Contribution of Ethylene Biosynthesis for Resistance to Blast Fungus Infection in Young Rice Plants

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The role of ethylene (ET) in resistance to infection with blast fungus (Magnaporthe grisea) in rice (Oryza sativa) is poorly understood. To study it, we quantified ET levels after inoculation, using young rice plants at the four-leaf stage of rice cv Nipponbare (wild type) and its isogenic plant (IL7), which contains the Pi-i resistance gene to blast fungus race 003. Small necrotic lesions by hypersensitive reaction (HR) were formed at 42 to 72 h postinoculation (hpi) in resistant IL7 leaves, and whitish expanding lesions at 96 hpi in susceptible wild-type leaves. Notable was the enhanced ET emission at 48 hpi accompanied by increased 1-aminoacyclopropane-1-carboxylic acid (ACC) levels and highly elevated ACC oxidase (ACO) activity in IL7 leaves, whereas only an enhanced ACC increase at 96 hpi in wild-type leaves. Among six ACC synthase (ACS) and seven ACO genes found in the rice genome, OsACS2 was transiently expressed at 48 hpi in IL7 and at 96 hpi in wild type, and OsACO7 was expressed at 48 hpi in IL7. Treatment with an inhibitor for ACS, aminooxyacetic acid, suppressed enhanced ET emission at 48 hpi in IL7, resulting in expanding lesions instead of HR lesions. Exogenously supplied ACC compromised the aminoxyacetic acid-induced breakdown of resistance in IL7, and treatment with 1-methylcyclopropene and silver thiosulfate, inhibitors of ET action, did not suppress resistance. These findings suggest the importance of ET biosynthesis and, consequently, the coproduction, cyanide, for HR-accompanied resistance to blast fungus in young rice plants and the contribution of induced OsACS2 and OsACO7 gene expression to it.

In monocot plants, the mechanism of disease resistance, including the roles of defense signal compounds for resistance (R)-gene-mediated resistance, such as salicylic acid (SA), ethylene (ET), and jasmonic acid (JA), has not been well elucidated. To study the mechanism, the Japonica rice (Oryza sativa) cv Nipponbare is an attractive model because of recent developments in genomic and molecular information, such as the Rice Genome Research Program (http://rgr.dna.affrc.go.jp; International Rice Genome Sequencing Project, 2005). Rice blast fungus (Magnaporthe grisea) is an extensively studied pathogen whose infection seriously affects rice yields worldwide. Genetic studies have identified 13 major R genes to blast fungi in rice plants, and standard rice cultivars with individual R genes and corresponding standard blast fungal races have been prepared to identify the race of blast fungus and R genes in a rice cultivar (Yamada et al., 1976; Kiyosawa, 1984). In Japan, attempts have been made to generate individual isogenic lines that contain a specific R gene with the same genetic background as practical rice cultivars, such as cv Nipponbare (Ise and Horisue, 1988). Actually, multilines containing compatible and incompatible lines were reported to be effective for disease control in the field (Browning and Frey, 1969) and, to our knowledge, there is no information to date on the breakdown of disease resistance by blast fungus races that have newly acquired the ability to infect all lines composing a multilime.

To analyze the resistant mechanism of rice plants to blast fungus infection, we used an isogenic line containing the R gene Pi-i to blast fungus race 003 in the background of cv Nipponbare and first focused on the levels of defense signal compounds after blast fungus infection. Koga (1994) reported that R-gene-mediated resistance to blast fungus infection was accompanied by the hypersensitive reaction or response (HR).

On resistance with the HR in dicot plants such as Tobacco mosaic virus (TMV) in tobacco (Nicotiana tabaquum; De Laat and van Loon, 1983; Malamy et al., 1990) and Cladosporium fulvum in tomato (Lycopersicon esculentum; Hammond-Kosack et al., 1996), SA accumulation and transient ET emission were accompanied by the formation of HR lesions (HRLs). Many reports indicated the importance of SA on HR-mediated resistance against pathogens such as TMV and Peronospora parasitica (Gaffney et al., 1993; Delaney et al., 1994); however, SA is reportedly not required for Cf-2- and Cf-9-dependent resistance of tomato to C. fulvum (Bradring et al., 2000). In rice plants, SA levels did not increase in the upper leaves in which the resistance to

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blast fungus was induced by *Pseudomonas syringae* D20 preinoculation to the under leaves (Silverman et al., 1995). We also detected no enhanced SA accumulation on Pi-i R-gene-mediated resistance to blast fungus in young rice plants (T. Iwai, S. Seo, I. Mitsuhara, and Y. Ohashi, unpublished data). Therefore, SA may not be the critical defense signal for induced or R-gene-mediated resistance in young rice plants, whereas SA may play an important role in modulating the redox balance protecting rice plants from oxidative stress (Yang et al., 2004). Although JA is known as another important defense signal compound, blast fungus infection did not alter the endogenous JA level and pretreatment with exogenous JA did not induce local resistance to blast fungus infection in a compatible interaction (Schweizer et al., 1997). Our preliminary experiment also showed that spraying JA solution before blast fungus inoculation did not induce a defensive response in a compatible host, resulting in a similar number of growing lesions in both JA-treated and -untreated leaves. Although JA pretreatment before fungal inoculation was reported to induce systemic resistance to blast fungus infection (Schweizer et al., 1998), JA likely does not contribute to local resistance.

On the other hand, the role of ET emission on resistance with the HR was poorly understood. Therefore, we were especially interested in the ET level after blast fungus inoculation in both susceptible and resistant rice lines. We studied the role of ET emission using susceptible wild-type Nipponbare and its isogenic resistant line, IL7, which contains the R gene Pi-i against blast fungus race 003 (Ise and Horisue, 1988). Because the level of pathogen resistance in adult rice plants is likely different from that in young rice plants (Kim et al., 1987; Yeh et al., 1989; Century et al., 1999), we used the fully developed fourth leaf of young rice plants at the four-leaf stage (about 16 d old) as the material. Our data presented here suggest the involvement of enhanced ET biosynthesis, which accompanies the production of not only ET, but also cyanide, for R-gene-mediated resistance to blast fungus and the possible contribution of specific types of genes for 1-aminocyclopropane-1-carboxylic acid (ACC) synthase (ACS) and ACC oxidase (ACO) to increased ET biosynthesis after blast fungus infection at a transcriptional level.

**RESULTS**

**Transient Increase in ET Emission during the Formation of HRLs in IL7**

First, we established an experimental system to analyze the mechanism of resistance to pathogen infection in rice plants. We used young rice plants at the four-leaf stage of wild-type Nipponbare and its isogenic line, IL7, which contains the R gene Pi-i (Ise et al., 1988). Wild type is susceptible and IL7 is resistant to infection by blast fungus race 003 (isolate Kyu89-241). In the fully expanded fourth leaf of IL7 plants, small HRLs were found at 42 h postinoculation (hpi) and gradually developed into dark-brown lesions up to 0.5 mm in diameter by 63 hpi, with no remarkable increase in size thereafter (Fig. 1), exhibiting a resistant response to race 003. In wild-type leaves, no detectable phenotype was found within 63 hpi, and whitish expanding lesions (ELs) 0.5 mm in diameter were first observed at 96 hpi, and the ELs

![Image](https://example.com/image.png)

**Figure 1.** Phenotypes of lesions in young rice leaves inoculated with blast fungus. Photographs of blast fungus-inoculated fourth leaves of IL7 and wild-type (WT) plants at the four-leaf stage. A conidial suspension of blast fungus race 003 was sprayed on an incompatible cultivar IL7 (top), which contains the R gene Pi-i against blast fungus race 003 in the Nipponbare background, and on wild-type Nipponbare, which is a compatible cultivar (bottom). In inoculated IL7 leaves, HRLs (black arrowhead) appeared at 42 hpi and matured by 63 hpi, turning dark brown. In inoculated wild-type leaves, whitish ELs (white arrowhead) were found at 96 hpi. Bar = 1 mm.
rapidly grew in size thereafter and infected leaves wilted by 144 hpi when inoculated with $1 \times 10^5$ conidia mL$^{-1}$; At lower concentrations, such as $1 \times 10^4$ conidia mL$^{-1}$, ELs grew to about $2 \times 1.5$ mm in size, developing conidia at the center of each lesion by 144 hpi (Fig. 1), indicating susceptibility of wild type to infection.

