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Methyl jasmonate-induced lateral root formation in rice: The role of heme oxygenase and calcium

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ABSTRACT

Lateral roots (LRs) play important roles in increasing the absorptive capacity of roots as well as to anchor the plant in the soil. Therefore, understanding the regulation of LR development is of agronomic importance. In this study, we examined the effect of methyl jasmonate (MJ) on LR formation in rice. Treatment with MJ induced LR formation and heme oxygenase (HO) activity. As well, MJ could induce *OsHO1* mRNA expression. Zinc protoporphyrin IX (the specific inhibitor of HO) and hemoglobin [the carbon monoxide/nitric oxide (NO) scavenger] reduced LR formation, HO activity and *OsHO1* expression. LR formation and HO activity induced by MJ was reduced by the specific NO scavenger 2-(4-carboxyphenyl)-4,4,5,5-tetramethylimidazoline-1-oxyl-oxide. The effects of Ca^{2+} chelators, Ca^{2+}-channel inhibitors, and calmodulin (CaM) antagonists on LR formation induced by MJ were also examined. All these inhibitors were effective in reducing the action of MJ. However, Ca^{2+} chelators and Ca^{2+} channel inhibitors induced HO activity when combining with MJ further. It is concluded that Ca^{2+} may regulate MJ action mainly through CaM-dependent mechanism.

Introduction

Lateral roots (LRs) originate in the pericycle, penetrate outward through the cortex and then appear on the surface of the parental roots and play important roles in increasing the root absorptive capacity of roots to absorb water and mineral nutrients as well as to anchor the plants in the soil (Hao and Ichii, 1999; López-Bucio et al., 2003; Wang et al., 2006). Thus, understanding the regulation of LR development is of agronomic importance.

Jasmonic acid (JA) and methyl jasmonate (MJ) are a class of plant hormones, which mediate various aspects of developmental and stress response (Wasternack, 2007). MJ and/or JA have been shown to be involved in inducing adventitious root formation and LR formation (Moons et al., 1997; Wang et al., 2002; Toro et al., 2003; Fattorini et al., 2009; Sun et al., 2009; Morquecho-Contreras et al., 2010). Recently, Raya-González et al. (2012) provided evidence to support that JA-promoted LR formation in *Arabidopsis* seedlings involved auxin-dependent and independent mechanisms.

Heme oxygenase (HO; EC 1.14.99.3) catabolizes heme into three products: carbon monoxide (CO), biliverdin (BV), and free iron. Three isoforms of mammalian HO protein have been identified: HO1, HO2, and HO3 (Synder and Baranano, 2001). In higher plants, much attention has been paid to HO1 because it is associated with the biosynthetic pathway leading to phytochrome chromophore formation (Davis et al., 1999; Emborg et al., 2006; Gisk et al., 2010), protection against oxidative damage (Noriega et al., 2004; Shen et al., 2011), and root development (Xuan et al., 2008; Guo et al., 2009; Han et al., 2012). The genes for HO1 have been identified in *Arabidopsis* (*AtHO1*; Muramoto et al., 1999), rice (*OsHO1*; Izawa et al., 2000), tomato (*LeHO1*; Terry and Kendrick, 1996), pea (*PsHO1*; Linley et al., 2006), rapeseed (*BnHO1*; Shen et al., 2011), cucumber (*CsHO1*; Li et al., 2011), and maize (*ZmHO1*; Han et al., 2012). The role of HO in regulating LR formation has been clarified in tomato (Guo et al., 2008), maize (Han et al., 2012), and rice (Chen et al., 2012). Plant *HO1* expression was reported to be induced by auxin (Xuan et al., 2008), abscisic acid (Cao et al., 2007b), cytokinin (Huang et al., 2011) and $CoCl_2$ (Xu et al., 2011b). However, it is not known whether MJ induces *HO1* expression. Neither do we know whether HO plays regulatory role in MJ-promoted LR formation.

