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Rice fatty acid α-dioxygenase is induced by pathogen attack and heavy metal stress: activation through jasmonate signaling

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Summary

Plant fatty acid α-dioxygenases (DOXs) catalyze the stereospecific conversion of fatty acids into the corresponding (*R*)-2-hydroperoxy fatty acids. In several plant species the corresponding gene was shown to be induced by pathogen infection, herbivore attack and environmental stresses. The precise signaling pathway accountable for the induction remains unidentified. In the present study, the effects of bacterial infection, oxidative- and heavy metal-stresses, and plant signaling molecules such as jasmonate, salicylic acid (SA), and ethylene (ET) on expression of a fatty acid α-*DOX* (*OsDOX*) gene in rice seedlings were investigated. The rice blight bacteria, *Xanthomonas oryzae*, elicited the accumulation of *OsDOX* transcripts in the leaves in both the incompatible and compatible interactions. Treating the seedling with $CuSO_4$ also significantly enhanced the *OsDOX* expression. The degree of induction was shown to be mostly parallel to the level of endogenous jasmonic acid (JA) in the leaves. In contrast, SA was little effective and ET down-regulated not only the *OsDOX* expression but also the endogenous level of JA in rice seedlings. These results suggested that the *OsDOX* gene expression by a variety of stress-related stimuli was activated through jasmonate signaling and was negatively regulated by ET.

Abbreviations: AOS, allene oxide synthase; DOX, dioxygenase; ET, ethylene; Fr wt, fresh weight; JA, jasmonic acid; MeJA, methyl jasmonate; MV, methyl viologen; SNP, sodium nitropruside; SA, salicylic acid

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Introduction

When plants encounter environmental stresses, a number of defense responsive systems begin to work to overcome them. One of these systems is the biosynthesis of oxygenated fatty acid derivatives (oxylipins) formed by a sequential action of a lipase, lipoxygenase, hydroperoxide lyase, and allene oxide synthase. The oxylipins such as jasmonic acid (JA) and C-6 aldehydes play important roles as signaling molecules that mediate the activation of genes required for stress-resistance (Creelman and Mullet, 1997; Mueller, 1997; Bate and Rothstein, 1998; Howe and Schilmiller, 2002).

In addition to lipoxygenases, α-dioxygenases (DOXs) were identified as another group of dioxygenases acting on fatty acids (Sanz et al., 1998; Hamberg et al., 2003). Plant α-DOX has a similarity to mammalian prostaglandin endoperoxide synthases in terms of their structures and enzymatic properties (Koeduka et al., 2002; Liu et al., 2004). The enzyme seems to be widespread in the plant kingdom. Tobacco (Hamberg et al., 2003), *Arabidopsis thaliana* (De Leon et al., 2002), rice (Koeduka et al., 2002), pea (Saffert et al., 2000), cucumber (Borge et al., 1999), and even a green marine alga, *Ulva pertusa* (Kajiwara et al., 1988), have been found to possess α-DOX. α-DOX catalyzes stereoselective introduction of one molecule of oxygen at the C-2 (α-) position of saturated and unsaturated fatty acids to afford unstable (*R*)-2-hydroperoxy derivatives. The formed 2-hydroperoxides of the fatty acids were spontaneously decarboxylated to give fatty aldehydes (Hamberg et al., 1999).

It has been reported that plant *α-DOX* is induced by pathogen and herbivore attacks in *Arabidopsis*, tobacco and hot pepper (Hermsmeier et al., 2001; De Leon et al., 2002; Kim et al., 2002). In *Arabidopsis*, in addition to the biotic stress, small α,β-unsaturated carbonyl compounds such as acrolein or methyl vinyl ketone, and oxidative- and drought-stresses are also potent stimulators of the expression of *α-DOX* (De Leon et al., 2002; Seki et al., 2002; Almeras et al., 2003). However, the distinct signaling pathway to activate *α-DOX* expression is still unclear. In particular, the signaling pathway of *α-DOX* in rice plants (*OsDOX*) is hardly known. Therefore, as a primary effort to elucidate the signaling pathway to induce *OsDOX* expression, we have examined the expression profile of rice *α-DOX* gene in response to oxidative- and heavy metal-stresses, bacterial infection and phytohormones.

Materials and methods

Plant materials

Rice plants (*Oryza sativa* L. cv. Nipponbare) were hydroponically grown with distilled water under white fluorescent light (14 h light period/day) at 28 °C and 50% relative humidity. Nine-day-old seedlings were used for the experiments. For pathogen infection, rice (cv. Kogyoku) plants were grown in soil for 2 weeks under the same conditions described above.

Oxidative- and heavy metal-stresses and phytohormone treatments

Nine-day-old seedlings were immersed in the solutions containing a given amount of sodium nitropruside, methyl viologen, $CuSO_4$, $NiSO_4$, methyl jasmonate, salicylic acid, or ethephone at 25 °C under continuous light. All treatments were performed and analyzed several times in independent experiments.

RT-PCR

Total RNA was prepared from rice shoots by TRIzol reagent (Invitrogen). After treatment with DNA-*free* kit (Ambion) to remove contaminating DNA, the RNA was quantified spectrophotometrically. Their qualities were checked by ethidium bromide staining after agarose gel electrophoresis. cDNA was synthesized with 20-mer oligo-dT primer and ThermoScript Reverse Transcriptase (Invitrogen), and used for PCR amplification. The gene-specific PCR primers used in this study were 5′-CATGCTATCCCTCGTCTCGACCT-3′ (sense) and 5′-CGCACTTCATGATGGAGTTGTAT-3′ (antisense) for rice actin-1 (*OsACT1*, accession number X16280), 5′-AATTGGTATGGATTATTGGGTAAGAAAA-TA-3′ (sense) and 5′-GTCACTTGTCAGATCC-TCCCAGC-3′ (antisense) for rice α-dioxygenase (*OsDOX*, accession number AF229813), 5′-GA-GAAGGCCAGGCAGAAGCTG-3′ (sense) and 5′-CCA-GAGAGGGCAACAATGTCCT-3′ (antisense) for rice ascorbate peroxidase (*OsAPX*, accession number D45423), 5′-CCCGGTCTTCCGCTCCAACTT-3′ (sense) and 5′-GACCTGGTCAGCTCCATCTTCT-3′ (antisense) for rice allene oxide synthase (*OsAOS*, accession number AY062258), 5′-CAGTTCAACTTCACCTCAGC-CAT-3′ (sense) and 5′-GTTGTAAGCGTCCGGGTTGGC-3′ (antisense) for rice jasmonate inducible pathogenesis-related class 10 protein gene (*OsJIOsPR10*, accession number AY062258).