We determined the level of ET emitted from the fourth leaves after blast fungus inoculation, using 16 fourth leaves as one sample (Fig. 2A). One gram of healthy leaf from both IL7 and wild type emitted about 0.9 nL of ET per hour just after detaching. In both mock- and fungus-inoculated IL7 plants, the first peak in ET emission was found at 24 hpi, which was also the case in wild type. This peak by mock inoculation may be caused by the inoculation itself, which involved incubation of plants under high humidity in the dark for 20 h just after spraying the inoculum. The peak at 24 hpi was higher after blast fungus inoculation than mock inoculation in both IL7 and wild type (Fig. 2A), indicating an additive enhancement of ET emission by fungal infection. The second peak of ET emission was detected only in fungus-inoculated IL7 plants at 48 hpi. The enhanced rate of ET emission by fungal inoculation at 48 hpi was $6.4$ nL g$^{-1}$ h$^{-1}$ fresh weight in inoculated IL7 and $0.36$ nL g$^{-1}$ h$^{-1}$ fresh weight in inoculated wild type, respectively. Because primary HRLs found at 42 hpi (black arrow) were mature by 63 hpi, the significant enhancement of ET emission found in IL7 was thought to be related to the formation of HRLs. During 63 to 96 hpi, emitted ET from inoculated IL7 was maintained at a high level ($4$ nL g$^{-1}$ h$^{-1}$ fresh

Figure 2. Profiles of ET emission from young rice leaves inoculated with blast fungus. A, ET emission in resistant IL7 leaves and susceptible wild-type leaves determined using 16 fourth leaves at the four-leaf stage for one sample. Solid lines show the level of ET emission after inoculation. Broken lines show the level after mock inoculation. B, ACC content determined using 12 fourth leaves at the four-leaf stage for one sample. Inoculated leaves were homogenized and the ACC in the extract was chemically converted to ET. An expansion at 48 to 72 hpi is shown in frame. C, ACO activity determined as the capacity for converting ACC to ET using 12 fourth leaves at the four-leaf stage for one sample. Values in A, B, and C are shown as the means ± SD based on three independent experiments. The experiment was repeated two times with similar results. The symbol without visible error bars indicates that bars are present inside the symbol.
Characterization of the Rice ACS Gene Family

What kinds of ACS and ACO contribute to ET biosynthesis in blast fungus-infected rice plants? We searched for ACS and ACO genes from expressed sequence tags, full-length cDNAs, and genome databases of rice cv. Nipponbare (http://riceblast.dna.affrc.go.jp; http://cdna01.dna.affrc.go.jp/cDNA).

At least five rice ACS genes are reported to exist in the rice genome (Zarembinski and Theologis, 1993); corresponding CDNs are OsACS1 (AK071011), OsACS2 (AK064250), OsACS3 (P0617H07.9 in AC135427), OsACS4 (OSbN0006B22.3 in AC136224), and OsACS5 (D46839). OsACS6 (AK065212) was newly identified as the ortholog of ACS1 (U35779) from wheat (Triticum aestivum). The homology in amino acid sequence between OsACS1 and OsACS2 to OsACS6 is 54%, 59%, 56%, 56%, and 46%, respectively. An alignment of the six putative OsACS polypeptides is shown in Figure 3A. All ACS isoforms contain the seven conserved domains (Fig. 3A, boxes), which were found in ACSVs from other plant species (Yamagami et al., 2003). The 11 invariant amino acid residues between ACS and amidotransferase in Arabidopsis (Arabidopsis thaliana; Yamagami et al., 2003) and tomato (Rottmann et al., 1991), which are shaded in Figure 3A, were also found in the members of the rice ACS gene family. In addition, the Tyr residue at 245 in OsACS1 (black inverted triangle), which is a part of the pyridoxal-5'-P-binding site, was conserved among OsACS1 to OsACS5, and it was replaced by Phe in OsACS6.

A phylogenetic analysis with ACS proteins from rice, Arabidopsis (Yamagami et al., 2003), tobacco (Liu and Zhang, 2004), and wheat (Subramaniam et al., 1996) revealed that the genes fall into three groups (Fig. 3B). ACSs from Arabidopsis in groups I and II exhibited ACS activity, but not in group III, which contains AtACS10 and 12 similar to Ala or Asp amidotransferases in Arabidopsis (Yamagami et al., 2003). Thus, OsACS1 to OsACS5 in groups I or II, but not III, might work in rice.

To elucidate the genomic organization of ACS in rice, genomic DNA from rice cv. Nipponbare (wild type) was subjected to Southern-blot analysis with mixed probes containing the cytoplasmic domain of ACS, which were prepared by PCR amplification using OsACS1, OsACS2, and OsACS5 as templates (Fig. 3C). Because the highest homology based on nucleotide sequence between the probe and OsACS1 to OsACS6 is 100%, 100%, 76%, 73%, 100%, and 51%, respectively, the mixed probes would detect five signals corresponding to OsACS1 to OsACS5 in the digests by EcoRI whose internal site was not found in OsACS6. These results suggested that five rice ACSs in groups I and II compose a functional ACS gene family.

Characterization of the Rice ACO Gene Family

As an ACO gene in deepwater rice, OS-ACO1 (X85747) has been reported in relation to submergence (Mehedov and Kende, 1996) and OS-ACO2 (AF049888) and OS-ACO3 (AF049889) in relation to hormonal cross talk (Chae et al., 2000). We searched for their orthologs...
and homologs in expressed sequence tags, full-length cDNAs, and genome databases of rice, and found seven possible ACO genes, designated OsACO1 (AK058296), OsACO2 (AK071557), OsACO3 (AK065039), OsACO4 (AK105491), OsACO5 (AK061064), OsACO6 (OJJ1504_G04.8 in AC105772), and OsACO7 (AK102472), respectively. OsACO6 was mapped next to OsACO5 in chromosome 5 and we found that OsACO6 is a pseudogene encoding a truncated ACO peptide. The homology in amino acid sequence between OsACO1 and OsACO2 to OsACO5 and OsACO7 is 93%, 73%, 47%, 48%, and 43%, respectively. An alignment of the six putative ACO polypeptides is shown in Figure 4A. ACO is a member of the Fe(II) ascorbate family of dioxygenases in which the nine amino acid residues shaded in Figure 4A are conserved (Lasserre et al., 1996). Three of these nine residues with an arrowhead contribute to the binding of Fe(II), namely, His at 183, Asp at 185, and His at 240 in OsACO1. OsACO4 lacks two and OsACO5 lacks one of the nine conserved amino acid residues with white and black circles, respectively. Therefore, OsACO4 and OsACO5 might not actually function.