Calcium (Ca^{2+}) has been suggested to act as a second messenger in signaling pathways for a diverse array of cellular responses to external and internal stimuli in plants (Sanders et al., 1999; Evans et al., 2001; White and Broadley, 2003). Lanteri et al. (2006) demonstrated that Ca^{2+} was downstream messenger in the

Abbreviations: ABA, abscisic acid; BAPTA/AM, 1,2-bis(*o*-aminopenoxy)ethane-*N*,*N*,*N*′,*N*′-tetraacetic acid tetra(acetoxymethyl) ester; CaM, calmodulin; CDPK, calcium-dependent protein kinase; cPTIO, 2-(4-carboxyphenyl)-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide; CPZ, chlorpromazine hydrochloride; DW, dry weight; EGTA, ethylene glycol-bis(2-amino ethylether-*N*,*N*,*N*′,*N*′-tetraacetic acid; Hb, hemoglobin; IP_3, inositol triphosphate; JA, jasmonic acid; LR, lateral root; MJ, methyl jasmonate; NEO, neomycin sulfate; NO, nitric oxide; RR, ruthenium red; TFP, trifluoperazine dihydrochloride; ZnPPIX, zinc protoporphyrin IX.

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signaling pathway triggered by auxins and nitric oxide (NO) to promote adventitious root formation. Recently, we reported that Ca^{2+} is involved in NO- and auxin-induced LR formation in rice (Chen and Kao, 2012). To date, it is not known whether Ca^{2+} is also involved in MJ-promoted LR formation in rice.

The role of HO in regulating LR formation by auxin and NO has been clarified in tomato (Guo et al., 2008) and rice (Chen et al., 2012). However, the role of HO in regulating MJ-induced LR formation in rice has not been examined. In this study, we examined the role of HO regulating MJ-promoted LR formation in rice. Furthermore, the involvement of Ca^{2+} was evaluated during the MJ-mediated LR formation in rice. We provide pharmacological evidence that both HO and Ca^{2+} are required for MJ-induced LR formation in rice.

Materials and methods

Plant material and growth conditions

Seeds of rice (*Oryza sativa* L., cv. Taichung Native 1, an Indica type) were sterilized with 3% sodium hypochlorite for 15 min and washed extensively with distilled water. To obtain more uniformly germinated seeds, rice seeds in a Petri dish (20 cm) containing distilled water were pretreated at 37 °C for 1 day under dark conditions. Uniformly germinated seeds were then selected and transferred to a Petri dish (20 cm) containing two sheets of filter paper moistened with distilled water for 2 days. Two-day-old seedlings were then transferred to Petri dishes (9 cm) containing distilled water, MJ, zinc protoporphyrin IX (ZnPPIX), hemoglobin (Hb), 2-(4-carboxyphenyl)-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide (cPTIO), Ca^{2+} chelators, Ca^{2+} channel inhibitors or calmodulin (CaM) antagonists at the desired concentration. Root growth of rice seedlings grown in distilled water is similar to that of grown in medium containing inorganic salts; thus, seedlings grown in distilled water were used as the control. Each Petri dish contained 5 seedlings and each treatment was replicated 4 times. The seedlings were allowed to grow at 27 °C in darkness. The seminal roots of rice seedlings at the times specified were used for analysis of lateral root (LR) formation, heme oxygenase (HO) activity, and *OsHO1* transcripts.

LR formation

To show LR formation in seminal roots for each treatment, the number of LRs longer than 1 mm per seedlings was counted.

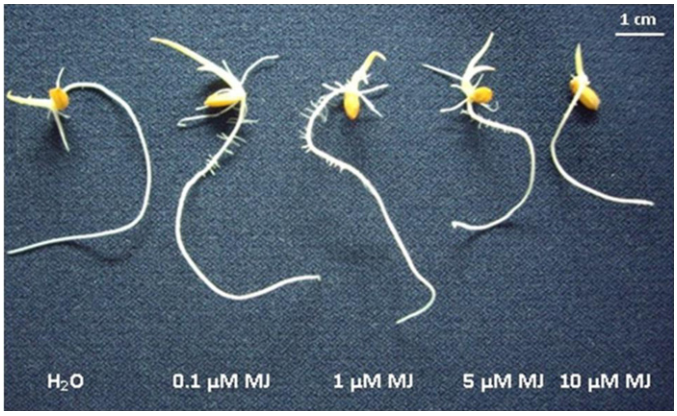

Fig. 1. Effect of MJ on LR formation in rice. Two-day-old rice seedlings were treated with MJ (0–10 μM) for 3 days. Experiment was repeated four times with similar results. Representative photograph of rice seedlings was shown. Bar = 1 cm.

