraction software (version 9.1; Agilent Technologies) at the default parameter settings.

The gene expression data were normalized and statistically analyzed using the CARMAweb package (Rainer et al. 2006), a comprehensive R language- and bioconductor-based web service. The parameter settings used were as follows. Data normalization: background correction method—subtract; within-array-normalization method—print tip loess; between-array-normalization method—quantile. Replicate handling: averaging replicated arrays and spots using the median value over the replicates. The signal used to determine differentially expressed genes: calculate test statistics on expression values. Test statistic analysis: the test statistic—paired moderated *t* statistics (limma); multiple testing corrections—adjusted *P* values for the Benjamini and Hochberg (1995) step-up FDR controlling procedure (independent and positive regression dependent test statistics).

Measurement of FA.

Approximately 0.35 g of rice seedling shoots were heated in 2.3 ml of 2-propanol at 85°C for 5 to 10 min and cooled on ice. Next, 5 µl of 2,6-di-*t*-butyl-4-methylphenol solution at 10 µg/ml was added to the sample to prevent oxidation and homogenized in 15 ml of a chloroform/methanol mixture (1:2, vol/vol). Total lipids were extracted according to the method of Bligh and Dyer (1959). After evaporation of the solvent, fractions of total lipids were dissolved in 2.5 ml of methanol containing 2.5% H₂SO₄ and 50 nM pentadecanoic acid (15:0) (Funakoshi, Tokyo) was added as the internal standard. Total lipids were then transesterified to FA methyl esters by heating at 80°C for 2.5 h. After cooling on ice, the FA methyl ester fraction was extracted with 2.5 ml of hexane, dried, and redissolved in 100 ml of hexane.

To detect the FA methyl esters, 1-ml aliquots of each sample were injected into the GC-MS instrument (GCmate II; JEOL, Tokyo). Separation was carried out on a Quadrex 007-23 column (0.25 mm i.d. by 50 m, 0.25-µm film thickness) (Quadrex, Woodbridge, CT, U.S.A.) under the following conditions: injector temperature, 250°C; carrier gas, helium; and flow rate, 0.7 ml/min. The oven temperature was programmed to increase from 70 to 185°C at 30°C/min and then was held at 185°C for 15 min. The conditions used for mass spectrometry were as follows: ionization mode, EI (70 eV); ion source temperature, 200°C; scan range, *m/z* 50–411; scan rate, 1 s/scan. The amounts of individual FA methyl esters were calculated by comparing the peak areas in the chromatograms with those of the standard samples.

Measurement of the endogenous SA content.

Approximately 0.1 g of each sample was extracted, and free SA and SAG were quantified, as described previously (Hennig et al. 1993; Malamy et al. 1992).

Glycerol and FA treatment.

Glycerol treatment was carried out essentially as described previously (Kachroo et al. 2008), with slight modifications. Rice plants at the fourth- or sixth-leaf stage were sprayed with 1% glycerol solution prepared in 0.02% Silwet L-77 once a day for 2 consecutive days. After 24 h of the last treatment, the plants were subjected to blast- (plants at fourth-leaf stage) and blight (plants at sixth-leaf stage) resistance tests.

For FA treatment, the leaf blade was cut into approximately 0.5-cm-long segments and vacuum infiltrated in solution containing 2 mM stearic acid (18:0) or oleic acid (18:1) prepared in 0.02% Silwet L-77. The leaf segments were then incubated under light for 12 h at 30°C.

ACKNOWLEDGMENTS

We thank K. Shimamoto (Nara Institute of Science and Technology, Nara, Japan) for providing the RNAi vector pANDA, Dr. H. Hirochika and A. Miyao (National Institute of Agrobiological Sciences [NIAS]), for providing Tos17 insertion mutant lines, N. Hayashi (NIAS) for providing the blast strain, S. Seo (NIAS) for technical advice in measuring SA content, the Rice Genome Resource Center at NIAS for the use of the rice microarray analysis system, and Y. Nagamura and R. Motoyama for technical support. This work was supported by a grant from the Ministry of Agriculture, Forestry, and Fisheries of Japan (Green Technology Project, IP-4006).