OsACO genes were classified into three major groups in a phylogenetic tree based on putative amino acid sequences (Fig. 4B). OsACO1, OsACO2, and OsACO3 are classified in group I, which contains NtACO1 and NtACO2, whose gene expression was accompanied by the formation of HRLs in tobacco (Liu and Zhang, 2004). OsACO7 is classified in group II, which contains STACO3, which was induced in the potato (Solanum tuberosum) tuber by inoculation with Fusarium eumartii and treatment of SA and indole acetic acid (Zanetti et al., 2002), and LEACO5, whose expression was anaerobically induced (Sell and Hehl, 2005).

Genomic Southern-blot analysis with mixed probes containing the catalytic domain of ACO, which were prepared by PCR amplification using OsACO1 to OsACO5 and OsACO7 as templates, detected six signals in both EcoRI and HindIII digests and seven signals in Ncol digests, indicating that seven OsACO genes, including the OsACO6 pseudogene, compose a gene family in rice (Fig. 4C).

Specific ACS and ACO Genes Are Induced Transiently at 48 hpi in IL7

As described, our results suggested the importance of ET biosynthesis for resistance to blast fungus infection. When the data on ACC content and ACO activity
after blast fungus inoculation in Figure 2 were reconstructed in Figure 5A, it became clearer that the levels before and during the formation of HRLs (36–63 hpi) were significantly higher in IL7 than those in wild type, suggesting an additive effect on ET biosynthesis in IL7 leaves. Thus, we studied the expression profiles of ACS and ACO genes in inoculated IL7 and wild-type plants.

The time-course expression profiles of six ACS genes were studied by one-step reverse transcription (RT)-PCR with specific primers for each ACS gene (Fig. 5B). Transient OsACS1 expression was found in mock-inoculated leaves at a low level and in blast fungus-inoculated leaves at a high level at 24 hpi in both plants, indicating OsACS1 may function for the first peak of ET emission at 24 hpi (Fig. 2A). OsACS2 was transiently induced at 48 hpi in fungus-inoculated IL7 plants, which is likely related to increased ACC content and dramatic ET emission at 48 hpi (Fig. 2A). A considerable level of OsACS2 transcript was found at
Figure 5. Expression profiles of ACS, ACO, and pathogenesis-related (PR) genes in young rice leaves infected with blast fungus.

A. Comparison of blast fungus-induced increase in ACC content and ACO activity in IL7 (black circle) and wild type (white circle). ACC content and ACO activity of fungus-inoculated leaves were subtracted by those in mock-inoculated leaves from the data in Figure 2, B and C, respectively, and new figures were reconstructed. B. Expression profiles of rice ACS and ACO genes by one-step RT-PCR using 0.5 μg of total RNA prepared from mock- and fungus-inoculated rice leaves at indicated time points. The sequences of the specific primers for each OsACS and OsACO gene and the length of amplified products, respectively, are shown in Table I. C. Induced expression of rice PR protein genes. Total RNA extracted from inoculated rice leaves was used for RNA-blot analysis. Specific probes for OsPR1a, PBZ1, and PAL were prepared by PCR using the 3'-untranslated region of each clone. Sequences of the specific primers are given in “Materials and Methods.”
96 hpi in wild type, but not IL7. It may be accompanied by the formation of ELs in wild-type plants. OsACS3, OsACS4, and OsACS6 were almost constitutively expressed in mock- and blast fungus-inoculated IL7 and wild-type leaves. No signal for the OsACSS transcript was found in fourth leaves under the same conditions (data not shown). These results suggest that OsACS1 contributes to dark- and high humidity-induced ET emission found at 24 hpi, and OsACS2 mainly acts to increase the ACC level during HRL formation at 48 hpi in IL7 and EL formation at 96 hpi in wild type.

Next, expression profiles of the six ACO genes, except for the pseudogene OsACO6, were determined using one-step RT-PCR with specific primers for each gene (Fig. 5B). OsACO1 was detected at 24 to 48 hpi in mock- and fungus-inoculated IL7 and wild-type plants. OsACO1 may be expressed under dark and high humidity conditions during inoculation, probably contributing to the first peak of ET emission at 24 hpi (Fig. 2A). OsACO2 was constitutively expressed in both plants, but down-regulated in blast fungus-inoculated IL7 at 72 and 96 hpi. OsACO3 was constitutively expressed in both plants not affected by the treatments. No signal for the OsACO4 transcript was found in either plant (data not shown). Expression of both OsACO5 and OsACO7 was inducible and enhanced by infection in both plants. Notably, expression of OsACO7 was very transient and strong at 48 hpi in IL7, but not wild-type plants. Whereas OsACO5 was also transiently enhanced to express at 48 hpi in IL7, the transcript was found in wild type at 48 to 96 hpi as well. From these results, transient expression of OsACO7 is thought to be most important for the increase of ACO activity at 48 to 72 hpi in infected IL7 leaves, possibly in cooperation with OsACO5 expression.

Blast fungus-induced OsACSs and OsACOs expression profiles were compared with control defense-related genes, such as OsPR1a (AJ278436; Agrawal et al., 2000) and PBZ1 (D38170; Midoh and Iwata, 1996) using each specific probe (Fig. 5C). The transcript for OsPR1a, but not PBZ1, was transiently and slightly accumulated at 24 h after mock and blast fungus inoculation in both host plants. In addition, in IL7, the transcript of OsPR1a was expressed again at 48 hpi, increasing to greater amounts thereafter, at which time the formation of HRLs was completed. In wild type, the OsPR1a transcript accumulated at 72 and 96 hpi accompanied by the formation of ELs. PBZ1 expression was found to be accompanied by the formation of lesions, but not mock inoculation. The transcript accumulated at 36 hpi and was almost saturated at 72 hpi in IL7. In wild type, it was found at 48 hpi, increasing at 96 hpi. Thus, expression of the two defense marker genes was found during and after the formation of lesions in both blast fungus-infected IL7 and wild-type plants. In IL7, these expression levels were remarkably higher after lesions had formed (72–96 hpi) than during their formation (48 hpi), indicating the downstream genes of defense signaling in rice. These expression profiles were clearly different from those of OsACS2 and OsACO7, which are very transient at 48 d postinfection in IL7. Expression of the Phe ammonia lyase (PAL) gene (X16099; Minami et al., 1989) was slightly down-regulated by the inoculation procedure and the level of expression induced by fungus inoculation was higher in wild type than in IL7.

