Induction of Resistance against Rice Bacterial Leaf Blight by 3-Chloro-1-methyl-1H-pyrazole-5-carboxylic Acid

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A pyrazole derivative, 3-chloro-1-methyl-1H-pyrazole-5-carboxylic acid (CMPA), exhibited high anti-rice blast activity without any significant antimicrobial activity. To assess the mode of action of CMPA, its effects on rice bacterial leaf blight caused by Xanthomonas oryzae pv. oryzae and the expression of a defense-related gene were examined. The treatment of CMPA reduced the disease symptoms in a dose-dependent manner, but CMPA did not exhibit any direct antibacterial activity against X. oryzae at concentrations up to 1 mg/ml. The treatment of CMPA induced the expression of PBZ1, a defense-related gene, which is evoked by several plant activators. This ability to induce PBZ1 expression and enhance disease resistance without antimicrobial activity suggests that CMPA can activate systemic acquired resistance in rice as well as in tobacco. © Pesticide Science Society of Japan

Keywords: systemic acquired resistance, disease resistance, rice, rice bacterial leaf blight, probenazole.

INTRODUCTION

We previously reported that a novel pyrazole derivative, 3-chloro-1-methyl-1H-pyrazole-5-carboxylic acid (CMPA), exhibited high anti-rice blast activity at 0.05 mg/pot in vivo. However, CMPA did not have any significant effects on the hyphal growth, spore germination, and appressorium formation of Pyricularia oryzae. These results suggest that the anti-rice blast activity of CMPA was caused by its effect on the rice plants and not on P. oryzae. On the other hand, CMPA induced the expression of pathogenesis related (PR) genes and a broad range of disease resistance without exhibiting antibacterial activity in tobacco, indicating that CMPA acts as an inducer of systemic acquired resistance (SAR) in tobacco like probenazole (PBZ; 3-allyloxy-1,2-benz[d]isothiazole 1,1-dioxide), acibenzolar-S-methyl (BTH; benzo[1,2,3]thiadiazole-7-carboxylic acid S-methyl ester), N-cyanomethyl-2-chloroisonicotinamide (NCI), and tiadinil (TDL). In tobacco, CMPA does not require the accumulation of salicylic acid (SA) to induce SAR, having a similar mode of action to BTH and NCI but not PBZ, however, the mechanism of SAR in rice plants, including the requirement of SA, remains to be clarified. In this paper, to elucidate in more detail the mode of action of CMPA in rice, we investigated the effect of CMPA on rice bacterial leaf blight. Furthermore, the expression of PBZ1, a defense-related gene, in the CMPA-treated rice was examined.

MATERIALS AND METHODS

1. Chemicals

CMPA was synthesized in Chemical Research Laboratories of Nissan Chemical Industries, Ltd. PBZ, BTH and NCI were obtained from Meiji Seika Kaisha, LTD, Syngenta Co., and Nippon Kayaku Co., Ltd., respectively.

2. Plant Materials and Bacterial Preparations

Rice seeds (Oryza sativa L. cv. Nipponbare) were sown on diluted soil in pots (80 cm³) and grown in a greenhouse at 25°C. Xanthomonas oryzae pv. oryzae race 003 was cultured on a YPDA (yeast extract 10 g, polypeptone 10 g, dextrose 20 g, agar 15 g/liter; pH 7.0) medium. The bacterial suspension was prepared in sterile distilled water to provide the proper density.

3. Rice Bacterial Leaf Blight Inoculation Assay

Rice seedlings at the 3-leaf stage were pretreated with CMPA at various concentrations. PBZ, BTH and NCI were used as reference compounds. Each chemical was applied as an emulsified solution with acetone using a soil-drenching method. Challenge inoculation with X. oryzae was performed 5 days after the pretreatment with chemicals. Plants were cut at about 4 cm from the tip of the 4th leaf, sprayed with a cell suspension (10⁶ colony-forming units (CFU) ml⁻¹) of X. oryzae, and kept in a greenhouse under the following conditions: 24°C and 70% humidity for 10 hr without light; 29°C and 70% humidity for 14 hr with light. The length of the bleached part of infected leaves was measured 12 days after the inoculation.
4. In vitro Activity of CMP A against X. oryzae

CMP A was dissolved in sterile distilled water to provide a concentration of 0.02–1 mg/ml and used for antimicrobial assays. The assay plate (9 cm in diameter) was prepared by overlaying YPDA medium (5 ml, 0.5% (w/v) agar) containing ca. 10^7 CFU of X. oryzae on YPDA medium (10 ml). A paper disk (8 mm in diameter) containing 10 μl of CMP A solution was placed on the assay plate. Antibacterial activity was evaluated by measuring the diameter of the halo that appeared around the disk after incubation at 28°C for 48 hr.

