Apoplastic Venom Allergen-like Proteins of Cyst Nematodes Modulate the Activation of Basal Plant Innate Immunity by Cell Surface Receptors

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ABSTRACT

Despite causing considerable damage to host tissue during the onset of parasitism, nematodes establish remarkably persistent infections in both animals and plants. It is thought that an elaborate repertoire of effectors proteins in nematode secretions suppresses damage-triggered immune responses of the host. However, the nature and mode of action of most immunomodulatory compounds in nematode secretions are not well understood. We have recently discovered that venom allergen-like proteins (VAPs) of plant-parasitic nematodes are uniquely conserved in secretions of all animal- and plant-parasitic nematodes studied to date, but their role during the onset of parasitism has thus far remained elusive. Knocking-down the expression of Gr-VAP1 severely hampered the infectivity of the potato cyst nematode Globodera rostochiensis. By contrast, heterologous expression of Gr-VAP1 and VAPs from the beet cyst nematode Heterodera schachtii in plants resulted in the loss of basal immunity to multiple pathogens. The modulation of basal immunity by ectopic VAPs involves extracellular protease-host defenses. Furthermore, the onset of programmed cell death was commonly suppressed by VAPs from G. rostochiensis, H. schachtii, and the root-knot nematode Meloidogyne incognita. Surprisingly, these VAPs only affected the programmed cell death mediated by surface-localized immune receptors. Furthermore, the delivery of VAPs into host tissue coincides with the enzymatic breakdown of plant cell walls by migratory nematodes. We, therefore, conclude that parasitic nematodes most likely utilize VAPs to suppress the activation of defenses by immunogenic breakdown products in damaged host tissue.

RESULTS

1. The venom allergen-like protein Gr-VAP1 is required for the onset of parasitism in host plants.

Fig. 1. (A) RNA interference specifically knocked down Gr-VAP1 expression in pre-parasitic second-stage juveniles of G. rostochiensis. Semi-quantitative reverse transcription-PCR of Gr-VAP1 in transgenic line Gr-VAP1 treated (−) and non-treated (+) juveniles. Controls indicate the cycles in the PCR. (B) The knockdown of Gr-VAP1 expression significantly reduces the number of infective juveniles of G. rostochiensis inside roots of tomato plants. C. Apopterpendicular second-stage juveniles were either treated with double stranded RNA matching the Gr-VAP1 or the Negative. Bars represent standard error of means of number of nematodes per plant at 7 days after inoculation over 10 replicates. Asterisk marks significance in a Student’s t-test (P-value < 0.05).

2. Apoplastic Gr-VAP1 suppresses immunity of potato plants to G. rostochiensis.

Fig. 2. (A) The expression of Gr-VAP1, as shown by semi-quantitative RT-PCR, is highly up-regulated in the migratory stages of G. rostochiensis (p2, J2, and males (♂)), while it declines after excision of the permanent feeding site in the sedentary juveniles (J3 and J4), and adult females (♀). Changes in expression of Gr-VAP1 were assessed using the constitutively expressed cAMP-dependent protein kinase (cAMP) gene in G. rostochiensis as reference. Reactions using uninoculated tomato roots as template (Root) and without reverse transcriptase (RT) were included as controls. (B) Transgenic potato plants stably overexpressing Gr-VAP1 in the apoplast show enhanced necrotrophy to G. rostochiensis. The number of nematodes per plant was compared at 6 weeks post inoculation for two independent transgenic lines harboring either Gr-VAP1 or the corresponding empty vector (EV) line. Bars represent standard error of the means. Different letters indicate statistically significant differences between plants (P-values < 0.05).

3. Ectopic venom allergen-like proteins suppress basal immunity in Arabidopsis thaliana.

Fig. 3. (A) Heterologous expression of Gr-VAP1 from G. rostochiensis, and Mel-VAP1 and Hs-VAP2 from Heterodera schachtii in the apoplast of transgenic Arabidopsis lines enhances their susceptibility to H. schachtii. Two independent lines per construct (A and B) were compared with corresponding transgenic empty vector (EV) line and wild type Col-0. Bars represent mean number of nematodes per plant ± standard error of the means. Different letters indicate statistically significant differences (P-values < 0.05). (B) Ectopic Hs-HVAP1 and Hs-HVAP2 enhancement development of disease symptoms of Arabidopsis in transgenic Arabidopsis. Symptoms on plants inoculated with B. cinerea, P. syringae, E. amylovora, and Phytophthora brassicae, or mock inoculated. (C) Ectopic Gr-VAP1 and Hs-VAP1 suppress feeding site development and expression of immune peptide fig22. Bars represent mean number ± standard error of the means. Different letters indicate statistically significant differences (P-values < 0.05).

4. Ectopic venom allergen-like proteins from cyst and root-knot nematodes selectively suppress defense-related programmed cell death.

Fig. 4. (A and B) Agroinfiltration assays in Nicotiana benthamiana showing the transient co-expression in the apoplast of receptor-like proteins (A) Cf-4 and (B) Cf-9 from tomato and their cognate effectors Avr4 and Avr9 from C. fulvum with venom allergen-like proteins from G. rostochiensis (Gr-VAP1), H. schachtii (Hs-HVAP1) and Hs-VAP2, and Meloidogyne incognita (Mi-VAP1). Co-expressions with the corresponding empty binary vector (EV) and green fluorescent protein (GFP) were included as controls. Photographs were taken 7 days post infiltration. (C and D) The bars represent the mean number of cells that have undergone apoptosis (P-value < 0.05). Different letters indicate a significant difference (P-value < 0.05).

5. A plant cell wall-associated subtilase and non-photochemical quenching in chloroplasts regulate immunity to plant-parasitic nematodes.

Fig. 5. (A) Global gene expression analyses as determined by RNA-seq in 2 weeks old Arabidopsis plants overexpressing Mi-VAP1 and Hs-Mi2P in the apoplast. The chart depict percentage products of genes significantly down-regulated by ectopic Hs-HVAP1 and Hs-2P in Arabidopsis, according to their significance in the Arabidopsis Information Resource (TAIR) database. (B) The lack of the VAP-regulated subtilisin-like serine protease Avr4 and the chlorophyll associated Photosystem II subunit S protein in homoygous Arabidopsis mutants (avr4-1 and npq1-1) results in increased necrotrophy of Arabidopsis, while no difference was observed in the H. schachtii. Bars represent mean number of nematodes per plant with standard error of mean. Different letters indicate statistically significant differences between homoygous knock out mutants and corresponding wild type Arabidopsis at 6 weeks after inoculation (P-values < 0.05).

DISCUSSION

- Plant-parasitic nematodes deliver venom allergen-like proteins into the apoplast of host cells to suppress basal immunity mediated by surface-localized immune receptors.
- VAMPs of plant-parasitic nematodes may specifically suppress the immune responses triggered by plant cell wall fragments released by the enzymatic breakdown of plant cell walls during nematode migration inside host plants.
- The modulation of basal immunity by venom allergen-like proteins in plants most likely involves at least two other classes of extracellular proteases i.e. cysteine proteases and subtilisin-like serine proteases.
- Most of the genes differentially regulated by the overexpression of venom allergen-like proteins in Arabidopsis are typically associated with innate immunity and non-photochemical quenching in chloroplasts.
- Ectopic Hs-VAP1 and Hs-VAP2 suppress innate immune responses in Arabidopsis, at least partly, through their regulation of PsbS which most likely reduces the formation of singlet oxygen under biotic stress.

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