**Inhibition of ET Biosynthesis, But Not ET Signaling, Results in Suppressed Resistance to Fungal Infection**

Enhanced ET emission during the formation of HRLs in IL7 depends on ET biosynthesis. To confirm the contribution of ET biosynthesis to resistance, the effect of aminooxyacetic acid (AOA), which is an inhibitor of pyridoxal-5’-P-related enzymes, such as ACS (Yu et al., 1979; Yang and Hoffman, 1984), was studied. The fourth leaves of 16 independent blast fungus-inoculated rice plants were detached at 24 hpi and a solution of AOA at 0.1, 0.5, or 1.0 mM was fed through the base of the detached leaf blades for 24 h, respectively. Then the leaves were put in an airtight vial and the level of ET released from the leaf was determined for 3 h (Fig. 6, A and B). Mock-inoculated leaves from IL7 and wild-type plants, which had been detached at 24 hpi and fed with water for 24 h, emitted ET at the rate of 7.5 and 8.5 nL g⁻¹ h⁻¹ fresh weight, respectively, whereas those from intact IL7 and wild-type plants were 1.7 and 3.1 nL g⁻¹ h⁻¹ fresh weight, respectively. These results indicate that detached leaves, which is a kind of wounding, enhanced ET emission from the leaf blade. When mock-inoculated leaves were treated with 0.1 mM AOA solution at 24 hpi, ET emission was diminished to about 1 nL g⁻¹ h⁻¹ fresh weight in both IL7 and wild type, which is similar to that at time 0 (see Fig. 2B), indicating that wound-induced ET emission was completely suppressed by 0.1 mM AOA. In fungus-inoculated leaves fed water at 24 hpi, the highest increase in ET emission (i.e. 14.8 nL g⁻¹ h⁻¹ fresh weight in IL7 and 10.6 nL g⁻¹ h⁻¹ fresh weight in wild type) was observed. When fed with 0.1, 0.5, or 1.0 mM AOA at 24 hpi, fungus-inoculated IL7 leaves emitted ET at the rate of 5.4, 1.1, and 1.0 nL g⁻¹ h⁻¹ fresh weight, which corresponded to a 64%, 92%, and 93% decrease of that emitted from water-treated control leaves, respectively. In fungus-inoculated wild type, AOA treatment at 0.1, 0.5, or 1.0 mM resulted in an 86%, 91%, and 92% decrease in the level of ET compared with the control, respectively. These results indicate that, for strong inhibition of fungus-induced ET emission, 0.1 mM AOA was not enough and 0.5 mM was required for IL7, whereas 0.1 mM was enough for wild type. Therefore, a higher level of ACS activity, which could not be inhibited by 0.1 mM AOA, seems to be induced in fungus-inoculated IL7, but not in wild type.

To confirm the effect of AOA on resistance to blast fungus in rice, infected leaf pieces were treated at 96 hpi with lactophenol-trypsin blue, which stains the mycelium blue. In 0.1 mM AOA-treated IL7 leaf pieces, HRLs remained dark brown and were not stained blue.
as in water-treated leaves (Fig. 6C), indicating that 0.1 mM AOA could not entirely inhibit the formation of HRLs, whereas 63% of ET emission at 48 to 51 hpi was inhibited. However, treatment with 0.5 and 1.0 mM AOA, which almost completely suppressed ET emission, resulted in larger lesions with blue-stained mycelia in IL7 plants. Thus, reduced ET emission possibly led to a breakdown of HRL formation, permitting enhanced mycelial growth in the inoculated IL7 leaves. In fungus-inoculated wild-type leaves treated with 0.1 to 1.0 mM AOA, similar ELs were formed in the water-treated control wild type and 0.5 to 1 mM AOA-treated IL7 leaves.

AOA is known as an inhibitor of ACS, but also that of deaminases and transaminases containing PAL (Amrhein and Gerhardt, 1979). To confirm that the effect of AOA treatment depended on the inhibition of ACS activity, we conducted the same AOA treatment in the presence of ACC, which is the product of ACS. Inoculated or mock-inoculated IL7 leaves were treated with a solution containing 0 or 0.5 mM AOA or both 0.5 mM AOA and 1 mM ACC at 24 hpi, and the rates of ET emission at 48 hpi and development of lesions at 96 hpi were determined (Fig. 7A). In fungus-inoculated control IL7 leaves fed with water, the rate of ET emission was 16.1 nL g⁻¹ h⁻¹ fresh weight and the formation of HRLs was observed by 96 hpi. In fungus-inoculated IL7 leaves fed with 1 mM ACC, the rate of ET emission was increased to 25.6 nL g⁻¹ h⁻¹ fresh weight (i.e., 1.6-fold higher compared with water-treated leaves), and similar HRLs were found. Similar to Figure 6, B and C, in the leaves fed with 0.5 mM AOA, the rate of ET emission was decreased to 1.73 nL g⁻¹ h⁻¹ fresh weight, and the formation of whitish ELs instead of HRLs was observed at 96 hpi. However, treatment with a solution containing both 0.5 mM AOA and 1 mM ACC compromised the breakdown of the resistance, recovering the rate of ET emission to 16.7 nL g⁻¹ h⁻¹ fresh weight and the formation of HRLs (Fig. 7). These results indicate that the reason for the reduced ACC level would be via AOA-originated inhibition of ACS activity; the main reason of the breakdown of the resistance is inhibition of ACS activity by AOA because exogenously added ACC complemented the resistance.

Does the necessity of ET biosynthesis, which accompanies the production of equal moles of cyanide for...
resistance, mean the importance of ET signaling? To elucidate, we examined the effect of the inhibitors for ET action, 1-methylcyclopropene (1-MCP) and silver thiosulfate (STS). Fungus-inoculated rice plants at the four-leaf stage were treated with gaseous 1-MCP 24 hpi for 16 h at 2 μL L⁻¹, which is enough concentration to disturb accelerated ripening and softening of climacteric fruit or accelerated senescence of cut flowers (Serek et al., 1995). Phenotypes of formed HRLs and ELs in the treated IL7 and wild-type leaves were very similar to these in nontreated control leaves at 96 hpi, respectively (Fig. 7B). Treatment with 2 mM STS solution through the base of detached leaves at 24 hpi for 1 h resulted in very similar phenotypes of HRLs and ELs compared with nontreated IL7 and wild-type leaves, respectively (Fig. 7B). These results indicated that inhibitors for ET action would not affect resistance to blast fungus infection, at least in our experimental condition, and the importance of cyanide production via ET biosynthesis.

DISCUSSION

We demonstrated here that ET biosynthesis, but not ET itself, is necessary for resistance to blast fungus infection in young rice plants. In Pi-i-mediated resistance in IL7 plants, enhanced ET emission at 48 hpi is distinctive because the phenomenon was not found in fungus-infected wild-type plants or mock-inoculated IL7 and wild-type plants. AOA treatment, which induced remarkable inhibition of ET emission, broke down resistance in IL7, preventing the formation of HRLs. Addition of ACC compromised the AOA-induced breakdown of resistance, indicating ACC synthesis by ACS is critical for resistance. Treatment of 1-MCP and STS, the inhibitors of ET action, did not induce the breakdown of resistance. From the characterization of the rice ACS and ACO gene families, we found that enhanced ET emission during the formation of HRLs would be supported by specific ACS and ACO genes at the transcriptional level.

Mechanism of ET Biosynthesis in Resistant Response

In our experimental system, the resistant cultivar IL7 exhibits strong resistance to blast fungus race 003; saturated numbers of solid HRLs small in size and dark brown in color were detected at about 63 hpi, with no remarkable enlargement thereafter. On the other hand, typical susceptibility is found in wild type, which lacks the Pi-i gene in almost the same genetic

**Figure 7.** Effects of exogenously supplied ACC and inhibitors of ET action on resistance. A, Exogenously supplied ACC canceled the suppressed resistance and reduced ET emission by AOA treatment in IL7. A solution containing 1 mM ACC, 0.5 mM AOA, or both 0.5 mM AOA and 1 mM ACC was supplied from the cut base of the freshly detached IL7 leaf at 24 hpi and incubated for a further 24 h. Emitted ET from these leaves was quantified at 48 hpi for 3 h using 16 leaves from each treatment. Phenotypes of HRLs in IL7 leaves were observed at 96 hpi. The levels of ET emission are shown as the mean ± SD based on three independent experiments. B, Inhibitors for ET action did not alter the formation and phenotypes of HRLs and ELs in IL7 and wild-type leaves, respectively. Treatments with gaseous 1-MCP at 2 μL L⁻¹ and 2 mM STS solution resulted in similar HRL and EL formation in IL7 and wild type at 96 hpi, respectively.

