OsLSD1, a Rice Zinc Finger Protein, Regulates Programmed Cell Death and Callus Differentiation

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The *Arabidopsis* LSD1 and LOL1 proteins both contain three conserved zinc finger domains and have antagonistic effects on plant programmed cell death (PCD). In this study, a rice (*Oryza sativa*) functional homolog of LSD1, designated *OsLSD1*, was identified. The expression of *OsLSD1* was light-induced or dark-suppressed. Overexpression of *OsLSD1* driven by the cauliflower mosaic virus 35S promoter accelerated callus differentiation in transformed rice tissues and increased chlorophyll b content in transgenic rice plants. Antisense transgenic rice plants exhibited lesion mimic phenotype, increased expression of *PR-1* mRNA, and an accelerated hypersensitive response when inoculated with avirulent isolates of blast fungus. Both sense and antisense transgenic rice plants conferred significantly enhanced resistance against a virulent isolate of blast fungus. Moreover, ectopic overexpression of *OsLSD1* in transgenic tobacco (*Nicotiana tabacum*) enhanced the tolerance to fumonisins B1 (FB1), a PCD-eliciting toxin. *OsLSD1* green fluorescent protein fusion protein was located in the nucleus of tobacco cells. Our results suggest that *OsLSD1* plays a negative role in regulating plant PCD, whereas it plays a positive role in callus differentiation.

Incompatible plant-pathogen interactions usually induce hypersensitive response (HR) and host defense mechanisms (Bent 1996; Hammond-Kosack and Jones 1996). The HR, characterized by rapid, localized cell death of the plant cells directly in contact with or close to the site of pathogen attack, is a form of programmed cell death (PCD) (Greenberg 1997; Morel and Dangl 1997). HR is often associated with a transient burst of reactive oxygen intermediates, fortification of cell walls, accumulation of antimicrobial phytoalexins, and activation of pathogenesis-related (PR) genes (Dangl et al. 1996; Hammond-Kosack and Jones 1996). Although multiple mediators involved in plant PCD have been identified (Gilchrist 1998; Hoeberichts and Woltering 2003; Loake 2001), the mechanisms and regulation of plant PCD remain poorly defined.

Recently, the characterization of lesion mimic mutants, termed by the spontaneous lesion formation in the absence of pathogens, has become a powerful tool to study the molecular mechanisms of PCD and its relationship with disease resistance

in plants. To date, more than 12 lesion mimic genes have been isolated from *Arabidopsis* (Lorrain et al. 2003; Mou et al. 2000), barley (*Hordeum vulgare*; Buschges et al. 1997), maize (*Zea mays*; Gray et al. 1997; Hu et al. 1998), and rice (Yamanouchi et al. 2002), of which *LSD1*, *Mlo*, and *Lls1* encode negative regulators of plant PCD. *LSD1* of *Arabidopsis* encodes a novel zinc finger protein that negatively regulates plant defense and cell death signaling pathways (Dietrich et al. 1994, 1997). The *Arabidopsis lsd1* mutant confers enhanced resistance to virulent bacteria and fungal pathogens before lesions occur. The barley *mlo* mutant confers broad-spectrum resistance to *Erysiphe graminis* f. sp. *hordei* and exhibits a spontaneous cell death phenotype. The *Mlo* protein has a dual, negative regulatory function in cell death and in the onset of pathogen defense (Buschges et al. 1997). Maize *Lls1* gene is another negative regulator of cell death, which encodes a chloroplast-localized protein that limits cell death by preventing reactive oxidative species formation or by removing a cell death mediator (Gray et al. 1997, 2002). Lesion mimic phenotypes have also been reported in a number of transgenic plants that express foreign or modified genes (Cheong et al. 2003; Fitzgerald et al. 2004; Hu et al. 2003; Li et al. 2002; Mittler and Rizhsky 2000). In a number of lesion mimic mutants, lesion formation was affected by environmental conditions such as temperature and light (Arase et al. 2000; Dietrich et al. 1994; Johal et al. 1994; Yamanouchi et al. 2002).

Recently, a novel zinc finger subfamily of plant-specific transcription factors has been shown to control plant PCD. This C2C2 class of zinc finger proteins contains multiple conserved zinc fingers with the majority of the consensus CxxCRxxLMYxxGASxVxCxxC (Dietrich et al. 1997). *Arabidopsis* LSD1 is a member of this subfamily that contains three zinc finger domains and regulates plant PCD in response to superoxide-dependent signal from cells undergoing HR cell death (Dietrich et al. 1997). It was speculated that LSD1 can limit the spread of cell death through up-regulating copper-zinc superoxide dismutase (CuZnSOD), leading to detoxification of accumulating superoxide (Kliebenstein et al. 1999). Cell death conditioned by *lsd1* depends on EDS1 and PAD4 (Rusterucci et al. 2001) and NIM1/NPR1 (Aviv et al. 2002). Two additional LSD1-like zinc finger proteins were identified in the genome of *Arabidopsis*. One of them, LOL1, was recently reported to determine the cell fate together with LSD1, through antagonistic regulation of CuZnSOD accumulation (Eppe et al. 2003). LOL1 and the transcription factor AtMYB30 both act as positive regulators of plant PCD. Overexpression of these two genes accelerates the appearance of HR in response to avirulent pathogens and increases resistance against virulent pathogens (Eppe et al. 2003; Vailleau et al. 2002). Conditional

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overexpression of *LOL1* to high levels is sufficient to induce cell death in both wild-type and *lsd1* plants (Epple et al. 2003). The other LSD1-like protein, *LOL2*, was suggested to integrate signals that impinge on ROI (reactive oxygen intermediates) homeostasis in plants (Epple et al. 2003).

Rice is one of the most important cereals and the monocot model plant. Annotation of the rice genomic sequence has identified six genes homologous to *Arabidopsis LSD1* (Goff et al. 2002). However, the function of these genes has not been studied.

Here, we report our structural and functional analyses of a rice *LSD1*-like zinc finger protein gene, *OsLSD1*. Antisense suppression of *OsLSD1* in rice evokes lesion mimic phenotype, accelerates HR cell death in response to avirulent blast fungus, and causes HR-like response to a virulent isolate of blast fungus. Overexpression of *OsLSD1* in rice promotes callus differentiation, increases the level of chlorophyll b, and significantly enhances resistance against a virulent isolate of blast fungus. In addition, overexpression of *OsLSD1* in tobacco (*Nicotiana tabacum*) enhances the tolerance to fumonisins B1 (FB1), a fungal toxin inducing cell death (Gilchrist 1997; Wang et al. 1996). Our results suggest that *OsLSD1* is a dual regulator of plant PCD as well as callus differentiation.

RESULTS

Isolation and characterization of a rice zinc finger protein gene.

By randomly sequencing a rice cDNA library constructed previously (He et al. 1999), a rice cDNA (GenBank accession number AY525368) encoding a putative protein homologous to *Arabidopsis LSD1* was obtained and designated as *OsLSD1* (for *Oryza sativa LSD1*). The cDNA is 988 bp in size, containing an open reading frame of 432 bp, a 5' untranslated region of 189 bp, and a 3' untranslated region of 367 bp including the poly (A) tail (Fig. 1). A protein of 143 amino acid residues with an estimated molecular mass of 14.8 kDa was predicted from the coding region. BLASTN search revealed that *OsLSD1* is located at a PAC clone P0672D01 of chromosome 8. The genomic sequence of *OsLSD1* was determined to be 2,971 bp long, consisting of six exons and five introns (Fig. 1).

The predicted *OsLSD1* protein contains three internally conserved zinc finger domains, defined by CxxCxxLLMYxxGxxSVxCxxC, and has 58 and 85% identity with *Arabidopsis LSD1* protein (accession number U87833; Dietrich et al. 1997) and *LOL1* protein (accession number AY349618; Epple et al. 2003), respectively (Fig. 2). Pfam algorithm revealed that *OsLSD1* does not have the putative N-terminal N-glycosylation site and the C-terminal phosphorylation site present in the *LOL1* protein (Fig. 2).

