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Regulation mechanisms of systemic acquired resistance induced by plant activators*

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Systemic acquired resistance (SAR) is a plant defense system against a broad range of pathogens and is induced through the salicylic acid (SA)-mediated pathway. We investigated the mode of action of SAR-inducible chemicals, *N*-cyanomethyl-2-chloroisonicotinamide (NCI), 3-chloro-1-methyl-1*H*-pyrazole-5-carboxylic acid (CMPA), and *N*-(3-chloro-4-methylphenyl)-4-methyl-1,2,3-thiadiazole-5-carboxamide (tiadinil, TDL), by analyzing disease resistance and the expression of SAR marker genes in tobacco and *Arabidopsis*. NCI, CMPA, TDL and its active metabolite 4-methyl-1,2,3-thiadiazole-5-carboxylic acid (SV-03) induced SAR by activating the site between SA accumulation and NPR1 in the SAR signaling pathway.

Keywords: systemic acquired resistance, *Arabidopsis thaliana*, tobacco, plant activator, *PR* genes.

Introduction

Systemic acquired resistance (SAR) is an inducible defense mechanism that plays an important role in defending plants from attack by pathogens. SAR is induced after a hypersensitive response including tissue necrosis caused by pathogens such as viruses, bacteria and fungi, and is effective against a broad spectrum of pathogens. SAR in tobacco and *Arabidopsis thaliana* has been well-characterized, and a set of pathogenesis-related (*PR*) genes has been identified as SAR marker genes. SAR development in these dicotyledonous plants is mediated by salicylic acid (SA), which is supported by the fact that SAR is not induced in NahG transgenic plants expressing bacterial salicylate dehydrogenase to convert SA into catechol. In contrast, the SAR mechanism in monocotyledonous rice plants remains to be clarified. Some synthetic compounds exhibit several essential criteria of SAR inducers: they induce a broad range of disease resistance; their effects are not due to their antimicrobial activities; and they induce an SAR molecular marker, *PR* genes, in plants.

Benzo(1,2,3)thiadiazole-7-carbothioic acid *S*-methyl ester (BTH) and 2,6-dichloroisonicotinic acid (INA) induce SAR without SA accumulation, whereas PBZ and its derivative BIT induce SAR through SA biosynthesis. Here we clarified the mode of action of NCI, CMPA, TDL and SV-03, recently developed anti-rice blast agents, in tobacco and *Arabidopsis*.

Analyis of SAR-inducing Activity

In tobacco and *Arabidopsis*, SAR induced by either chemical treatment or pathogen infection is associated with enhanced resistance to a variety of pathogens and the expression of SAR marker genes, such as acidic *PR-1*, *PR-2* (β-1,3-glucanase) and *PR-5* (thaumatin-like). To determine whether NCI, CMPA, TDL or SV-03 acts as an SAR activator in tobacco and *Arabidopsis*, we analyzed the ability to enhance disease resistance and induce *PR* gene expression.

In tobacco, the abilities of chemicals to enhance resistance against diseases caused by viral, bacterial and fungal pathogens were assessed. Tobacco plant, *Nicotiana tabacum* cv. Xanthi nc, possessing *N* gene, the resistance gene against tobacco mosaic virus (TMV), forms necrotic lesions in response to TMV infection. SAR activators enhance this resistance and reduce the size of lesions. The average lesion size in NCI-, CMPA-, TDL- or SV-03-treated plants was markedly smaller than that of water-treated control plants, indicating that these chemicals enhanced *N* gene-mediated resistance. Next, we tested the effect of NCI, CMPA, TDL or SV-03 on the interaction between tobacco plants and a virulent bacterial pathogen, *Pseudomonas syringae* pv. *tabaci* (*Pst*). Susceptibility was estimated by measuring bacterial growth in leaf tissues after challenge infection. Treatment with either of these

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chemicals by soil drenching suppressed the growth of *Pst* in infected tissues relative to water-treated control plants. This result indicated that NCI, CMPA, TDL or SV-03 induced resistance against *Pst* in tobacco plants. Powdery mildew, *Oidium lycopersici*, is a virulent fungal pathogen for *N. tabacum*. Treating tobacco plants with NCI or other chemicals also reduced the symptoms of disease caused by infection with *O. lycopersici*. In *Arabidopsis*, the ability of chemicals to enhance resistance to infection by the virulent bacterial pathogen *P. syringae* pv. *tomato* DC3000 (*Pst* DC3000) was assessed. Growth of *Pst* DC3000 was reduced in plants treated with these chemicals by soil drenching. By 4 days'-post inoculation, these plants contained about 10-fold lower titers of bacteria than were detected in water-treated control leaves. These results indicate that NCI, CMPA, TDL and SV-03 are able to induce SAR-like disease resistance to a broad range of pathogens.

RNA blot analysis indicated that transcripts for SAR marker genes, *PR-1*, *PR-2* and *PR-5*, accumulated in the leaves of tobacco and *Arabidopsis* treated with NCI, CMPA, TDL or SV-03. The abilities of these chemicals to induce SAR marker gene expression and enhance disease resistance confirmed that these chemicals could activate SAR.

SAR Induction Mechanisms by NCI, CMPA and TDL

To determine the activation site in the SAR signaling pathway by these chemicals, we used NahG transgenic plants and *npr1*-deficient *Arabidopsis* mutants. Whether the defense-related hormone SA is required for resistance induction by these chemicals was investigated in NahG transgenic plants. NahG transgenic *Arabidopsis* and tobacco plants are unable to accumulate SA due to the expression of an SA degrading enzyme. NahG transgenic tobacco plants treated with NCI, CMPA, TDL or SV-03, exhibited enhanced resistance to TMV and *Pst* and the *PR* gene expression. Enhanced resistance and *PR* gene expression by these chemicals were observed also in NahG transgenic *Arabidopsis*. These results indicated that the induction of SAR by NCI, CMPA, TDL and SV-03 is independent of SA biosynthesis.

Genetic analyses have identified NPR1 protein, which is an essential key component of SAR induction in *Arabidopsis*. We therefore tested whether SAR induction by these chemicals requires NPR1. No *PR-1* gene expression was detected in the *npr1* mutant treated with NCI or other chemicals. The *npr1* mutant also failed to develop enhanced resistance to *Pst* DC3000 following treatment with these SAR-inducible chemicals. Thus, NCI, CMPA, TDL and SV-03 require a functional *NPR1* gene to induce SAR in *Arabidopsis*.

To assess the role of other defense-related hormones, ethylene and jasmonic acid (JA), in the activation of SAR by these chemicals, *PR* expression was examined in ethylene-insensitive mutants *ein2-1* and *etr1-1* and JA-insensitive mutant *jar1-1*. Nearly wild-type levels of *PR-1* transcripts were detected in either of these mutants treated with SAR inducible chemicals, indicating that these chemicals appear to activate *PR* gene expression independently of ethylene and JA. These results indicated that NCI, CMPA, TDL and SV-03 induce SAR by activation the site between SA and NPR1 in the SAR signaling pathway.