HO extraction and assay

Heme oxygenase activity was analyzed basically as described (Xuan et al., 2008). For extraction of HO, 25 roots were homogenized with 3 mL of 25 mM HEPES–Tris (pH 7.4) containing 250 mM mannitol, 1 mM EDTA, 1% (w/v) polyvinylpyrrolidone, 10% (v/v) glycerol, and 1 mM dithiothreitol. The whole isolation procedure was carried out at 4 °C. The homogenate was centrifuged at 15,000 × *g* for 30 min, and resulting supernatant was used for determination of HO activity as described (Xuan et al., 2008). The reaction mixture (1 mL) contained 2 mM deferrioxamine in 100 mM HEPES–NaOH (pH 7.2), 100 μM NADPH, 10 μM Hm, 0.15 mg mL^{-1} bovine serum albumin, 50 μg mL^{-1} (4.2 μM) spinach ferredoxin, 0.025 units mL^{-1} spinach ferredoxin-$NADP^+$ reductase, 5 mM ascorbate and enzyme extract (250 μL). The reaction was started by adding NADPH and allowed to proceed at 37 °C for 30 min. The absorbance of BV was measured at 650 nm. The increase in BV concentration was determined by the extinction coefficient 6.25 mM^{-1} cm^{-1} at 650 nm. One unit of activity for HO was defined as the amount of enzyme that produced 1 μmol of BV per 30 min. Rice roots contained very low protein. Thus, HO activity was expressed on a dry weight (DW) basis.

Semi-quantitative RT-PCR

Total RNA was isolated from the roots of seedlings by the TRIzol reagent method (Invitrogen, CA, USA). To prevent DNA contamination, RNA was treated with Turbo DNase I (Ambion, TX, USA) for 30 min at 37 °C before RT-PCR. Control PCR amplifications involved RNA used as a template after DNase I treatment to verify the elimination of contaminated DNA. Reverse-transcription reactions

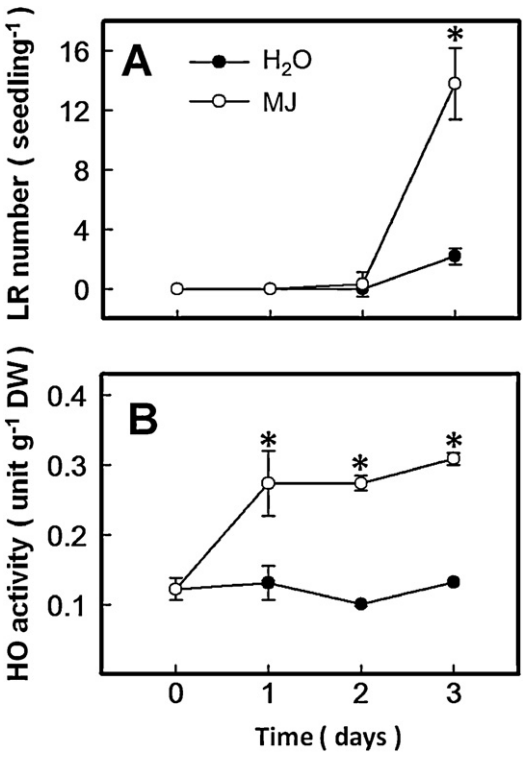

Fig. 2. Changes in the LR number (A) and HO activity (B) in rice seedlings treated with MJ. Two-day-old rice seedlings were treated with 1 μM MJ for 1, 2, and 3 days. Bars show stand errors ($n = 20$ or 4). Asterisks represent that values that are significantly different between H_2O and MJ treatment at $P < 0.05$.