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Research

Expression of tomato prosystemin gene in *Arabidopsis* reveals systemic translocation of its mRNA and confers necrotrophic fungal resistance

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Summary

- Systemin (SYS), an octadecapeptide hormone processed from a 200-amino-acid precursor (prosystemin, PS), plays a central role in the systemic activation of defense genes in tomato in response to herbivore and pathogen attacks. However, whether *PS* mRNA is transferable and its role in systemic defense responses remain unknown.
- We created the transgenic tomato *PS* gene tagged with the green fluorescent protein (*PS-GFP*) using a shoot- or root-specific promoter, and the constitutive *35S* promoter in *Arabidopsis*. Subcellular localization of PS-/SYS-GFP was observed using confocal laser scanning microscopy and gene transcripts were determined using quantitative real-time PCR.
- In *Arabidopsis*, PS protein can be processed and SYS is secreted. Shoot-/root-specific expression of *PS-GFP* in *Arabidopsis*, and grafting experiments, revealed that the *PS* mRNA moves in a bi-directional manner. We also found that ectopic expression of *PS* improves *Arabidopsis* resistance to the necrotrophic fungus *Botrytis cinerea*, consistent with substantial upregulation of the transcript levels of specific pathogen-responsive genes.
- Our results provide novel insights into the multifaceted mechanism of SYS signaling transport and its potential application in genetic engineering for increasing pathogen resistance across diverse plant families.

Introduction

Higher plants have a complex array of defense mechanisms for protection against microbial pathogens and herbivorous insects. Some of these defense mechanisms are pre-existing or constitutive, while others are only activated upon pathogen or insect attack. These inducible defensive responses occur both at the site of stress exposure (local response) and in undamaged tissues far from the initial site of attack (systemic response), indicating that mobile signals can induce defense responses over long distance. Several phloem-mobile signaling compounds have been identified, including salicylic acid (SA) and jasmonic acid (JA) (Glazebrook, 2005; Shah & Zeier, 2013). In general, SA-mediated defenses more strongly affect biotrophic pathogens and JA-mediated defenses more strongly affect necrotrophic pathogens and herbivorous insects (Glazebrook, 2005; Pieterse *et al.*, 2012).

Systemin (SYS), an octadeca-peptide isolated from tomato, was the first bioactive peptide hormone identified in plants (Pearce *et al.*, 1991). Upon wounding, SYS is thought to be released from its 200-amino-acid precursor protein prosystemin (PS) and exported out of the cell by an unknown mechanism (McGurl *et al.*, 1992). PS was shown to be cleaved by proteases present in tomato foliar apoplastic wash fluid or in medium of cultured tomato cells (Dombrowski *et al.*, 1999). However, an engineered PS precursor in which the SYS-encoding region was replaced by synthetic sequence encoding TMOF (trypsin-modulating oostatic factor) was found to be mainly processed in the cytoplasm of tobacco cells (Tortiglione *et al.*, 2003). SYS can bind to membrane-bound receptor(s) to initiate an intracellular