To determine the copy number of *OsLSD1*, genomic DNA of rice cultivars CO39, C101A51, Nipponbare, IR24, and IR36 was digested with restriction enzymes *Bam*HI, *Eco*RI, and *Hind*III, respectively. Southern hybridization revealed single hybridizing fragments (14, 3, and 3 kb for *Bam*HI, *Eco*RI, and *Hind*III enzyme digestion, respectively), indicating that *OsLSD1* is a single-copy gene in rice. No restriction fragment length polymorphism was detected among the five tested rice cultivars, using the above enzymes (data not shown).

Expression

of *OsLSD1* was light-induced or dark-suppressed.

The *Arabidopsis LSD1* gene is required in light acclimatization (Mateo et al. 2004). To investigate *OsLSD1* transcript accumulation in response to light, rice plants (grown under light conditions) were placed in the dark for 24 h and then moved back to the light. Northern blot analysis showed that the expression of *OsLSD1* was reduced significantly at 4 and 24 h after the dark treatment. After moving back to the light, an accumulation of *OsLSD1* transcripts was observed at 4 h, and the transcript level was almost restored at 5 h after exposure to light (Fig. 3). These results demonstrated that the expression of *OsLSD1* was light-induced or dark-suppressed.

Reverse transcription polymerase chain reaction (PCR) using RNA from root, stem, and leaf tissues demonstrated that the *OsLSD1* gene was expressed constitutively (data not shown).

Establishment

of transgenic rice plants and analysis of gene expression.

To facilitate functional study of *OsLSD1*, we constructed the gene in sense (pCOsL-S) and antisense (pCOsL-AS) orientations driven by cauliflower mosaic virus 35S promoter in plant

1	TAGAGAGAGCTGTACTAAGTGAAGTCTCTCATGCAAGCCAGATTACCATAAAGATT	
61	GGGTCATAAGCACTGTTGTTAGGGTTTCTTTCTTGTAGGGAAAGGAAAGTGATTTCAGT	
121	TGCCTGACTGCTGCCAGAATCTGCGAAGCATCTACAAGCGACGCCGTCGTTACGGCAGCC	
181	GGCACGGCAATGCCGGTTCCTTGTCTCCATACCCAAACCCGCGCTGTGCCATTTACACCT	
	M P V P L A P Y P T P P V P F T P	17
241	CCCAATGGGGCTCAAAGCCAGCTCGTGTCTGCGTGGGTGCCGTAATCTGCTCATGTATCCA	
	P N G A Q S Q L V C S G C R N L L M Y P	37
301	GCCGGAGCAACGTCGGTTTGTCTGCGCAGTTTGCAGTACCGTGACTGCTGTGCCTGCTCCT	
	A G A T S V C C A V C S T V T A V P A P	57
361	GGAACCGAGATGGCGCAGTTAGTTTGGCGAGGATGCCACACTCTCCTGATGTATATACGC	
	G T E M A Q L V C G G C H T L L M Y I R	77
421	GGTGGCACAAGTGTGCAATGTTCTTGTCTGTCACACTGTCAACCTGGCTATGGAAGCAAAT	
	G A T S V Q C S C C H T V N L A M E A N	97
481	CAAGTTGCGCATGTAACTGCGGAAGTGGCGTATGCTGCTCATGTACCAGTATGGCGCA	
	Q V A H V N C G N C R M L L M Y Q Y G A	117
541	AGGTCTGTCAAATGCGCAGTCTGCAATTTTGTGACATCAGTTGGGGCCTCACCTGGTATA	
	R S V K C A V C N F V T S V G A S P G I	137
601	GACCAGAAGCCAGCAGCTGAAGTGCATAAATATTGAGAAAGAGCTTCTC	
	D Q K P S S *	143
661	AAACCGATGCTTTGAGTGCACATGTATAGAGTTCTTCCCTGAAGCATTTTTTTTCTCCCT	
721	TTTTTATCTCCCTTTCCCTCCCAATTTTCCACCTTTCTTCTTGGATATGGAGAGAGCCT	
781	GCGGAATAAATGAGAGTTTGTCTAAATACCTAGTGCTCAAAATTCGTCGAATCGTCTGTA	
841	TGACCGAACAAGATTGACAGATTATATACATAAACCTATGATAATATTATGTCATTGCTC	
901	ATTGTTTCTGAAT (A) ₇₅	

Fig. 1. *OsLSD1* cDNA sequence and deduced amino acid sequence. Nucleotide position 1 is the 5' end of the cDNA and position 988 is the 3' end of the cDNA. Amino acid residues are numbered at the right. The cysteine residues of the three zinc finger domains are in bold. Single underlines are splice junctions. Intron sizes in bp are: intron 1 = 676, 2 = 430, 3 = 320, 4 = 460, and 5, 168.

transformation vector pCAMBIA1301 (CAMBIA, Canberra, Australia). Totals of 235, 161, and 106 independent transgenic rice lines for pCOsL-S, pCOsL-AS, and pCAMBIA1301 were obtained, respectively.

Some regenerated seedlings transformed with the antisense gene displayed small, brown lesions in the middle of lower leaves, which led to leaf curling and finally the death of the whole leaf in culture medium (data not shown). A normal leaf phenotype was observed in plants transformed with the sense gene and the control vector.

The primary transformants (T_0) of pCOsL-S and pCOsL-AS were first identified by PCR using hygromycin phosphotransferase (*hpt*) and *OsLSD1* gene-specific primers. A total of 14 sense lines and 16 antisense lines were further confirmed by Southern blot analysis, using the *hpt* gene fragment as a probe. These transgenic lines have either one or two copies of *OsLSD1* transgene (Table 1). Northern blot analysis was performed to evaluate the levels of *OsLSD1* mRNA in these transgenic plants. Three sense lines and five antisense lines exhibited

>30-fold higher *OsLSD1* mRNA or antisense RNA accumulation than that of control plants (nontransgenic rice cv. Nipponbare and a transgenic line P6 with pCAMBIA1301) detected by Northern blot were chosen for further study (data not shown). No reduction of endogenous mRNA was detected in these five antisense lines with Northern blot analysis (Fig. 4A). Seeds obtained from these eight T_0 self-pollinated plants and P6 control plant were analyzed for segregation of hygromycin resistance, and homozygous lines of the third generation (T_2) plants were used for further analysis.

Overexpression of *OsLSD1* in transgenic calli accelerated callus differentiation.

During the initial process of rice tissue transformation, calli transformed with pCOsL-S, pCOsL-AS, and pCAMBIA1301 did not show obvious difference in morphology or growth during cocultivation with *Agrobacterium tumefaciens*, subculture of hygromycin-resistant calli, and predifferentiation. However, expression of *OsLSD1* in sense displayed signifi-

Table 1. Transgene copy number and chlorophyll contents of *OsLSD1* transgenic plants

Transformant no.	Gene copy no. ^a	Chlorophyll content (mg per g of tissue fresh weight)			Mean
		Chlorophyll a	Chlorophyll b	Chlorophyll a+b	
S9	1	0.546 ± 0.012	0.444 ± 0.004	0.990 ± 0.008	0.935 ± 0.050
S12	1	0.456 ± 0.011	0.413 ± 0.008	0.868 ± 0.007	
S15	1	0.446 ± 0.011	0.501 ± 0.009	0.947 ± 0.020	
AS2	2	0.543 ± 0.008	0.193 ± 0.006	0.736 ± 0.014	0.694 ± 0.025
AS4	1	0.454 ± 0.009	0.227 ± 0.004	0.681 ± 0.013	
AS6	2	0.461 ± 0.014	0.239 ± 0.007	0.700 ± 0.010	
AS10	1	0.455 ± 0.013	0.206 ± 0.006	0.661 ± 0.018	0.683 ± 0.013
AS11	1	0.456 ± 0.010	0.235 ± 0.010	0.691 ± 0.007	
P6	1	0.459 ± 0.006	0.237 ± 0.004	0.696 ± 0.010	
WT	0	0.461 ± 0.011	0.209 ± 0.006	0.670 ± 0.011	

^a Transgene copy number was determined from the number of bands hybridizing to the *hpt* gene probe on a genomic Southern blot.

