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Article Addendum

The *AtNFXL1* gene functions as a signaling component of the type A trichothecene-dependent response

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Phytopathogenic *Fusarium* species produce the trichothecene family of phytotoxins, which function as a virulence factor during infection of plants. Trichothecenes are classifiable into four major groups by their chemical structures. Recently, the *AtNFXL1* gene was reported as a type A trichothecene T-2 toxin-inducible gene. The *AtNFXL1* gene encodes a putative transcription factor with similarity to the human transcription repressor NF-X1. The *atnfxl1* mutant exhibited hypersensitivity phenotype to T-2 toxin but not to type B deoxynivalenol (DON) in comparison with wild type when *Arabidopsis thaliana* grew on agar medium containing trichothecenes. The absence or presence of a carbonyl group at the C8 position distinguishes type A and type B. Growth defect by another type A trichothecene diacetoxyscirpenol (DAS), was weakly enhanced in the atnfxl1 mutant. Diacetoxyscirpenol is distinguishable from T-2 toxin only by the absence of an isovaleryl group at the C8 position. Correspondingly, the *AtNFXL1* promoter activity was apparently induced in T-2 toxin-treated and DAS-treated plants. In contrast, DON failed to induce the *AtNFXL1* promoter activity. Consequently, the *AtNFXL1* gene functions as a signaling component of the type A trichothecene-dependent response in Arabidopsis. In addition, the C8 position of trichothecenes might be closely related to the function of *AtNFXL1* gene.

Phytotoxins represent a diverse group of secondary fungal metabolites, which vary widely in their chemistry and toxicology. Trichothecene phytotoxins are produced by necrotrophic phytopathogens such as *Fusarium* species.[1] Trichothecene-producing *Fusarium* species have strain-specific trichothecene metabolite profiles, suggesting that these chemotypes play a role in the virulence of individual *Fusarium* strains.[2] Trichothecenes are classifiable into four groups by their characteristic functional groups. Type A [T-2 toxin and DAS] and type B [nivalenol (NIV) and DON] trichothecenes, which are distinguishable by the absence or presence of a carbonyl group at the C8 position, have frequently contaminated in cereal crops and processed grains. Trichothecenes inhibit peptidyl transferase activity in eukaryotic cells by binding to the 60S ribosomal subunit.[3] Therefore, trichothecenes are considered to inhibit the defense response of host plants. However, we showed that type A trichothecenes, such as T-2 toxin and DAS, induce an elicitor-like signaling pathway and cell death in *Arabidopsis thaliana* at a concentration of 1 μM.[4] It is likely that type A trichothecene-induced cell death contributes directly to virulence of necrotrophic fungi. In contrast, 5–10 μM DON apparently inhibits protein translation in Arabidopsis cells, but fails to activate the elicitor-like signaling pathway.[4] These results suggest that Fusarium species use DON as a non-defense-inducing translational inhibitor during disease spread in host plants. The role of trichothecene in virulence is likely to differ greatly among its molecular species.

Furthermore, we performed a comparative analysis of the phytotoxic action of representative trichothecenes when Arabidopsis grew on media containing these compounds.[4] Both DON and DAS) preferentially inhibited root elongation. Preferential inhibition of root elongation was also observed in plants treated with another phytotoxin, coronatine.[5] In addition, T-2 toxin-treated seedlings exhibited dwarfism with aberrant morphological changes (e.g., petiole shortening, curled dark-green leaves and reduced cell size). These results imply that the phytotoxic action of trichothecenes differed among their molecular species. Seedlings treated with another translational inhibitor, cycloheximide (CHX), did not display these features. Although the DAS structure closely resembles that of T-2 toxin, DAS and T-2 toxin are distinguished by the presence and absence, respectively, of an isovaleryl group at the C8 position. In addition, phytotoxic effects of HT-2 toxin (type A with the isovaleryl group) are comparable to those of T-2 toxin, whereas T-2 tetraol (type A without the isovaleryl group) did not have these effects (data not shown). It might be that the isovaleryl group at the C8 position affects the mode of action of trichothecenes in host plants, causing morphological change of Arabidopsis shoots.

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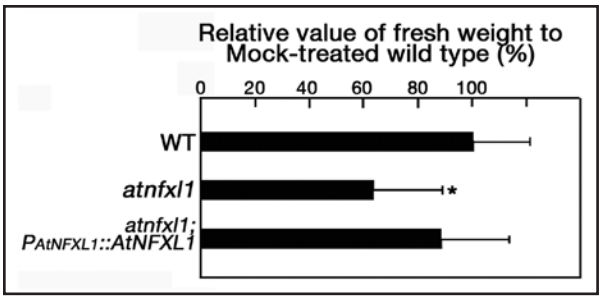

Figure 1. The *atnfxl1* mutant exhibited hypersensitivity phenotype to DAS. The fresh weight of each DAS-treated plant expressed relative (%) to mock-treated wild-type plants. Plants were treated with 2.5 μM DAS or without trichothecenes. The data are representative of three independent experiments. Significant difference between the 2.5 μM DAS-treated *atnfxl1* mutant and wild type/complementation line 5 was observed (*$p < 0.01$, based on Student's t-test). Similar results were obtained for another six independent complementation lines.

Recently, we isolated an *AtNFXL1* gene as a T-2 toxin-inducible gene in Arabidopsis.[6] The *AtNFXL1* gene encodes a zinc finger type of transcription factor with similarity to the human transcription repressor *NF-X1*. We examined GUS activities of the *AtNFXL1* promoter::β-glucuronidase (*GUS*) transgenic plants treated with some trichothecenes. The *AtNFXL1* promoter activity was apparently induced by T-2 toxin and DAS in Arabidopsis plants.[7] The fold increase of promoter activities of T-2 toxin-treated plants was higher than that of DAS-treated plants. In contrast, DON only weakly induced promoter activity in *AtNFXL1* promoter::*GUS* plants.[7] Therefore, *AtNFXL1* gene exhibited type A trichothecene-dependent expression pattern. Correspondingly, an *atnfxl1* mutant exhibited a severe growth defect on MS medium containing 0.1 μM T-2 toxin compared to wild type.[7] Microarray analysis suggested that the atnfxl1 mutant could not appropriately repress the defense response induced by T-2 toxin, resulting in severe growth defects in T-2 toxin-treated Arabidopsis seedlings.[7] As presented in Figure 1, growth defect by DAS was also enhanced in the *atnfxl1* mutant. Hypersensitivity phenotype of the T-2 toxin-treated *atnfxl1* mutant is more severe than that of the DAS-treated mutant (Fig. 1).[7] In contrast, growth defects of DON-treated *atnfxl1* mutant were similar to those of DON-treated wild type plants.[7] Therefore, *AtNFXL1* gene functions as a signaling component of the type A trichothecene-dependent response in Arabidopsis. In particular, the group at the C8 position of trichothecenes might be important for the molecular function of *AtNFXL1* gene.

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