5. PBZ1 Gene Expression in Rice Plants Treated with CMP A

Rice seedlings at the 3-leaf stage were pretreated with chemicals at various concentrations by the soil-drenching method, and the 4th leaves were harvested 5 days after application. Total RNA was extracted from frozen leaf tissue samples by using TRIzol reagent (Life Technologies, Rockville, MD, USA) following the manufacturer's instructions.15 P-Labeled PBZ1 cDNA probe was synthesized as previously described.16 Total RNA samples were subjected to 1.2% agarose-1.1% formaldehyde gel electrophoresis and transferred to a nylon membrane (Hybond N+, Amersham, Buckinghamshire, UK). After the transfer, RNA was cross-linked to the membrane using an UV linker (GS GENE LINKER, Buckinghamshire, UK). After the transfer, RNA was cross-linked to the membrane using an UV linker (GS GENE LINKER, Bio-Rad, Hercules, CA, USA). Hybridization and washing were performed as described by Church and Gilbert (1984).15 Prehybridization and hybridization were performed at 68°C for 1 hr or longer and 8 hr or longer, respectively. The membrane was washed twice with 2×SSC containing 0.1% SDS for 30 min at 68°C and then washed twice with 0.1×SSC containing 0.1% SDS for 15 min at 68°C. The detection was performed with a BAS2500 image analyzer (Fujifilm).

RESULTS AND DISCUSSION

CMP A induced PR gene expression and a broad range of disease resistance without antibacterial activity in tobacco.25 CMP A also exhibited an anti-rice blast effect without direct antifungal activity.11 To determine whether CMP A induces disease resistance in rice and protects against various pathogens, the effect of the treatment on rice bacterial leaf blight was examined. Treating O. sativa cv. Nipponbare plants with CMP A (0.05, 0.5, 5 mg/pot) reduced disease symptoms caused by infection with the virulent pathogen X. oryzae pv. oryzae race 003. The effect of CMP A was dose-dependent, with 5 mg/pot resulting in about 99.5% protection from the pathogen (Fig. 1). CMP A exhibited a slightly higher level of protection against this disease than PBZ and BTH, which is similar to the protective efficacy against rice blast disease.11 However, CMP A did not exhibit any direct antibacterial activity against X. oryzae at concentrations up to 1 mg/ml (data not shown). Thus, in addition to the anti-rice blast effect reported previously,11 CMP A provided resistance against a disease caused by a bacterial pathogen, without showing direct antibacterial activity. These findings suggest that CMP A acts on the rice plants to induce resistance against diseases.

In tobacco, some PR proteins are coordinately expressed during the induction and maintenance of SAR.16) CMP A induced the expression of PRI, 2 and 5 genes without the accumulation of SA in tobacco plants.21 To determine whether CMP A induces the expression of defense-related genes in rice, the expression of PBZ1, which is induced by PBZ treatment, was examined. Northern blot analysis indicated that transcripts for PBZ1 moderately accumulated in rice leaves treated with CMP A in a dose-dependent manner as well as in PBZ-, BTH- or NCI-treated plants (Fig. 2). In contrast, no transcript for PBZ1 was detected in the leaves of the water-treated control plants. The ability of CMP A to induce the PBZ1 gene expression and enhance disease resistance in the absence of antimicrobial activity strongly supports that CMP A can activate a disease resistance system like systemic acquired resistance in rice plants. CMP A induced a lower level of accumulation of the PBZ1 transcript than did PBZ or NCI (Fig. 2), whereas these chemicals exhibited the same level of induction of disease resistance (Fig. 1), suggesting that the mode
of action of CMPA is different from that of PBZ or NCI. This paper demonstrates that CMPA activates the innate immunity system of rice plants like PBZ. At present, the exact mode of action of CMPA to induce disease resistance in rice is unknown, because the mechanisms of rice SAR have not been determined yet. However, it was demonstrated in tobacco plants that CMPA activated the SAR signaling pathway by stimulating a point downstream of SA accumulation. Understanding the detailed mechanism of CMPA-induced disease resistance will help to clarify SAR in rice, which is under investigation.

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REFERENCES