OsLSD1	1	MEVPLAPYPTFPVPETFP-----NG---A
LOL1	1	MEVPLAPYPTFPAPALAPSYNTFPANGSTSG
LSD1	1	MKVADM-----
OsLSD1	22	QSQLVCSGCRNLLMPAGATSVCCAVCSTVTAVPAPG--TE
LOL1	32	QSQLVCSGCRNLLMPVGATSVCCAVCNAVTAVPAPG--TE
LSD1	7	QDQLVCHGCRNLLMPRGASNVRCALCNTINMVPPPPPPH
OsLSD1	61	MAQLVCGGCHTLLMYIRGATSVQCSCCHTVNLALEAN-----
LOL1	71	MAQLVCGGCHTLLMYIRGATSVQCSCCHTVNLALEAN-----
LSD1	48	MAHIICGGCRTMLMYTRGASSVRCSCCQTTLNLPVPAHSNQVAHAPSS
OsLSD1	98	QVAHVNCGNCRMLLMYQYGARSVKCAVCNEFTSVGASPG--TDQKPSS
LOL1	108	QVAHVNCGNCRMLLMYQYGARSVKCAVCNEFTSVGGSTSTTDSKFN
LSD1	86	QVAQINCGHCRTTLLMPYGAASSVKCAVCQFVTNVNMSNGRVPLPTNR
OsLSD1		-----
LOL1		-----
LSD1	141	PNGTACPPSTSTSTPPSQTQTVVVENPMSVDESGKLVSNNVVGVTTDKK

Fig. 2. Alignment of the deduced OsLSD1 protein sequence with *Arabidopsis* LOL1 and LSD1. Conserved amino acid residues among the three proteins are shaded. The numbers on left side indicate the amino acid positions of each protein. Gaps were induced to maximize alignment. Single underline is the predicted N-glycosylation site at the N-terminal of LOL1. Double underline is the putative phosphorylation site at the C-terminal of LOL1.

cant effects on callus differentiation and plant regeneration. The differentiation time for hygromycin-resistant calli was reduced from an average of 7 to 10 days for control vector to 3 to 5 days for *OsLSD1* overexpression after transfer to differentiation medium (Fig. 4B). The regeneration efficiency for the sense construct was 68.5% whereas, for antisense construct and control vector, regeneration efficiency was 46.1 and 49.8%, respectively.

To confirm whether *OsLSD1* could accelerate callus differentiation and plant regeneration, a regeneration experiment was carried out. Mature seeds from homozygous lines S12-2, AS2-1 (antisense), and P6-1 were sterilized and cultured to induce hygromycin-resistant calli. When the calli were transferred to the differentiation medium, S12-2 calli turned green as early as two days after transfer, while AS2-1 and P6-1 varied from four to seven days. After one month, S12-2 calli showed a regeneration rate of 83.3%, approximately twofold higher than that for AS2-1 (41.7%) and control P6-1 (42.9%). These observations clearly demonstrated that overexpression of *OsLSD1* promotes callus differentiation and plant regeneration.

Fig. 3. Northern blot analysis of *OsLSD1* transcript accumulation after dark and light treatment. Total RNAs (20 µg each lane) were isolated from rice leaf tissues (3 weeks old) following dark treatment for 4 (D4h) and 24 h (D24h) and then in light for 5 (L5m) and 30 min (L30m) and 1 (L1h), 4 (L4h), and 5 h (L5h). The RNA blot was probed with the cDNA fragment of *OsLSD1*. rRNA stained with ethidium bromide is shown as a control.

Overexpression

of *OsLSD1* increased chlorophyll b content.

In addition to the shorter differentiation time and higher regeneration rate, overexpression of *OsLSD1* resulted in a phenotype characterized by obvious dark green transgenic plants. Quantification of the total chlorophyll (a and b) content of the plant tissues revealed a marked increase from the average of 0.683 milligrams of chlorophyll per gram of fresh weight of tissues in control and 0.694 mg/g in the antisense plants to 0.935 mg/g in the sense lines (Table 1). The increase in chlorophyll content was mainly due to chlorophyll b, whereas the level of chlorophyll a remained unaltered. Similar results were obtained from transgenic tobacco plants overexpressing *OsLSD1* (data not shown).

OsLSD1 antisense transgenic rice plants developed a lesion mimic phenotype accompanied by increased *PR-1* gene expression.

When the T1 generation of these nine transgenic lines were grown in a greenhouse in Hainan, P. R. China (latitude 19.5N, longitude 100.18E) during February through April, three of five antisense lines (AS2, AS4, and AS10) developed discrete and brown lesions on mature leaves in the absence of pathogen attack at about the six-week-old stage, which further developed to cause death of the whole leaf (Fig. 4C). However, when the same seeds were grown in a greenhouse in Beijing (latitude 29.39N, longitude 116.20E) during June through September, no visible necrotic lesion was observed in any plants. The major differences between growth conditions in Hainan and Beijing were temperature and day length. Generally, temperature is higher and day length is longer in Beijing during June to September than those in Hainan during February to April (average of 25.7°C and 15 to 16 h vs. 21.9°C and 11 to 12 h, respectively).

HR-like cell death is usually correlated with increased PR protein expression and an increased resistance to pathogens

Fig. 4. The phenotype and Northern blot analysis of transgenic rice plants. **A**, Northern blot analysis of *OsLSD1* mRNA in transgenic rice lines (T₀ generation) used in this study. Total RNA (20 µg each lane), isolated from sense (S9, S12, S15) and antisense lines (AS2, AS4, AS6, AS10, AS11), control (P6) and wild-type (WT) plants were hybridized with a ³²P-labeled *OsLSD1* antisense RNA probe. The sense transcripts were approximately the same size as the endogenous *OsLSD1* transcripts (approximately 1 kb). **B**, Photograph of resistant calli 12 days after transfer to regeneration medium to show the accelerated callus differentiation by overexpression of *OsLSD1* gene. **C**, Lesions formed on *OsLSD1* antisense transgenic rice leaves (AS2, AS4, and AS10) at the 6-week-old stage. **D**, Expression of *PR-1* gene in antisense plants. Total RNAs were extracted from wide-type (WT) plants or *OsLSD1* antisense transgenic plants at the 8-week-old stage with lesion formation.

(Dangl et al. 1996). Expression of the *PR-1* gene was analyzed in eight-week-old antisense transgenic plants with spontaneous lesions. Figure 4D shows that the *PR-1* mRNA was accumulated to a high level in the eight-week-old antisense plants with lesions, whereas no hybridization was detected for control nontransgenic plants. In addition, the high accumulation of *PR-1* mRNA was not detected in three-week-old leaves (no lesion) of antisense plants (Fig. 5C). These results indicate that suppression of *OsLSD1* triggers HR-like cell death at the adult stage and enhances *PR-1* gene expression.

Both overexpression and antisense transgenic rice of *OsLSD1* enhanced resistance to virulent blast fungus.