16 individual plants at the four-leaf stage. Phenotypes of HRLs in IL7 leaves were observed at 96 hpi. The levels of ET emission are shown as the mean ± SD based on three independent experiments. B, Inhibitors for ET action did not alter the formation and phenotypes of HRLs and ELs in IL7 and wild-type leaves, respectively. Treatments with gaseous 1-MCP at 2 μL L⁻¹ and 2 mM STS solution resulted in similar HRL and EL formation in IL7 and wild type at 96 hpi, respectively.
HRLs and ELs in rice plants. ACO activity was dramatically increased at the area of HRLs (De Laat and Van Loon, 1983), suggesting localization of increased ACC at the area of HRLs (De Laat and Van Loon, 1983), as well as increased ACC at 36 to 72 hpi was reportedly restricted to fungus (Fig. 5A). In only HRLs but also ELs in rice leaves infected with blast fungus race 003. The first peak at 24 hpi was likely a result of the darkness and high humidity in both host plants, and the second peak at 48 hpi was specific for the formation of HRLs in infected IL7 plants (Fig. 2A).

The level of the second peak in ET emission was almost proportional to the number and size of developing HRLs. When the conidial suspension (1 × 10⁵ conidia mL⁻¹) was sprayed onto IL7 plants at the four-leaf stage, about 150 HRLs were detected on the fourth leaf accompanied by ET emission at the rate of 7 nL g⁻¹ fresh weight at 48 hpi. This level of ET emission is similar to the formation of HRLs mediated by the N gene (18 nL g⁻¹ h⁻¹ fresh weight) in TMV-infected tobacco plants (De Laat and Van Loon, 1981). The sharp peak in ET emission similar to the second peak in infected IL7 plants was observed during the formation of HRLs in N-gene-mediated resistance in TMV-infected tobacco plants (De Laat and Van Loon, 1981, 1983) as well as Cf-gene-mediated resistance in C. fulvum-infected tomato plants (Hammond-Kosack et al., 1996). Enhanced ET emission at 48 to 72 hpi, when HRLs developed and matured in infected IL7 plants, was accompanied by infection-enhanced ACC accumulation and ACO activity (Figs. 2 and 5A). The amount of ACC at 36 to 72 hpi was slightly, but significantly, higher in infected IL7 than infected wild-type leaves, and it reversed at 96 hpi, at which time formation of ELs is going on, indicating that ACC synthesis was accompanied by the formation of not only HRLs but also ELs in rice leaves infected with blast fungus (Fig. 5A). In N-gene-mediated resistance, accumulation of increased ACC was reportedly restricted to the area of HRLs (De Laat and Van Loon, 1983), suggesting localization of increased ACC at the area of HRLs and ELs in rice plants. ACO activity was dramatically and specifically elevated during the formation of HRLs in infected IL7 plants (Fig. 2C), suggesting enhanced ACO activity and increased levels of ACC at 36 to 63 hpi are important for ET emission from HRLs.

In deepwater rice, Os-ACS1, whose product shares 99% homology with OsACS1 from Nipponbare wild type, was induced by partial submergence at the uppermost elongating internode and involved in stem elongation (Zarembski and Theologis, 1993, 1997). Lowland rice plants, such as Nipponbare wild type and IL7, possibly recognized the dark and high humidity conditions as partial submergence, and ET emission might be enhanced at 24 hpi in both mock- and blast fungus-inoculated leaves. OsACS1 was classified into group I, which contains auxin-responsive AtACS4 and AtACS5 (Tsuchioka and Theologis, 2004), suggesting involvement in plant hormone-mediated responses. OsACS2 was classified into group II. Group II contains AtACS6, which was rapidly induced by ozone exposure-induced cell death (Overmyer et al., 2000) and NtACS1, NtACS2, and NtACS3, whose gene expression was induced during an N-gene-mediated TMV-resistant response in tobacco (Kim et al., 2003). Thus, ACS proteins in group II would function in response to biotic stresses, including pathogen infection. Phosphorylation was also reported to be important for ACS proteins belonging to group II, such as AtACS2 and AtACS6, which are very similar to OsACS2. These AtACS proteins are stabilized by phosphorylation at their C-terminal regions by Arabidopsis mitogen-activated protein kinase 6, the ortholog of tobacco SA-induced protein kinase, whose activation confers TMV resistance (Liu and Zhang, 2004). Thus, studies on posttranscriptional and posttranslational regulation of OsACS2 would also be important to elucidate the dynamic regulation of ET biosynthesis for disease resistance.

Maintenance of a high level of ACO activity at 48 to 96 hpi may guarantee a considerable level of ET biosynthesis during this period. Two newly characterized rice ACO genes, OsACO5 and OsACO7, out of seven ACO members, were transiently induced by blast fungus in IL7 plants (Fig. 5B). Because OsACO5 lacks one of nine conserved amino acid residues, it might confer weaker or no ACO activity (Fig. 4A). OsACO5

Table 1. DNA sequence of primers for RT-PCR

| Target Gene | Accession No. | Forward | Reverse | Product Length |
|-------------|---------------|---------|---------|----------------|
| OsACS1      | AK071011      | TCGCCCAAGACCGCTCAGGACG | CGAAAGGAATCTCGACGCTGTC | 300            |
| OsACS2      | AK064250      | ACCTGGCGCAAATGCGGCGG  | GAACGACGGCGCTCTCTGCG  | 293            |
| OsACS3      | AC135427      | CCTGTCGCCATCAAGAATGGGACG | GATGGAAGCATAGGGGAGGAC | 255            |
| OsACS4      | AC136224      | CCTGACAGCCTGTCAGGGAAAGC | GTCCTCTTCTTCTCTCAATTCTCG  | 271            |
| OsACS5      | AU065424      | CACGGTACCAGGGCAAGATGC | CGTCTCATGAGGCGAAGCTTATCCTG  | 670            |
| OsACS6      | AK065212      | GACGCCAACATGATACCCTTGA  | GCTCTCTATGGGTAAGAGG  | 217            |
| OsACO1      | AK065039      | CAGATTGCCGATGACCCAGGAC | CAGATTGACGCAGCGGCCAG | 254            |
| OsACO2      | AK058296      | AGCAACCCCGGCCCTCCCTCTCCTCAG  | AGGACTTGGCTATGAGACCGG  | 242            |
| OsACO3      | AK071557      | CGCCGGCGAGTGGCTCCACG  | GCCGGCACACACCTTGGAG  | 242            |
| OsACO4      | AK105491      | CCGGGAGATAGGCTCTCCGCT  | GTCGGCGGCGGGCTGGAACC  | 723            |
| OsACO5      | AK061064      | CGGAAGAGAGCCCTGTATGCGG | ATTTTGGCGCCCTTGACGCCC  | 793            |
| OsACO7      | AK102472      | GTGATCCGCGGCGACGGCGGC | GGGGAAACCTTGCATGAC  | 257            |
expression was induced by blast fungus infection in both IL7 and wild type in a similar manner, suggesting no relation to resistance. Interestingly, the maximal level of the OsACO7 transcript was found at 48 hpi in infected IL7, but not wild type, indicating a transcriptional contribution of OsACO7 to ET emission during the formation of HRLs in IL7. It was also increased at 24 hpi in both IL7 and wild type, possibly indicating a contribution to the first peak of ET emission at 24 hpi. OsACO7 belongs to group II, as well as StACO3, which was induced by infection with F. eumartii in potato (Fig. 4B). On the other hand, information on transcriptional regulation of plant ACO genes is limited and we could find no evidence of posttranscriptional regulation of ACO in plants. Thus, the mechanism on the regulation of ACO for disease resistance remains to be solved.