LITERATURE CITED

- Babu, R. M., Sajeena, A., Vijayasamundeeswari, A., Sreedhar, A., Vidhyasekaran, P., and Reddy, M. S. 2003. Induction of bacterial blight (*Xanthomonas oryzae* pv. *oryzae*) resistance in rice by treatment with acibenzolar-S-methyl. *Ann. Appl. Biol.* 143:333-340.
- Benjamini, Y., and Hochberg, Y. 1995. Controlling the false discovery rate: A practical and powerful approach to multiple testing. *J. R. Stat. Soc. [Ser. A]* 57:289.
- Bligh, E. G., and Dyer, W. J. 1959. A rapid method of total lipid extraction and purification. *Can. J. Biochem. Physiol.* 37:911-917.
- Bostock, R. M. 2005. Signal crosstalk and induced resistance: straddling the line between cost and benefit. *Annu. Rev. Phytopathol.* 43:545-580.
- Chandra-Shekara, A. C., Venugopal, S. C., Barman, S. R., Kachroo, A., and Kachroo, P. 2007. Plastidial fatty acid levels regulate resistance gene-dependent defense signaling in *Arabidopsis*. *Proc. Natl. Acad. Sci. U.S.A.* 104:7277-7282.
- Chaturvedi, R., Krothapalli, K., Makandar, R., Nandi, A., Sparks, A. A., Roth, M. R., Welti, R., and Shah, J. 2008. Plastid omega3-fatty acid desaturase-dependent accumulation of a systemic acquired resistance inducing activity in petiole exudates of *Arabidopsis thaliana* is independent of jasmonic acid. *Plant J.* 54:106-117.
- Chern, M. S., Fitzgerald, H. A., Yadav, R. C., Canlas, P. E., Dong, X., and Ronald, P. C. 2001. Evidence for a disease-resistance pathway in rice similar to the NPR1-mediated signaling pathway in *Arabidopsis*. *Plant J.* 27:101-113.
- Chern, M., Canlas, P. E., Fitzgerald, H. A., and Ronald, P. C. 2005a. Rice NRR, a negative regulator of disease resistance, interacts with *Arabidopsis* NPR1 and rice NH1. *Plant J.* 43:623-635.
- Chern, M., Fitzgerald, H. A., Canlas, P. E., Navarre, D. A., and Ronald, P. C. 2005b. Overexpression of a rice NPR1 homolog leads to constitutive activation of defense response and hypersensitivity to light. *Mol. Plant-Microbe Interact.* 18:511-520.
- Dong, X. 2004. NPR1, all things considered. *Curr. Opin. Plant Biol.* 7:547-552.
- Durrant, W. E., and Dong, X. 2004. Systemic acquired resistance. *Annu. Rev. Phytopathol.* 42:185-209.
- Felton, G. W., and Korth, K. L. 2000. Trade-offs between pathogen and herbivore resistance. *Curr. Opin. Plant Biol.* 3:309-314.
- Fitzgerald, H. A., Chern, M. S., Navarre, R., and Ronald, P. C. 2004. Overexpression of (At)NPR1 in rice leads to a BTH- and environment-induced lesion-mimic/cell death phenotype. *Mol. Plant-Microbe Interact.* 17:140-151.
- Fitzgerald, H. A., Canlas, P. E., Chern, M. S., and Ronald, P. C. 2005. Alteration of TGA factor activity in rice results in enhanced tolerance to *Xanthomonas oryzae* pv. *oryzae*. *Plant J.* 43:335-347.
- Heil, M., and Bostock, R. M. 2002. Induced systemic resistance (ISR) against pathogens in the context of induced plant defences. *Ann. Bot. (Lond.)* 89:503-512.