To assess the effect of *OsLSD1* on disease response, 24 plants of each transgenic line were inoculated with a virulent blast isolate, Y34. Surprisingly, leaves of antisense transgenic plants developed a large number of small, brown, HR-like lesions as early as 24 h after inoculation, while the sense transgenic lines exhibited fewer and smaller lesions than the antisense plants two days after inoculation. Visible disease lesions appeared on leaves of control plants three days after inoculation. Seven days after inoculation, sparse, reduced-size lesions were observed on the leaves of sense transgenic plants, in con-

trast to the many dispersed disease lesions on P6 and Nipponbare control plants (Fig. 5A). All antisense transgenic lines revealed many but limited-size lesions, as if a HR had occurred (Fig. 5A). The disease index was scored and is illustrated in Figure 5B. Over 87.5% of plants of each transgenic line developed lesions ranged from 0 to 1, whereas most of the lesions formed on control plants were at 5. The significant higher resistance to rice blast fungus conferred by *OsLSD1* sense and antisense transgenic rice plants was confirmed by three independent experiments.

To examine the cellular events in association with the blast resistance, the expressions of *PR-1* and *PBZ1* genes were examined by Northern blot analysis. *PR-1* and *PBZ1* mRNA accumulation were induced to maximal levels at 48 h postinoculation in nontransgenic control rice cv. Nipponbare, S15-2, and AS2-1 plants (Fig. 5C). *PR-1* and *PBZ1* transcripts abundance in S15-2 and AS2-1 plants was higher than that in control plants, with a more pronounced increase in the S15-2 plants than in the AS2-1 plants. Similar *PR-1* and *PBZ1* expression results were observed in S9-37, AS4-3, and AS10-2 plants (data not shown). These results demonstrated that the increased expression of defense-related genes is associated with the enhanced resistance to blast disease in *OsLSD1* sense and antisense transformants.

Fig. 5. Disease response to virulent and avirulent isolates of blast fungus in transgenic rice plants. **A**, Typical symptoms on different transgenic lines seven days after inoculation with a virulent isolate Y34. **B**, Disease severity expressed as the percentage of disease lesions at each level (index 0 to 5) in 24 plants of each transgenic line. **C**, Expression of two defense-related genes in *OsLSD1* transgenic sense (S15-2), antisense (AS2-1), and wild-type (WT) plants. Total RNAs were isolated at different times after inoculation with isolate Y34. **D**, Representative leaves stained with trypan blue. Leaves inoculated with avirulent isolate P06-6 were removed and stained at 48 h after inoculation.

Antisense suppression of *OsLSD1* leads to accelerated and intensified HR to avirulent pathogens.

To test whether alteration of *OsLSD1* expression affects the HR caused by avirulent pathogens, the leaves of transgenic lines were infected with three avirulent isolates of rice blast fungus: P06-6, 97-55-2, and ML8. Infected leaves 48 h post-inoculation were stained with trypan blue, a histochemical indicator of cell death. Figure 5D shows rice leaves inoculated with P06-6. Dark-blue staining was observed on many leaves of antisense transgenic lines but not the control plants and sense transgenic plants. Leaves inoculated with isolates 97-55-2 and ML8 produced similar responses (data not shown). Numbers of HR lesions formed on sense transgenic plants were slightly reduced compared with those of control plants (data not shown). These observations were observed in repeated experiments, suggesting that the appearance of the HR in response to avirulent blast fungal attack was accelerated by antisense expression of *OsLSD1* in rice.

Overexpression of *OsLSD1* in tobacco enhanced the tolerance to FB1.

The pCOsL-S vector was also transformed into tobacco. Four of six transgenic lines showing high levels of *OsLSD1* mRNA accumulation (data not shown) were used for the analysis of response to FB1. Tobacco cv. SR1 is insensitive to the sphinganine-analog mycotoxins (SAM) (Brandwagt et al. 2001). While infiltration with 10 μ M FB1 solution can elicit macroscopic lesions on the *Arabidopsis* leaves (Stone et al. 2000), no necrosis can be detected on the leaves of four-week-old tobacco plants treated with a higher concentration of FB1 (100 μ M). FB1 treatment also causes growth arrest of plant seedlings (Lamprecht et al. 1994; Stone et al. 2000). The transgenic tobacco seedlings (second generation) overexpressing *OsLSD1* grown on $1/2$ Murashige and Skoog (MS) medium containing 20 μ g of hygromycin per milliliter for 10 days were transferred to medium supplemented with 2 or 5 μ M FB1. During the first two weeks after the FB1 treatment, there was no obvious difference between the *OsLSD1* transgenic plants and control plants (with vector pCAMBIA1301). However, the difference between them was gradually visible thereafter. After one month, four *OsLSD1* transgenic lines displayed higher

biomass than that of control plants (Fig. 6). FB1 at both concentrations inhibited shoot and leaf growth, root elongation, and root-hair formation to a similar extent. This result demonstrates that transgenic tobacco plants overexpressing *OsLSD1* are more tolerant to FB1.

OsLSD1-GFP fusion protein is localized in the nucleus.

Arabidopsis LSD1 and LOL1 are both C2C2 zinc finger proteins and may act as transcription factors (Dietrich et al. 1997; Epple et al. 2003). However, the localization of these two proteins has not been determined. We speculate that *OsLSD1* protein functions as a transcript factor, which could be located in the nucleus. To determine the subcellular localization of *OsLSD1*, an *OsLSD1*-GFP fusion gene was cloned into the binary vector pCAMBIA1300 and then was transformed into tobacco Bright Yellow-2 (BY-2) suspension cells. The green fluorescent signal was detected mainly in the nuclei of transgenic cells (data not shown). In contrast, BY-2 cells transformed with the control construct pCGFP showed green fluorescent protein (GFP) fluorescence throughout the cytoplasm and the nucleus (data not shown). Stable transgenic tobacco plants expressing *OsLSD1*-GFP and GFP were obtained. Examination of the transgenic tobacco root tips with a confocal microscope also revealed the nuclear localization of *OsLSD1*-GFP (data not shown). However, no conventional nuclear localization signal (NLS) was found in *OsLSD1*. Recently, it was reported that rice MOC1 was located in the nucleus without NLS, indicating there may be an unidentified NLS in MOC1 or MOC1 may be imported into the nucleus independent of NLS (Li et al. 2003). It is possible that similar mechanisms or interactions between *OsLSD1* and other proteins containing an NLS result in the localization of *OsLSD1* in nucleus.

DISCUSSION

No obvious reduction of endogenous *OsLSD1* mRNA was detected by Northern blot (Fig. 4B) and real-time PCR (data not shown) in the *OsLSD1* antisense plants, despite the fact that *OsLSD1* antisense RNAs were accumulated to a high level in these transgenic plants. Nevertheless, the antisense expression of *OsLSD1* in transformed rice plants led to distinct biological effect, including evoking lesion mimic phenotype, enhancing resistance to a virulent blast isolate, and accelerating HR-like cell death caused by avirulent pathogens. These characteristics resemble very much the phenotype displayed by the *Arabidopsis* *lsd1* mutant plant, suggesting the suppression of the *OsLSD1* gene function. A possible explanation to this seemingly contradictory result is that antisense *OsLSD1* RNA does not lead to efficient degradation of the *OsLSD1* sense RNA. Yet, *OsLSD1* may be so critical that a minor reduction causes distinct effects.