Role of ET Biosynthesis in the Resistance Response

In this article, we proposed the involvement of ET biosynthesis, but not ET itself, in resistance to blast fungus infection in rice plants. This indicates the positive role of ET biosynthesis in blast fungus-dependent HRL formation and suppression of subsequent fungal growth. AOA treatment at 24 hpi clearly inhibited ACC synthesis and subsequent ET emission in IL7 at 48 hpi, inducing EL-like lesions with vigorous hyphal growth instead of HRLs, which are observed in the absence of AOA (Fig. 6, B and C). Addition of ACC canceled the inhibitory effect of AOA, recovering enhanced ET emission and formation of HRLs in IL7 (Fig. 7A). These results indicate that ET biosynthesis is essential for Pi-i (R-gene)-mediated resistance. In the cytological studies reported by Koga (1994), hypersensitive cell death occurred during fungal penetration and little growth of invading hyphae was permitted after the host cell had died. At the final step of ET biosynthesis, endogenous ACC is converted by ACO to equal moles of ET and cyanide, which is an inhibitor for the mitochondrial respiratory chain (by blocking cytochrome oxidase in complex IV). Thus, the role of ET itself for disease resistance should be separately elucidated from the role of ET biosynthesis, which accompanies cyanide production. Using inhibitors for ET action, such as 1-MCP and STS, we evaluated the role of ET itself for resistance to blast fungal infection. Treatment of gaseous 1-MCP (2 μL L⁻¹) for 16 h or STS (2 mM) for 1 h at 24 hpi could not significantly affect the formation and the phenotypes of HRLs in inoculated IL7 leaves and ELs in inoculated wild-type leaves (Fig. 7B). These results indicate the importance of cyanide production for resistance rather than ET production. This evidence is coincident with the information that HRLs were generated in response to avirulent fungi and bacteria such as C. fulvum, P. parasitica, and P. syringae pv gicinica in ET-insensitive mutants or transgenic plants with modified ET receptors (Brading, 1997; Van Loon et al., 2006). The contribution of cyanide for blast fungus protection was also suggested by the studies using an effective fungicide metominostrobin (SSF126) in rice plants (Mizutani et al., 1996). The agrochemical is a derivative of stroblurin A, which covers a wide range of antifungal spectra. SSF126 strongly inhibited mycelial growth of blast fungus in vitro by prohibiting the mitochondrial oxidative respiration chain at the earlier period. However, 20 min after the treatment, the mycelia again began to respire, inducing cyanide-resistant respiration, which is sensitive to salicyl hydroxamic acid (Mizutani et al., 1995). On the other hand, cyanide-resistant respiration of the fungus was inhibited by flavonoid compounds such as flavone, flavanone, and naringenin, which widely exist in the plant kingdom, proposing the following mechanism that the inhibition of cyanide-sensitive respiration by SSF-126 and inhibition of cyanide-resistant respiration by flavonoids cooperatively suppress fungal growth (Mizutani et al., 1996). Actually, blast fungus-induced accumulation of a flavanone phytoalexin, sakuranetin, was detected at 40 hpi, and the level was increased thereafter only in resistant rice plants (Kodama et al., 1992). The importance of cyanide production via ET biosynthesis to resistance was emphasized by our results presented here; however, the possible cooperation of ET, which is produced from ACC at the same time, could not be excluded for the resistance mechanism.

The analysis of rice ACS and ACO gene families pointed out that specific members, such as OsACS2 and OsACO7, are transcriptionally activated during the formation of HRLs in fungus-infected IL7 leaves. The expression profile of a gene often suggests its function, but the studies about modification of the gene products such as dynamic activation/inhibition or stabilization/unstabilization would also be important. Loss- or gain-of-function studies about OsACS2 and OsACO7 would clearly indicate the roles of these genes in the defense against blast fungus in relation to ET biosynthesis. Such experiments have been started in our laboratory.

MATERIALS AND METHODS

Plant Materials

Rice (Oryza sativa cv. Nipponbare) and the isogenic line IL7 (Jue and Horisue, 1988), which carries the R gene Pi-i against blast fungus (Magnaporthe grisea) race 003 (isolate, Kyu89-241; Yamada et al., 1976), were grown for about 3 weeks in a greenhouse at 25°C. The fourth leaf of 3-week-old young plants at the four-leaf stage was used as the material in all experiments. For ET analysis, 24 and 16 fourth leaves at the four-leaf stage were used as one sample, respectively.

Inoculation with Rice Blast Fungus

Blast fungus race 003 was grown on oatmeal medium (Difco) for 2 weeks at 26°C in the dark, and then spores were induced to form under a 20-W BLB light (FL20S BLB; Toshiba) for 2 to 3 d at 24°C. A spore suspension (1 × 10⁸ conidia mL⁻¹) containing 0.05% (v/v) Tween 20 was sprayed onto rice plants. The inoculated plants were incubated at 25°C with high humidity in the dark for 20 h and then moved to a greenhouse.

Light Microscopy

Blast fungus-inoculated leaves, cut 0.5 cm in length, were vacuum infiltrated with water and then stained with a lactophenol-trypsin blue

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solution containing 10 mL of lactic acid, 10 mL of glycerol, 10 g of phenol, and 10 mg of trypan blue dissolved in 10 mL of distilled water (Koch and Süssarenko, 1980). Leaf segments were boiled for 3 min in the stain solution and decolorized in a chloral hydrate solution containing 2.5 g of chloral hydrate dissolved in 1 mL of distilled water for at least 24 h. They were mounted in the chloral hydrate solution and viewed under a microscope.

Chemical Treatments

AOA was purchased from Sigma-Aldrich. AOA was dissolved in water and the pH adjusted to 7.0 with NaOH solution. 1-MCP was provided by Rohm and Haas. Two millimolar STS solution was prepared by adding 20 mL of 0.01 M silver nitrate (Sigma-Aldrich) solution to 80 mL of 0.01 M STS (Sigma-Aldrich) solution. Inoculated fourth leaves were detached at 24 hpi and fed with solutions of 0.1, 0.5, and 1.0 mM AOA, 1 mM ACC, both 0.5 mM AOA and 1 mM ACC, or 2 mM STS, respectively, from the cut base of freshly detached leaf blades. Inoculated rice plants at the four-leaf stage were treated with gaseous 1-MCP in airtight containers at 24 hpi for 16 h, following the manufacturer’s instructions.

Measurement of ET Emission

At 0, 24, 36, 48, 63, 72, and 96 h after the inoculation, fourth leaves were detached from the base of the leaf blade. Sixteen leaves were put into 52-mL glass vials with 5 mL of water, sealed with a gas-proof septum, and left in a growth cabinet at 24°C for 3 h under light. One milliliter of gas was withdrawn from the airspace of each tube using a gas-tight syringe (Hamilton) and injected into a gas chromatograph (Shimadzu GC-14B) equipped with an aluminum column (Shumapke-A; Shimazu) and a flame-ionization detector for ET determination.