- Hennig, J., Malamy, J., Gryniewicz, G., Indulski, J., and Klessig, D. F. 1993. Interconversion of the salicylic acid signal and its glucoside in tobacco. *Plant J.* 4:593-600.
- Hirochika, H. 2001. Contribution of the Tos17 retrotransposon to rice functional genomics. *Curr. Opin. Plant Biol.* 4:118-122.
- Hirochika, H., Guiderdoni, E., An, G., Hsing, Y. I., Eun, M. Y., Han, C. D., Upadhyaya, N., Ramachandran, S., Zhang, Q., Pereira, A., Sundaresan, V., and Leung, H. 2004. Rice mutant resources for gene discovery. *Plant Mol. Biol.* 54:325-334.
- Iwai, T., Miyasaka, A., Seo, S. and Ohashi, Y. 2006. Contribution of ethylene biosynthesis for resistance to blast fungus infection in young rice plants. *Plant Physiol.* 142:1202-1215.
- Jump, D. B. 2004. Fatty acid regulation of gene transcription. *Crit. Rev. Clin. Lab. Sci.* 41:41-78.
- Jwa, N. S., Agrawal, G. K., Tamogami, S., Yonekura, M., Han, O., Iwahashi, H., and Rakwal, R. 2006. Role of defense/stress-related marker genes, proteins and secondary metabolites in defining rice self-defense mechanisms. *Plant Physiol. Biochem.* 44:261-273.
- Kachroo, A., and Kachroo, P. 2007. Salicylic acid-, jasmonic acid- and ethylene-mediated regulation of plant defense signaling. *Genet. Eng. (N. Y.)* 28:55-83.
- Kachroo, P., Shanklin, J., Shah, J., Whittle, E. J., and Klessig, D. F. 2001. A fatty acid desaturase modulates the activation of defense signaling pathways in plants. *Proc. Natl. Acad. Sci. U.S.A.* 98:9448-9453.
- Kachroo, A., Lapchuk, L., Fukushige, H., Hildebrand, D., Klessig, D., and Kachroo, P. 2003a. Plastidial fatty acid signaling modulates salicylic acid- and jasmonic acid-mediated defense pathways in the *Arabidopsis* *ssi2* mutant. *Plant Cell* 15:2952-2965.
- Kachroo, P., Kachroo, A., Lapchuk, L., Hildebrand, D., and Klessig, D. F. 2003b. Restoration of defective cross talk in *ssi2* mutants: role of salicylic acid, jasmonic acid, and fatty acids in *SSI2*-mediated signaling. *Mol. Plant-Microbe Interact.* 16:1022-1029.
- Kachroo, A., Venugopal, S. C., Lapchuk, L., Falcone, D., Hildebrand, D., and Kachroo, P. 2004. Oleic acid levels regulated by glycerolipid metabolism modulate defense gene expression in *Arabidopsis*. *Proc. Natl. Acad. Sci. U.S.A.* 101:5152-5157.
- Kachroo, P., Venugopal, S. C., Navarre, D. A., Lapchuk, L., and Kachroo, A. 2005. Role of salicylic acid and fatty acid desaturation pathways in *ssi2*-mediated signaling. *Plant Physiol.* 139:1717-1735.
- Kachroo, A., Fu, D. Q., Havens, W., Navarre, D., Kachroo, P., and Ghabrial, S. A. 2008. An oleic acid-mediated pathway induces constitutive defense signaling and enhanced resistance to multiple pathogens in soybean. *Mol. Plant-Microbe Interact.* 21:564-575.