Spontaneous formation of HR-like lesions has been produced by the overexpression of foreign genes such as cholera toxin (Beffa et al. 1995), bacterio-opsin (Mittler et al. 1995), oxalate oxidase (Hu et al. 2003), BWMK1 (Cheong et al. 2003), and (At)NPR1 (Fitzgerald et al. 2004), or inhibition of catalase (Takahashi et al. 1997), ascorbate peroxidase (Orvar and Ellis 1997), and protoporphyrinogen oxidase (Molina et al. 1999). These results indicate that perturbations of both signal transduction and metabolic genes can cause cell death in plants. The mechanism underlying lesion formation in *OsLSD1* antisense plants is not immediately understood, but the formation of lesions appeared to be triggered by certain environmental conditions. Environmental regulation on lesion formation has been reported in several lesion mimic plants. For example, high temperature was reported to inhibit lesion

Fig. 6. Response of *OsLSD1* transgenic tobacco to FB1 and subcellular localization of *OsLSD1*. Ten-day-old tobacco seedlings were transferred to $1/2$ Murashige and Skoog plates supplemented with 5 μ M FB1 and were photographed four weeks later. pCOsL-S refers to representative *OsLSD1* transgenic tobacco plants. pCAMBIA1301 refers to control plants transformed with vector pCAMBIA1301.

formation in transgenic plants overexpressing tomato (*Lycopersicon esculentum*) disease resistance gene *Pto* (Li et al. 2002), bacterial proton pump (Abad et al. 1997), and pyruvate decarboxylase (Tadege et al. 1998). Lesion formation was also affected by light intensity and day length in a number of lesion mimic mutants of *Arabidopsis* (Dietrich et al. 1994), maize (Johal et al. 1994), and rice (Arase et al. 2000; Yamanouchi et al. 2002). *lsd1* plants develop lesions under long-day growth conditions, whereas *lsd2* and *lsd5* develop lesions under short-day environments (Dietrich et al. 1994). Lesion formation in rice *spl7* mutants requires high temperature and UV solar radiation (Yamanouchi et al. 2002). In the case of *OsLSD1* antisense plants, lesion formation seems to be inhibited by high temperatures, long day conditions, or both.

OsLSD1 antisense plants conferred significant resistance against a virulent isolate of blast fungus. The experiment of blast inoculation was performed on three-week-old transgenic plants before lesion formation. Enhanced resistance upon virulent pathogens has been observed in many lesion mimic mutants, most of which show elevated resistance to pathogens only when lesions occur (Dietrich et al. 1994; Greenberg et al. 1994; Yin et al. 2000). So far, only four lesion mimic mutants appear to exhibit elevated resistance before lesion development. The *Arabidopsis lsd1* mutant was resistant to virulent pathogens at a state when no lesions were observed on the leaves (Dietrich et al. 1994). The barley *mlo* mutant conferred non-race-specific resistance to the powdery mildew fungus prior to the appearance of lesions (Buschges et al. 1997). Finally, the rice lesion-mimic mutants *spl13* and *spl14* also displayed enhanced resistance to rice blast and bacterial blight in the absence of lesions (Mizobuchi et al. 2002). Different from *spl13* and *spl14*, which express PR genes before the onset of lesion formation, and similar to the *Arabidopsis lsd1* mutant (Dietrich et al. 1994), *OsLSD1* antisense transgenic plants did not express *PR-1* in leaves free of lesions (Fig. 5C). However, upon pathogen infection, defense-related genes are quickly induced to a higher level in the *OsLSD1* antisense plants than in the control plants. The higher expression of the defense-related genes is positively correlated with the enhanced disease resistance in the *OsLSD1* antisense plants.

Like the *OsLSD1* antisense transgenic plants, *OsLSD1* sense transgenic plants also showed enhanced resistance to virulent blast fungus. We propose that *OsLSD1* mediates resistance to virulent blast fungus through different signal pathways in these two systems. *OsLSD1* antisense plants may acquire blast resistance by alleviating the inhibition on hypersensitive cell death and defense-related genes. Our results suggested that *OsLSD1* is a negative regulator of PCD. Many inhibitors of PCD have been proved to enhance the plant disease resistance. For example, transgenic tobacco plants constitutively expressing animal anti-apoptotic genes *Bcl-2*, *Bcl-xl*, and *CED-9* confer resistance to several necrotrophic pathogens (Dickman et al. 2001). Constitutive expression of anti-apoptotic baculovirus p35 gene in tomato blocks PCD and confers broad-spectrum resistance to disease (Lincoln et al. 2002). Therefore, if *OsLSD1* indeed functions as a negative regulator of PCD, sense plants may confer resistance by protection from cell death or enhancing the cell vigor. The better performance of rice calli overexpressing *OsLSD1* during tissue culture and elevated tolerance of tobacco transgenic plants overexpressing *OsLSD1* to FB1 support this speculation.

FB1 elicits PCD in both plants and animal tissue culture cells, presumably through competitive inhibition of sphinganine *N*-acyltransferase, a key enzyme in sphingolipid biosynthesis (Gilchrist 1997; Wang et al. 1996; Yoo et al. 1996). The sphingolipid ceramides influence key aspects of cell growth, regulation, differentiation, and death and are postulated to be a

key regulator of apoptosis (Obeid et al. 1993; Venkataraman et al. 2002). Recent studies demonstrated that the yeast longevity assurance gene (*LAG1*) is required for acyl-CoA-dependent ceramide synthesis (D'Mello et al. 1994). A tomato gene, *ASC-1*, homolog of *LAG1*, mediates FB1 resistance, presumably through encoding a SAM-insensitive sphinganine *N*-acyltransferase in plants (Brandwagt et al. 2000). Overexpression of *ASC-1* in SAM-sensitive tomato hairy roots mediates a high insensitivity to SAM (Brandwagt et al. 2002). In addition, FB1 inhibition can be bypassed by formation of ceramide via the reverse activity of a rat brain ceramidase (El Bawab et al. 2001). It remained to be tested whether *OsLSD1* positively regulates sphinganine *N*-acyltransferase and whether overexpression of the *OsLSD1* gene elevates sphingolipid biosynthesis.

Overexpression of *OsLSD1* increased the level of chlorophyll b. The elevated chlorophyll production may promote vegetative growth via an increase in light-absorbing capacity and photosynthesis, thus accelerating the growth and differentiation of calli in sense-transgenic lines. The metabolism of chlorophyll plays a role in disease response and cell death. Several studies have shown that chlorophyll b is important in stabilizing apoproteins in the thylakoid membrane (Meehan et al. 1996). The blocks of chlorophyll metabolism resulted in lesion mimic phenotype in *Arabidopsis* plants (Hu et al. 1998; Mach et al. 2001). Whether increased chlorophyll b content correlates with disease response in *OsLSD1* sense transformants is unclear. However, with the pleiotropic effects on rice, including promoted callus differentiation, increased resistance to blast fungus, and enhanced tolerance to FB1, *OsLSD1* could be used as a candidate gene for engineering crops with useful traits.

The deduced *OsLSD1* protein contains three internal conserved zinc finger domains, which share the consensus sequence with *LSD1* and *LOL1*. Although, at the amino acid level, *OsLSD1* is more homologous to *LOL1* than to *LSD1* (Fig. 2), functionally *OsLSD1* is more closely related to *LSD1*. *OsLSD1* antisense transgenic plants share several common features with the *Arabidopsis lsd1* mutant. First, dysfunction of both *OsLSD1* and *LSD1* genes leads to lesion mimic phenotype accompanied by elevated *PR-1* gene expression. Second, the lesion mimic phenotype on both plants is light-dependent and the enhanced resistance to virulent pathogens is induced before lesion formation. *LOL1* appears to be a positive regulator of plant PCD. Constitutive overexpression of *LOL1* in *Arabidopsis* only increased two- to threefold mRNA accumulation because of the lethal effect of *LOL1* (Epple et al. 2003). In the contrast, *OsLSD1* acts as a negative regulator of cell death. *OsLSD1* antisense transgenic rice plants i) developed lesion mimic phenotype with increased expression of *PR-1* mRNA, ii) showed HR-like response to a virulent isolate of blast fungus, and iii) accelerated and intensified the appearance of the HR in response to different isolates of avirulent blast fungus. In addition, three sense transgenic lines with 30- to 90-fold increases in the *OsLSD1* mRNA exhibited no sign of cell death (Fig. 4B). The more vigorous tissue growth, higher regeneration rate, and the enhanced tolerance to the cell death-inducing toxin FB1 of the *OsLSD1* sense plants also support the negative regulatory role of *OsLSD1* in cell death. Furthermore, we also generated *OsLSD1* sense transgenic *Arabidopsis* lines with high accumulation of *OsLSD1* transcript. These transgenic *Arabidopsis* plants grew normally without a sign of lesion development (C. He, unpublished data). All these results indicated that *OsLSD1* functions differently from *LOL1*, although *OsLSD1* is the rice gene with the best homology to the *Arabidopsis LOL1*. Such functional difference may attribute to the N-glycosylation and the phosphorylation sites that are present in *LOL1* but absent from *OsLSD1*. These sites are known to be important for the protein functions.