Determination of ACC Content

Leaf material frozen in liquid nitrogen was ground with a mortar and pestle and stirred with a 5% (w/v) sulfosalicylic acid solution (2 mL g⁻¹ fresh weight) for 30 min at room temperature. The concentration of ACC in the supernatant after centrifugation at 30,000 rpm for 30 min was determined directly by chemical conversion to ET according to Lizada and Yang (1979), with modifications by De Laat and Van Loon (1983).

Determination of ACO Activity

The ACO assay was performed as described by Mekhedov and Kende (1996). Twelve mock-inoculated or blast fungus-inoculated fourth leaves from 12 individual plants (about 0.5 g fresh weight) were homogenized with 1.0 mL of extraction buffer (100 mM Tris-Cl, pH 7.2, containing 30 mM sodium ascorbate and 10% [v/v] glycerol) in triplicate. ACO activity was analyzed by incubation of a total of 2.0 mL of reaction mixture (1.7 mL of extraction buffer, 50 μM of 4-MCP, 0.5 μM of ACC, 10 μg of trypan blue dissolved in 10 mL of distilled water (Koch and Thompson, 1980). DNA-blot analysis was performed using 5 μg of genomic DNA denatured in 1 mL of 1 N NaOH for at least 24 h. They were then neutralized overnight at 4°C with 1 N HCl, washed twice with water, and decolorized in a chloral hydrate solution containing 2.5 g of chloral hydrate and 10 mg of trypan blue dissolved in 1 mL of distilled water. They were mounted in the chloral hydrate solution and viewed under a microscope.

DNA- and RNA-Blot Analyses

DNA- and RNA-blot analyses were performed using the digoxigenin nonradioactive nucleic acid labeling and detection system (Roche), following the manufacturer’s instructions.

Genomic DNA was isolated from rice seedlings as described by Murray and Thompson (1980). DNA-blot analysis was performed using 5 μg of genomic DNA from rice cv Nipponbare after digestion with EcoRI, HindIII, and NcoI and a common probe for rice ACO or ACC genes using the SuperScript One-Step RT-PCR system with Platinum Taq (Invitrogen). Specific primers were designed based on DNA sequences reported to GenBank and displayed the primer DNA sequence listed in Table I. The specificity of the primers was checked by excising the RT-PCR products after electrophoresis. Among 0.1 to 1 μg of total RNA as a template for RT-PCR, amplification products for each gene increased linearly. PCR amplification conditions were 50°C for 30 min, 96°C for 2 min, followed by 30 cycles of 96°C for 30 s, 56°C for 30 s, and 72°C for 1 min, and then one cycle of 72°C for 5 min.

The relative transcript amounts were visualized by using a luminescent image analyzer LAS-1000plus (Fujifilm) from the images of agarose gels after electrophoresis.

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LITERATURE CITED

Agrawal GK, Jwa NS, Rakwal R (2000) A novel rice (Oryza sativa L.) acidic PRI gene highly responsive to cut, phytohormones, and protein phoshatase inhibitors. Biochem Biophys Res Commun 274: 157–165

Amrhein N, Gerhardt J (1979) Supernuduction of phenylalanine ammonia-lyase in gherkin hypocotyls caused by the inhibitor, l-alpha-aminoxy-benzylpropionic acid. Biochim Biophys Acta 383: 434–442

Brading PA (1997) Functional analysis of Cf gene-dependent defense responses in tomato. PhD thesis. University of East Anglia, Norwich, UK

Brading PA, Hammond-Kosack KE, Parr A, Jones JD (2000) Salicylic acid is not required for Cf-2 and Cf-9-dependent resistance of tomato to Cladosporium fulvum. Plant J 23: 305–318

Browning JA, Frey KJ (1969) Multiline cultivars as a means of disease control. Annu Rev Phytopathol 7: 355–382

Century KS, Lagman RA, Adkisson M, Morlan J, Tobias R, Schwartz K, Smith A, Love J, Ronald PC, Whalen MC (1999) Short communication: developmental control of Xa21-mediated disease resistance in rice. Plant J 26: 231–236

Chae HS, Cho YG, Park MY, Lee MC, Eun MY, Kang RG, Kim WT (2000) Hormonal cross-talk between auxin and ethylene differentially regulates the expression of two members of the 1-aminoacyclopropane-1-carboxylate oxidase gene family in rice (Oryza sativa L.). Plant Cell Physiol 41: 354–362