- Kauffman, H. E., Reddy, A. P. K., Hsieh, S. P. Y., and Merca, S. D. 1973. An improved technique for evaluating resistance to rice varieties of *Xanthomonas oryzae* pv. *oryzae*. *Plant Dis. Rep.* 57:537-541.
- Kunkel, B. N., and Brooks, D. M. 2002. Cross talk between signaling pathways in pathogen defense. *Curr. Opin. Plant Biol.* 5:325-331.
- Lee, M. W., Qi, M., and Yang, Y. 2001. A novel jasmonic acid-inducible rice *myb* gene associates with fungal infection and host cell death. *Mol. Plant-Microbe Interact.* 14:527-535.
- Malamy, J., Hennig, J., and Klessig, D. F. 1992. Temperature-dependent induction of salicylic acid and its conjugates during the resistance response to tobacco mosaic virus infection. *Plant Cell* 4:359-366.
- Mei, C., Qi, M., Sheng, G., and Yang, Y. 2006. Inducible overexpression of a rice allene oxide synthase gene increases the endogenous jasmonic acid level, PR gene expression, and host resistance to fungal infection. *Mol. Plant-Microbe Interact.* 19:1127-1137.
- Miki, D., and Shimamoto, K. 2004. Simple RNAi vectors for stable and transient suppression of gene function in rice. *Plant Cell Physiol.* 45:490-495.
- Miki, D., Itoh, R., and Shimamoto, K. 2005. RNA silencing of single and multiple members in a gene family of rice. *Plant Physiol.* 138:1903-1913.
- Nandi, A., Krothapalli, K., Buseman, C. M., Li, M., Welti, R., Enyedi, A., and Shah, J. 2003. *Arabidopsis* *sfd* mutants affect plastidic lipid composition and suppress dwarfing, cell death, and the enhanced disease resistance phenotypes resulting from the deficiency of a fatty acid desaturase. *Plant Cell* 15:2383-2398.
- Napier, J. A. 2007. The production of unusual fatty acids in transgenic plants. *Annu. Rev. Plant Biol.* 58:295-319.
- Pieterse, C. M., and Van Loon, L. C. 2004. NPR1: the spider in the web of induced resistance signaling pathways. *Curr. Opin. Plant Biol.* 7:456-464.
- Profotova, B., Burketova, L., Novotna, Z., Martinec, J., and Valentova, O. 2006. Involvement of phospholipases C and D in early response to SAR and ISR inducers in *Brassica napus* plants. *Plant Physiol. Biochem.* 44:143-151.
- Rainer, J., Sanchez-Cabo, F., Stocker, G., Sturm, A., and Trajanoski, Z. 2006. CARMAweb: comprehensive R- and bioconductor-based web service for microarray data analysis. *Nucleic Acids Res.* 34:W498-503.
- Rohilla, R., Singh, U. S., and Singh, R. L. 2002. Mode of action of acibenzolar-S-methyl against sheath blight of rice, caused by *Rhizoctonia solani* Kuhn. *Pest Manage. Sci.* 58:63-69.
- Schweizer, P., Schlagenhauf, E., Schaffrath, U., and Dudler, R. 1999. Different patterns of host genes are induced in rice by *Pseudomonas syringae*, a biological inducer of resistance, and the chemical inducer benzothiadiazole (BTH). *Eur. J. Plant Pathol.* 105:659-665.
- Sekine, K. T., Nandi, A., Ishihara, T., Hase, S., Ikegami, M., Shah, J., and Takahashi, H. 2004. Enhanced resistance to Cucumber mosaic virus in