MATERIALS AND METHODS

Southern and Northern hybridization.

Rice genomic DNA was isolated as described by Dellaporta and associates (1984). About 2 µg of rice genomic DNA was digested with appropriate restriction enzymes, was resolved on a 0.8% agarose gel, and then was transferred onto hybond-N⁺ membrane (Amersham, Buckinghamshire, U.K.). Total RNA was isolated with the Trizol total RNA isolation reagent (Invitrogen, Carlsbad, CA, U.S.A.). Total RNA (20 µg per lane) was fractionated in a 1.2% (wt/vol) formaldehyde-agarose gel and was transferred onto hybond-N⁺ membrane. The *OsLSD1* antisense RNA probe used in Figure 4B was prepared with a Riboprobe System-T7 (Promega, Madison, WI, U.S.A.) with [α -³²P]dCTP. The other probes were labeled using the Primer-a-Gene labeling system (Promega) with [α -³²P]dCTP. Hybridization and washing were carried out using standard procedures (Sambrook et al. 1989). Relative amounts of the transcript were determined using a Phosphor Imager Storm 840 (Amersham), following the manufacturer's instructions.

Constructs preparation and rice transformation.

OsLSD1 cDNA was amplified by PCR and was introduced in sense and antisense orientation into *NcoI* and *BstEII* sites of pCambia1301 vector, downstream of the cauliflower mosaic virus 35S promoter, resulting in pCosl-S and pCosl-AS, respectively. *A. tumefaciens* EHA105 transformants carrying pCosl-S and pCosl-AS were used to transform rice cv. Nipponbare. Rice tissue transformation was performed according to Hiei and associates (1994). Transformed calli were selected on medium containing 50 µg of hygromycin per milliliter. Regenerated plants were transferred to soil and were grown in a greenhouse.

Construction of *OsLSD1-GFP* fusion protein gene vector and transformation of tobacco BY-2 suspension cells.

The *OsLSD1* open reading frame was amplified by PCR and was introduced into *XhoI* and *KpnI* sites of pGFP2 vector, to form the recombinant plasmid pGFP2-*OsLSD1*. The in-frame fusion between *OsLSD1* and *GFP* was confirmed by sequencing in the junction region between the *OsLSD1* and *GFP* coding regions in pGFP2-*OsLSD1*. The DNA fragment within the *EcoRI* and *PstI* of pGFP2-*OsLSD1* and pGFP2, respectively, were subcloned into the binary vector pCambia1300, to produce the final constructs pCosl-GFP and pCGFP. Tobacco BY-2 suspension culture cells were transformed with *A. tumefaciens* EHA105 bearing pCosl-GFP or pCGFP, according to Granger and associates (2000). GFP expression was visualized with a confocal microscope (MRC 1024; Bio-Rad, Hercules, CA, U.S.A.).

Rice blast inoculation.

Isolates of *Magnaporthe grisea*, P06-6 (from the International Rice Research Institute, Manila, The Philippines), Y34 (collected in Yunnan, China), 97-55-2 (collected in Jiangsu, China), and ML8 (from Mali) were used in the inoculation experiments. Three-week-old rice plants were inoculated with blast suspension spores in a concentration of 1×10^5 spores per ml. The inoculated plants were placed in a dark, dew chamber for 24 h at 25°C and subsequently were transferred to a growth chamber under 12 h light, 25°C, and 90% relative humidity. Leaf tissues were harvested at different timepoints after inoculation. Disease reaction of the inoculated plants was scored seven days after inoculation with the scoring system described by Bonman and associates (1986).

Chlorophyll quantification.

Chlorophyll concentrations were detected according to Lichtenthaler (1987).

Histochemical analysis.

Leaves of transgenic and control plants were harvested two days after inoculation with avirulent blast isolates and were stained with trypan blue as previously described (Bowling et al. 1997).

Transformation of tobacco and treatment of the transgenic plants with FB1.

The transgenic tobacco plants were generated following a standard leaf disk transformation method (Horsch et al. 1988) and were selected by hygromycin resistance. Seeds of pCosl-S transgenic tobacco plants were germinated axenically on 1/2 MS medium containing 20 µg of hygromycin per ml and were maintained in a temperature-controlled growth room (25°C) with 16 h of light. Tobacco seedlings were grown for 10 days before being transferred to fresh 1/2 MS plates supplemented with 2 or 5 µM FB1 (Sigma, St. Louis). The growth and development of these tobacco seedlings were observed every three days.

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LITERATURE CITED

- Abad, M. S., Hakimi, S. M., Kaniewski, W. K., Rommens, C. M. T., Shulaev, V., Lam, E., and Shah, D. M. 1997. Characterization of acquired resistance in lesion mimic transgenic potato expressing bacteriopsin. *Mol. Plant-Microbe Interact.* 10:635-645.
- Arase, S., Fujita, K., Uehara, T., Honda, Y., and Isota, J. 2000. Light-enhanced resistance to *Magnaporthe grisea* infection in the rice Segikuchi lesion mutants. *J. Phytopathol.* 148:197-203.
- Aviv, D. H., Rusterucci, C., Iii, B. F., Dietrich, R. A., Parker, J. E., and Dangel, J. L. 2002. Runaway cell death, but not basal disease resistance, in *Isd1* is SA- and *NIM1/NPR1*-dependent. *Plant J.* 29:381-391.
- Beffa, R., Szell, M., Meuwly, P., Pay, A., Vogeli-Lange, R., Mettraux, J. P., Neuhaus, G., Meins, F., Jr., and Nagy, F. 1995. Cholera toxin elevates pathogen resistance and induces pathogenesis-related gene expression in tobacco. *EMBO (Eur. Mol. Biol. Organ.) J.* 14:5753-5761.
- Bent, A. F. 1996. Plant disease resistance genes: Function meets structure. *Plant cell* 8:1757-1771.
- Bonman, J. M., Vergel de Dios, T. I., and Khin, M. M. 1986. Physiologic specialization of *Pyricularia oryzae* in the Philippines. *Plant Dis.* 70:767-769.
- Bowling, S. A., Clarke, J. D., Liu, Y., Klessig, D. F., and Dong, X. 1997. The *cpr5* mutant of *Arabidopsis* expresses both NPR1-dependent and NPR1-independent resistance. *Plant Cell* 9:1573-1584.
- Brandwagt, B. F., Mesbah, L. A., Takken, F. L. W., Laurent, P. L., Kneppers, T. J. A., Hille, J., and Nijkamp, H. J. 2000. A longevity assurance gene homolog of tomato mediates resistance to *Alternaria alternata* f. sp. *lycopersici* toxins and fumonisin B1. *Proc. Natl. Acad. Sci. U.S.A.* 97:4961-4966.
- Brandwagt, B. F., Kneppers, T. J. A., Van der Weerden, G. M., Nijkamp, H. J. J., and Hille, J. 2001. Most AAL toxin-sensitive *Nicotiana* species are resistant to the tomato fungal pathogen *Alternaria alternata* f. sp. *lycopersici*. *Mol. Plant-Microbe Interact.* 14:460-470.
- Brandwagt, B. F., Kneppers, T. J. A., Nijkamp, H. J. J., and Hille, J. 2002. Overexpression of the tomato *Asc-1* gene mediates high insensitivity to AAL toxins and fumonisin B1 in tomato hairy roots and confers resistance to *Alternaria alternata* f. sp. *lycopersici* in *Nicotiana umbratica* plants. *Mol. Plant-Microbe Interact.* 15:35-42.
- Buschges, R., Hollricher, K., Panstruga, R., Simons, G., Wolter, M., Frijters, A., Van Daelen, R., Van der Lee, T., Dergaarde, P., Vos, P., Salamini, F., and Schulze-Lefert, P. 1997. The barley *Mlo* gene: A novel control element of plant pathogen resistance. *Cell* 88:695-705.
- Cheong, Y. H., Moon, B. C., Kim, J. K., Kim, C. Y., Kim, M. C., Kim, I. H., Park, C. Y., Kim, J. C., Park, B. O., Koo, S. C., Yoon, H. W., Chung, W. S., Lim, C. O., Lee, S. Y., and Cho, M. J. 2003. BWMK1, a rice mitogen-activated protein kinase, locates in the nucleus and mediates pathogenesis-related gene expression by activation of a transcription