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De Laat AMM, van Loon LC (1983) Regulation of ethylene biosynthesis in virus-infected tobacco leaves. Plant Physiol 68: 256–260
De Laat AMM, van Loon LC (1983) The relationship between stimulated乙烯 production and symptom expression in virus-infected tobacco leaves. Physiol Plant Pathol 22: 261–273
Delaney TP, Uknes S, Vernooij B, Friedrich L, Weymann K, Negrotto D, Klessig DF, Silverman P, Raskin I (1994) A central role of salicylic acid in plant disease resistance. Science 266: 1247–1250
Gaffney T, Gardner M, Kessmann H, Ward E, et al (1994) A central role of salicylic acid in the induction of systemic acquired resistance. Science 261: 754–756
Hammond-Kosack KE, Silverman P, Raskin I, Jones J (1994) Race-specific elicitors of Cladosporium fulvum induce changes in cell morphology and the synthesis of ethylene and salicylic acid in tomato plants carrying the corresponding Cf disease resistance gene. Plant Physiol 100: 1381–1394
International Rice Genome Sequencing Project (2005) The map-based sequence of the rice genome. Nature 436: 793–800
Ise K, Horisue N (1998) Characteristics of several near-isogenic lines of rice for blast resistance gene. Breed Sci 38: 404–405
Kikuchi S, Satoh K, Nagata T, Kawagashira N, Doi K, Kishimoto N, Yazaki J, Ishikawa M, Yamada H, Ooka H, et al (2003) Collection, mapping, and annotation of over 28,000 cDNA clones from japonica rice. Science 318: 376–379
Kim CY, Liu Y, Thorne ET, Yang H, Fujishige H, Gassmann W, Hildebrand D, Sharp RE, Zhang S (2003) Activation of a stress-responsive mitogen-activated protein kinase cascade induces the biosynthesis of ethylene in plants. Plant Cell 15: 2707–2718
Kim KD, Hwang BK, Koh YJ (1987) Evaluation of rice cultivars under greenhouse conditions for adult-plant resistance to Pyricularia oryzae. J Phytopathol 120: 310–316
Kiyosawa S (1984) Establishment of differential varieties for pathogenicity test of rice blast fungus. Rice Genet Newsl 11: 95–97
Koch E, Slusarenko A, Kiyosawa S (1993) Collection, mapping, and annotation of over 28,000 cDNA clones from japonica rice. Nature 362: 81–90
Koga H (1994) Hypersensitive death, autofluorescence, and ultrastructural change in cells of leaf sheaths of susceptible and resistant near-isogenic lines of rice (cv. sashihara) in relation to penetration and growth of Pyricularia oryzae. Can J Bot 72: 1463–1477
Lasserre E, Bouquin T, Hernandez JA, Bull J, Pech JC, Balague C (1996) Regulation of ethylene biosynthesis in Arabidopsis thaliana by root-predominant expression of their genes with root-predominant expression. Plant Mol Biol 31: 1009–1020
Tsuchisaka A, Theologis A (2004) Unique and overlapping expression patterns among the Arabidopsis 1-aminocyclopropane-1-carboxylate synthase gene family members. Plant Physiol 136: 2982–3000
Van Loon LC, Geraats BP, Linnstra H, Yu Y-B, Adams DO, Yang SF, Hoffman NE (1995) Salicylic acid in rice (biosynthesis, conjugation, and possible role). Plant Physiol 108: 633–639
Sokal RR, Michener CD (1958) A statistical method for evaluating systematic relationships. University Kansas Sci Bull 28: 1409–1438
Subramaniam K, Abbo S, Ueng P (1996) Isolation of two differentially expressed wheat ACC synthase cDNAs and the characterization of one of their genes with root-predominant expression. Plant Mol Biol 31: 1002–1004
Mekhedov SI, Kende H (1996) Submergence enhances expression of a gene encoding 1-aminocyclopropane-1-carboxylate oxidase in deepwater rice. Plant Cell Physiol 37: 531–537
Midoh N, Iwata M (1996) Cloning and characterization of a probenazole-inducible gene for an intracellular pathogenesis-related protein in rice. Plant Cell Physiol 37: 793–798
Minami E, Sako M, Koizumi K, Tanaka Y (1993) Structure and some characterization of the gene for phenylalanine ammonia-lyase from rice plants. Eur J Biochem 195: 185–195
Mizutani A, Miki N, Yukioka H, Tamura H, Masuko M (1996) A possible mechanism of control of rice blast disease by a novel alkoxyiminoacetamide fungicide. Crop Sci 36: 1021–1027
Mizutani A, Miki N, Yukioka H, Tamura H, Masuko M (1996) A possible mechanism of control of rice blast disease by a novel alkoxyiminoacetamide fungicide. Jap J Bot 72: 1463–1477
Murray MG, Thompson WF (1980) Rapid isolation of high molecular weight plant DNA. Nucleic Acids Res 8: 4321–4325
Nagy E, Kay SA, Chua NN (1988) Plant Molecular Biology Manual, Vol B4. Kluwer Academic Publishers, Dordrecht, The Netherlands
Overmyer K, Tuominen H, Kettnun R, Betz C, Langelberchts C, Sandermann H Jr, Kangasjarvi J (2000) Oxone-sensitive Arabidopsis rcdl1 mutant reveals opposite roles for ethylene and jasmonate signaling pathways in regulating superoxide-dependent cell death. Plant Cell 12: 1849–1862
Peiser GD, Wang T-T, Hoffman NE, Yang SF, Liu H-W, Walsh CT (1984) Formation of cyanide from carbon 1 of 1-aminocyclopropane-1-carboxylate acid during its conversion to ethylene. Proc Natl Acad USA 81: 3059–3063
Rottmann WH, Peter GF, Oeller PW, Keller JA, Shen NF, Nagy BP, Taylor LP, Campbell AD, Theologis A (1991) A 1-aminocyclopropane-1-carboxylate synthase in tomato is encoded by a multigene family whose transcription is induced during fruit and floral senescence. J Mol Biol 222: 937–961
Schweizer P, Buchala A, Dutler R, Metraux JP (1998) Induced systemic resistance in wounded rice plants. Plant J 14: 475–481
Schweizer P, Buchala A, Silverman P, Seskar M, Raskin I, Metraux JP (1997) Jasmonate-inducible genes are activated in rice by pathogen attack without a concomitant increase in endogenous jasmonic acid levels. Plant Physiol 114: 79–88
Sell S, Hehl R (2005) A fifth member of the tomato 1-aminocyclopropane-1-carboxylic acid (ACC) oxidase gene family harbours a leucine zipper and is anaerobically induced. DNA Seq 16: 80–82
Serek M, Sticher L, Reid MS (1995) Effect of 1-MCP on the vase life and ethylene response of cut flowers. Plant Growth Regul 16: 93–97
Silverman P, Seskar M, Kanter D, Schweizer P, Metraux JP, Raskin I (1995) Salicylic acid in rice (biosynthesis, conjugation, and possible role). Plant Physiol 108: 633–639
Sokal RR, Michener CD (1958) A statistical method for evaluating systematic relationships. University Kansas Sci Bull 28: 1409–1438
Subramaniam K, Abbo S, Ueng P (1996) Isolation of two differentially expressed wheat ACC synthase cDNAs and the characterization of one of their genes with root-predominant expression. Plant Mol Biol 31: 1002–1020
Tsuchisaka A, Theologis A (2004) Unique and overlapping expression patterns among the Arabidopsis 1-aminocyclopropane-1-carboxylate synthase gene family members. Plant Physiol 136: 2982–3000
Van Loon LC, Geraats BP, Linnstra H, Yu Y-B, Adams DO, Yang SF, Hoffman NE (1995) Salicylic acid in rice (biosynthesis, conjugation, and possible role). Plant Physiol 108: 633–639
Yamada M, Kiyosawa S, Yamaguchi T, Hirano T, Kobayashi T, Kusuhirki C, Watanabe S (1976) Proposal of a new method for differentiating races of Pyricularia oryzae Cavara in Japan. Ann Phytopathol Soc Jpn 42: 216–219
Yamagami T, Tsuchisaka A, Yamada K, Haddon LF, Warden LA, Theologis A (2003) Biochemical diversity among the 1-aminocyclopropane-1-carboxylate synthase isoforms encoded by the Arabidopsis gene family. J Biol Chem 278: 49102–49112
Yang SF, Hoffman NE (1984) Ethylene biosynthesis and its regulation in higher plants. Annu Rev Plant Physiol 35: 155–189
Yang Y, Qi M, 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
Yeh WH, Bonman JM, Lee EJ (1989) Effects of temperature, leaf wetness duration and leaf age on partial resistance to rice blast. J Plant Prot Trop 6: 223–230
Yu Y, Adams DO, Yang SF (1979) 1-Aminocyclopropane-1-carboxylate synthase, a key enzyme in ethylene biosynthesis. Arch Biochem Biophys 198: 455–465
Zanetti ME, Terrile MC, Arce D, Godoy AV, Segundo BS, Casalongue C (2002) Isozyme characterization of a potato cDNA corresponding to a 1-aminocyclopropane-1-carboxylate (ACC) oxidase gene differentially activated by stress. J Exp Bot 53: 2455–2457
Zarembski TJ, Theologis A (1993) Anaerobiosis and plant growth hormones induce two genes encoding 1-aminocyclopropane-1-carboxylate synthase in rice (Oryza sativa L.). Mol Biol Cell 4: 363–373
Zarembski TJ, Theologis A (1997) Expression characteristics of Os-AACS1 and Os-ACS2, two members of the 1-aminocyclopropane-1-carboxylate synthase gene family in rice (Oryza sativa L. cv. Habigan Aman II) during partial submergence. Plant Mol Biol 33: 71–77
CORRECTIONS

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Iwai T., Miyasaka A., Seo S., and Ohashi Y. Contribution of Ethylene Biosynthesis for Resistance to Blast Fungus Infection in Young Rice Plants.

The accession numbers for OsACO genes are listed incorrectly at the top right of p. 1206 and under the “One-Step RT-PCR” header in the “Materials and Methods” section on p. 1214. In both instances, OsACO1 (AK058296) should be listed as OsACO1 (AK065039), OsACO2 (AK071557) should be listed as OsACO2 (AK058296), and OsACO3 (AK065039) should be listed as OsACO3 (AK071557).

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García-Mata C. and Lamattina L. Nitric Oxide Induces Stomatal Closure and Enhances the Adaptive Plant Responses against Drought Stress.

The name of the first author, Carlos García-Mata, was incorrectly published without a hyphen. The online version of the article has been revised.