- the *Arabidopsis thaliana* *ssi2* mutant is mediated via an SA-independent mechanism. *Mol. Plant-Microbe Interact.* 17:623-632.
- Shah, J. 2003. The salicylic acid loop in plant defense. *Curr. Opin. Plant Biol.* 6:365-371.
- Shah, J. 2005. Lipids, lipases, and lipid-modifying enzymes in plant disease resistance. *Annu. Rev. Phytopathol.* 43:229-260.
- Shah, J., Kachroo, P., Nandi, A., and Klessig, D. F. 2001. A recessive mutation in the *Arabidopsis* *SSI2* gene confers SA- and NPR1-independent expression of PR genes and resistance against bacterial and oomycete pathogens. *Plant J.* 25:563-574.
- Shimono, M., Sugano, S., Nakayama, A., Jiang, C. J., Ono, K., Toki, S., and Takatsui, H. 2007. Rice *WRKY45* plays a crucial role in benzothiadiazole-inducible blast resistance. *Plant Cell* 19:2064-2076.
- Silverman, P., Seskar, M., Kanter, D., Schweizer, P., Metraux, J. P., and Raskin, I. 1995. Salicylic acid in rice: biosynthesis, conjugation, and possible role. *Plant Physiol.* 108:633-639.
- Spoel, S. H., Koornneef, A., Claessens, S. M., Korzelius, J. P., Van Pelt, J. A., Mueller, M. J., Buchala, A. J., Metraux, J. P., Brown, R., Kazan, K., Van Loon, L. C., Dong, X., and Pieterse, C. M. 2003. NPR1 modulates cross-talk between salicylate- and jasmonate-dependent defense pathways through a novel function in the cytosol. *Plant Cell* 15:760-770.
- Toki, S., Hara, N., Ono, K., Onodera, H., Tagiri, A., Oka, S., and Tanaka, H. 2006. Early infection of scutellum tissue with *Agrobacterium* allows high-speed transformation of rice. *Plant J.* 47:969-976.
- Vale, R. D. 2000. AAA proteins. Lords of the ring. *J. Cell Biol.* 150:F13-19.
- Vallad, G. E., and Goodman R. M. 2004. Systemic acquired resistance and induced systemic resistance in conventional agriculture. *Crop Sci.* 44:1920-1934.
- Vergne, E., Ballini, E., Marques, S., Sidi Mammar, B., Droc, G., Gaillard, S., Bourot, S., DeRose, R., Tharreau, D., Notteghem, J. L., Lebrun, M. H., and Morel, J. B. 2007. Early and specific gene expression triggered by rice resistance gene Pi33 in response to infection by ACE1 avirulent blast fungus. *New Phytol.* 174:159-171.
- Wang, C., and Wang, X. 2001. A novel phospholipase D of *Arabidopsis* that is activated by oleic acid and associated with the plasma membrane. *Plant Physiol.* 127:1102-1112.
- Wang, D., Amornsiripanitch, N., and Dong, X. 2006. A genomic approach to identify regulatory nodes in the transcriptional network of systemic acquired resistance in plants. *PLoS Pathog.* 2:e123.
- Weber, H. 2002. Fatty acid-derived signals in plants. *Trends Plant Sci.* 7:217-224.
- Yang, Y., Qi, M., and Mei, C. 2004. Endogenous salicylic acid protects rice plants from oxidative damage caused by aging as well as biotic and abiotic stress. *Plant J.* 40:909-919.
- Yara, A., Yaeno, T., Hasegawa, M., Seto, H., Montillet, J. L., Kusumi, K., Seo, S., and Iba, K. 2007. Disease resistance against *Magnaporthe grisea* is enhanced in transgenic rice with suppression of omega-3 fatty acid desaturases. *Plant Cell Physiol.* 48:1263-1274.
- Yu, D., Liu, Y., Fan, B., Klessig, D. F., and Chen, Z. 1997. Is the high basal level of salicylic acid important for disease resistance in potato? *Plant Physiol.* 115:343-349.
- Yuan, Y., Zhong, S., Li, Q., Zhu, Z., Lou, Y., Wang, L., Wang, J., Wang, M., Li, Q., Yang, D., and He, Z. 2007. Functional analysis of rice NPR1-like genes reveals that *OsNPR1/NH1* is the rice orthologue conferring disease resistance with enhanced herbivore susceptibility. *Plant Biotechnol. J.* 5:313-324.
- Zhang, W., Wang, C., Qin, C., Wood, T., Olafsdottir, G., Welti, R., and Wang, X. 2003. The oleate-stimulated phospholipase D, PLD?, and phosphatidic acid decrease H₂O₂-induced cell death in *Arabidopsis*. *Plant Cell* 15:2285-2295.
- Zhang, W., Yu, L., Zhang, Y., and Wang, X. 2005 Phospholipase D in the signaling networks of plant response to abscisic acid and reactive oxygen species. *Biochim. Biophys. Acta* 1736:1-9.

AUTHOR-RECOMMENDED INTERNET RESOURCES

KEGG: Kyoto Encyclopedia of Genes and Genomes:
www.genome.jp/kegg
 Rice Genome Resource Center: www.rgrc.dna.affrc.go.jp/index.html.en
 TargetP server: www.cbs.dtu.dk/services/TargetP
 Tyrolean Cancer Research Institute CARMAweb:
carmaweb.genome.tugraz.at
 WoLF PSORT server: wolfsort.seq.cbrc.jp/aboutWoLF_PSORT.html.en