- factor. *Plant Physiol.* 132:1961-1972.
- Dangl, J. L., Dietrich, R. A., and Richberg, M. H. 1996. Death don't have no mercy: Cell death programs in plant-microbe interactions. *Plant Cell* 8:1793-1807.
- Dellaporta, S. L., Wood, J., and Hicks, J. B. 1984. Maize DNA miniprep. Pages 36-37 in: *Molecular Biology of Plants: A Laboratory Course Manual*. R. Maimberg, ed. Cold Springs Harbor Laboratory, Cold Springs Harbor, NY, U.S.A.
- Dickman, M. B., Park, Y. K., Oltersdorf, T., Li, W., Clemente, T., and French, R. 2001. Abrogation of disease development in plants expressing animal antiapoptotic genes. *Proc. Natl. Acad. Sci. U.S.A.* 98:6957-6962.
- Dietrich, R. A., Delaney, T. P., Uknes, S. J., Ward, E. R., Ryals, J. A., and Dangl, J. L. 1994. *Arabidopsis* mutants simulating disease resistance response. *Cell* 77:565-577.
- Dietrich, R. A., Richberg, M. H., Schmidt, R., Dean, C., and Dangl, J. L. 1997. A novel zinc finger protein is encoded by the *Arabidopsis LSD1* gene and functions as a negative regulator of plant cell death. *Cell* 88:685-694.
- D'Mello, N. P., Childress, A. M., Franklin, D. S., Kale, S. P., Pinswasdi, C., and Jazwinski, S. M. 1994. Cloning and characterization of LAG1, a longevity-assurance gene in yeast. *J. Biol. Chem.* 269:15451-15459.
- El Bawab, S., Birbes, H., Roddy, P., Szulc, Z. M., Bielawska, A., and Hannun, Y. A. 2001. Biochemical characterization of the reverse activity of rat brain ceramidase. *J. Biol. Chem.* 276:16758-16766.
- Eppe, P., Mack, A. A., Morris, V. R. F., and Dangl, J. L. 2003. Antagonistic control of oxidative stress-induced cell death in *Arabidopsis* by two related, plant-specific zinc finger proteins. *Proc. Natl. Acad. Sci. U.S.A.* 100:6831-6836.
- 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.
- Gilchrist, D. G. 1997. Mycotoxins reveal connections between plants and animals in apoptosis and ceramide signaling. *Cell Death Differ.* 4:689-698.
- Gilchrist, D. G. 1998. Programmed cell death in plant disease: The purpose and promise of cellular suicide. *Annu. Rev. Phytopathol.* 36:393-414.
- Goff, S. A., Rieke, D., Lan, T. H., Presting, G., Wang, R., Dunn, M., Glazebrook, J., Sessions, A., Oeller, P., Varma, H., Hadley, D., Hutchison, D., Martin, C., Katagiri, F., Lange, B. M., Moughamer, T., Xia, Y., Budworth, P., Zhong, J., Miguel, T., Paszkowski, U., Zhang, S., Colbert, M., Sun, W. L., Chen, L., Cooper, B., Park, S., Wood, T. C., Mao, L., Quail, P., Wing, R., Dean, R., Yu, Y., Zharkikh, A., Shen, R., Sahasrabudhe, S., Thomas, A., Cannings, R., Gutin, A., Pruss, D., Reid, J., Tavtigian, S., Mitchell, J., Eldredge, G., Scholl, T., Miller, R. M., Bhatnagar, S., Adey, N., Rubano, T., Tusneem, N., Robinson, R., Feldhaus, J., Macalima, T., Oliphant, A., and Briggs, S. 2002. A draft sequence of the rice genome (*Oryza sativa* L. ssp. *japonica*). *Science* 296:92-100.
- Granger, C. L., and Cyr, R. J. 2000. Microtubule reorganization in tobacco BY-2 cells stably expressing GFP-MBD. *Planta* 210:502-509.
- Gray, J., Close, P. S., Briggs, S. P., and Johal, G. S. 1997. A novel suppressor of cell death in plants encoded by the *L1sl* gene of maize. *Cell* 89:25-31.
- Gray, J., Janick-Buckner, D., Buckner, B., Close, P. S., and Johal, G. S. 2002. Light-dependent death of maize *l1sl* cells is mediated by mature chloroplasts. *Plant Physiol.* 130:1894-1907.
- Greenberg, J. T. 1997. Programmed cell death in plant-pathogen interactions. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 48:525-545.
- Greenberg, J. T., Guo, A., Klessig, D. F., and Ausubel, F. M. 1994. Programmed cell death in plants: A pathogen-triggered response activated coordinately with multiple defense functions. *Cell* 77:551-563.
- Hammond-Kosack, K. E., and Jones, J. D. G. 1996. Resistance gene-dependent plant defense responses. *Plant Cell* 8:1773-1791.
- He, C., Fong, S. H., Yang, D., and Wang, G. L. 1999. BWMK1, a novel MAP kinase induced by fungal infection and mechanical wounding in rice. *Mol. Plant-Microbe Interact.* 12:1064-1073.
- Hiei, Y., Ohta, S., Komari, T., and Kumashiro, T. 1994. Efficient transformation of rice (*Oryza sativa* L.) mediated by *Agrobacterium* and sequence analysis of the boundaries of the T-DNA. *Plant J.* 6:271-282.
- Hoebrechts, F. A., and Woltering, E. J. 2003. Multiple mediators of plant programmed cell death: Interplay of conserved cell death mechanisms and plant-specific regulators. *Bioessays* 25:47-57.
- Horsch, R. B., Fry, J., Hoffmann, N., Neidermeyer, J., Rogers, S. G., and Fraley, R. T. 1988. Leaf disc transformation. Pages 1-9 in: *Plant Molecular Biology Manual*. S. B. Gelvin, R. A. Schilperoot, eds. Kluwer Academic Publishers, Dordrecht, The Netherlands.
- Hu, G., Yalpani, N., Briggs, S. P., and Johal, G. S. 1998. A porphyrin pathway impairment is responsible for the phenotype of a dominant disease lesion mimic mutant of maize. *Plant Cell* 10:1095-1105.
- Hu, X., Bidney, D. L., Yalpani, N., Duvick, J. P., Crasta, O., Folkerts, O., and Lu, G. 2003. Overexpression of a gene encoding hydrogen peroxide-generating oxalate oxidase evokes defense responses in sunflower. *Plant Physiol.* 133:170-181.
- Johal, G. S., Hulbert, S. H., and Briggs, S. P. 1994. Disease lesion mimics of maize: A model for cell death in plants. *Bioessays* 17:685-692.
- Kliebenstein, D. J., Dietrich, R. A., Martin, A. C., Last, R. L., and Dangl, J. L. 1999. LSD1 regulates salicylic acid induction of copper zinc superoxide dismutase in *Arabidopsis thaliana*. *Mol. Plant-Microbe Interact.* 12:1022-1026.
- Lamprecht, S. C., Marasas, W. F. O., Alberts, J. F., Cawood, M. E., Gelderblom, W. C. A., Shepherd, G. S., Thiel, P. G., Calitz, F. J. 1994. Phytotoxicity of fumonisins and TA-toxin to corn and tomato. *Phytopathology* 84:383-391.
- Li, J. X., Shan, L. B., Zhou, J. M., and Tang, X. Y. 2002. Overexpression of Pto induces a salicylate-independent cell death but inhibits necrotic lesions caused by salicylate-deficiency in tomato plants. *Mol. Plant-Microbe Interact.* 15:654-661.
- Li, X. Y., Qian, Q., Fu, Z. M., Wang, Y. H., Xiong, G. S., Zeng, D. L., Wang, X. Q., Liu, X. F., Teng, S., Hiroshi, F., Yuan, M., Luo, D., Han, B., and Li, J. Y. 2003. Control of tillering in rice. *Nature* 422:618-621.
- Lichtenthaler, H. K. 1987. Chlorophylls and carotenoids: Pigments of photosynthetic biomembranes. *Methods Enzymol.* 148:350-382.
- Lincoln, J. E., Richael, C., Overduin, B., Smith, K., Bostock, R., and Gilchrist, D. G. 2002. Expression of the antiapoptotic baculovirus p35 gene in tomato blocks programmed cell death and provides broad-spectrum resistance to disease. *Proc. Natl. Acad. Sci. U.S.A.* 99:15217-15221.
- Loake, G. 2001. Plant cell death: Unmasking the gatekeepers. *Curr. Biol.* 11:R1028-R1031.
- Lorrain, S., Vaillau, F., Balague, C., and Roby, D. 2003. Lesion mimic mutants: Keys for deciphering cell death and defense pathways in plants? *Trends Plant Sci.* 8:263-271.
- Mach, J. M., Castillo, A. R., Hoogstraten, R., and Greenberg, J. T. 2001. The *Arabidopsis*-accelerated cell death gene *ACD2* encodes red chlorophyll catabolite reductase and suppresses the spread of disease symptoms. *Proc. Natl. Acad. Sci. U.S.A.* 98:771-776.
- Mateo, A., Muhlenbock, P., Rusterucci, C., Chang, C., Misalski, Z., Karpinska, B., Parker, J. E., Mullineaux, P. M., Karpinski, S. 2004. LESION SIMULATING DISEASE 1 is required for acclimation to conditions that promote excess excitation energy. *Plant Physiol.* 136:2818-2130.
- Meehan, L., Harkins, K., Chory, J., and Roderick, S. 1996. *Lhcb* transcription is coordinated with cell size and chlorophyll accumulation. *Plant Physiol.* 112:953-963.
- Mittler, R., and Rizhsky, L. 2000. Transgene-induced lesion mimic. *Plant Mol. Biol.* 44:335-344.
- Mittler, R., Shulaev, V., and Lam, E. 1995. Coordinated activation of programmed cell death and defense mechanisms in transgenic tobacco plants expressing a bacterial proton pump. *Plant Cell* 7:29-42.
- Mizobuchi, R., Hirabayashi, H., Kaji, R., Nishizawa, Y., Satoh, H., Ogawa, T., and Okamoto, M. 2002. Differential expression of disease resistance in rice lesion-mimic mutants. *Plant Cell Rep.* 21:390-396.
- Molina, A., Volrath, S., Guyer, D., Maleck, K., Ryals, J., and Ward, E. 1999. Inhibition of protoporphyrinogen oxidase expression in *Arabidopsis* causes a lesion-mimic phenotype that induces systemic acquired resistance. *Plant J.* 17:667-678.
- Morel, J. B., and Dangl, J. L. 1997. The hypersensitive response and the induction of cell death in plants. *Cell Death Differ.* 4:671-683.
- Mou, Z., He, Y., Dai, Y., Liu, X., and Li, J. 2000. Deficiency in fatty acid synthase leads to premature cell death and dramatic alterations in plant morphology. *Plant Cell* 12:405-418.
- Obeid, L. M., Linardic, C. M., Karolak, L. A., Hannun, Y. A. 1993. Programmed cell death induced by ceramide. *Science* 259:1769-1771.
- Orvar, B. L., and Ellis, B. E. 1997. Transgenic tobacco plants expressing antisense RNA for cytosolic ascorbate peroxidase show increased susceptibility to ozone injury. *Plant J.* 11:1297-1305.
- Rusterucci, C., Aviv, D. H., Holt, B. F. 3rd, Dangl, J. L., and Parker, J. E. 2001. The disease resistance signaling components *EDS1* and *PAD4* are essential regulators of the cell death pathway controlled by *LSD1* in *Arabidopsis*. *Plant Cell* 13:2211-2224.
- Sambrook, J., Fritsch, E. F., and Maniatis, T. 1989. *Molecular cloning: A Laboratory Manual*. 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory, NY, U.S.A.
- Stone, J. M., Heard, J. E., Asai, T., and Ausubel, F. M. 2000. Simulation of fungal-mediated cell death by fumonisin B1 and selection of fumonisin B1-resistant (*fbr*) *Arabidopsis* mutants. *Plant Cell* 12:1811-1822.
- Tadege, M., Bucher, M., Stähli, W., Suter, M., Dupuis, I., and Kuhlmeier, C. 1998. Activation of plant defense responses and sugar efflux by ex-

- pression of pyruvate decarboxylase in potato leaves. *Plant J.* 16:661-671.
- Takahashi, H., Chen, Z. X., Du, H., Liu, Y. D., and Klessig, D. F. 1997. Development of necrosis and activation of disease resistance in transgenic tobacco plants with severely reduced catalase levels. *Plant J.* 11:993-1005.
- Vailleau, F., Daniel, X., Tronchet, M., Montillet, J. L., Triantaphylides, C., and Roby, D. 2002. A R2R3-MYB gene, *AtMYB30*, acts as a positive regulator of the hypersensitive cell death program in plants in response to pathogen attack. *Proc. Natl. Acad. Sci. U.S.A.* 99:10179-10184.
- Venkataraman, K., Riebeling, C., Bodennec, J., Riezman, H., Allegood, J. C., Sullards, M. C., Merrill, A. H., Futerman, A. H. 2002 Upstream of growth and differentiation factor 1 (*uog1*), a mammalian homolog of the yeast longevity assurance gene 1 (*LAG1*), regulates *N*-stearyl-sphinganine (C18-(dihydro)ceramide) synthesis in a fumonisin B1-independent manner in mammalian cells. *J. Biol. Chem.* 277:35642-35649.
- Wang, H., Jones, C., Ciacci-Zanella, J. R., Holt, T., Gilchrist, D. G., and Dickman, M. B. 1996. Fumonisin and *Alternaria alternata lycopersici* toxins: Sphinganine analog mycotoxins induce apoptosis in monkey kidney cells. *Proc. Natl. Acad. Sci. U.S.A.* 93:3461-3465.
- Yamanouchi, U., Yano, M., Lin, H., Ashikari, M., and Yamada, K. 2002. A rice spotted leaf gene, *Spl7*, encodes a heat stress transcription factor protein. *Proc. Natl. Acad. Sci. U.S.A.* 99:7530-7535.
- Yin, Z. C., Chen, J., Zeng, L. R., Goh, M., Leung, H., Khush, G. S., and Wang, G. L. 2000. Characterizing rice lesion mimic mutants and identifying a mutant with broad-spectrum resistance to rice blast and bacterial blight. *Mol. Plant-Microbe Interact.* 13:869-876.
- Yoo, H. S., Norred, W. P., Showker, J., and Riley, R. T. 1996. Elevated sphingoid bases and complex sphingolipid depletion as contributing factors in fumonisin-induced toxicity. *Toxicol. Appl. Pharmacol.* 138:211-218.

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