A novel pore-forming toxin triggers apoptotic cell death *via* a C_2-domain containing protein in *Ditylenchus destructor*

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ABSTRACT

Plant parasitic nematodes (PPNs) cause enormous losses of agricultural crops, which up to about $157 billion globally. PPNs are difficult to prevent for they mainly live in the soil or plant roots with a protrusible stylet to withdraw nutrients from their host plant. *Bacillus thuringiensis* (Bt) produces a variety of crystal proteins, and some of which show high toxicity against PPNs. However, there is no report on the mechanism of nematicidal crystal protein on plant parasitic nematode. Here, the nematicidal crystal protein Cry55Aa and *D. destructor*, a plant parasitic nematode, were used to reveal the mechanism of nematicidal crystal protein against plant parasitic nematodes. It was found that Cry55Aa interacts with nematode target protein Dd_12442, a C_2 domain-containing putative protein, and this interaction activates apoptotic cell death.

RESULT 1 Cry55Aa exhibits high toxicity to PPNs by forming pores in the intestinal cells

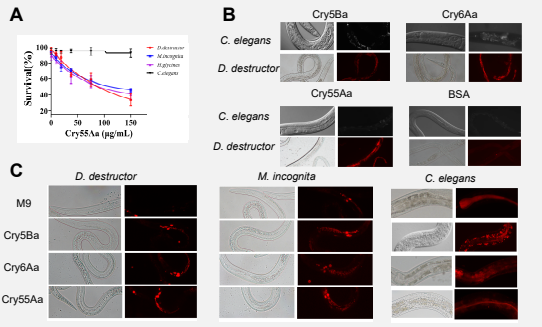


Fig.1 Cry55Aa induced PPNs death by forming pores in the intestinal cells(A)The dose response curves of Cry55Aa against *D. destructor*, *M. incognita*, *H. glycindes* J2 and *C. elegans* L4. (B)Detection of Rhodamin labeled Cry5Ba, App6Aa, Cry55Aa and BSA in nematode intestinal.(C) *In vivo* pore formation.

RESULT 2 Cry55Aa activates caspase-dependent apoptosis in *D. destructor*

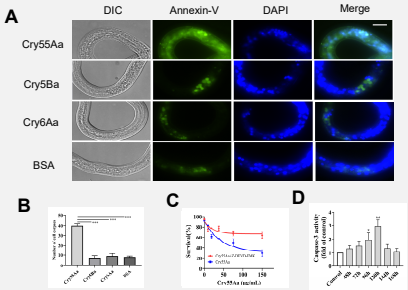


Fig.2 Cry55Aa activated apoptotic cell death in *D. destructor* (A)Toxin treated animals were stained by Annexin-V and DAPI.(B) Numbers of cell corpses per nematode were counted after toxin treated.(C) Dose-dependent mortality bioassay were performed using Cry55Aa and pan-caspase inhibitor Z-DEVD-FMK.(D) Cry55Aa induced the increase of caspase-3 activity in *D. destructor*

RESULT 3 Cry55Aa treatment disrupts the mitochondria in *D. destructor*, resulting in caspase-dependent death.

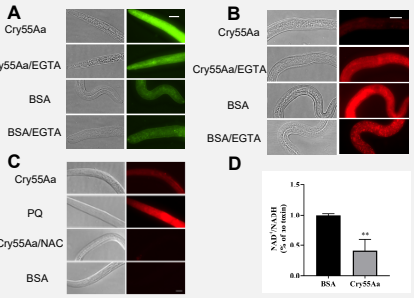


Fig.3 Cry55Aa caused mitochondrial disruption by increasing the calcium concentration in the cytoplasm. (A) Cry55Aa-induced an increase in cytoplasm calcium concentration. (B) Cry55Aa treated induce the decrease of mitochondrial membrane potential.(C) Cry55Aa-induced the level of ROS in *D. destructor*.(D) Cry55Aa inhibited the activity of mitochondrial complex I in *D. destructor*

RESULT 4Dd_12442 is required for Cry55Aa-mediated nematode death

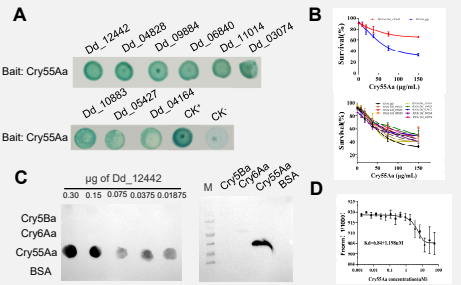


Fig.4 Dd 12442, a C_2-domain-containing protein, is essential for Cry55Aa-mediated worm death.(A) Cry55Aa interacts with preys. (B) Dose-dependent mortality bioassay of Cry55Aa against wild type and candidate genes knock-down mutant.(C) Cry55Aa interacts with Dd_12442 detected by Dot Blot and Ligand Blot.(D) Binding affinity of Cry55Aa to Dd_12442 was determined by MST.

RESULT 5 Dd_12442 is involved in Cry55Aa-mediated apoptotic cell death

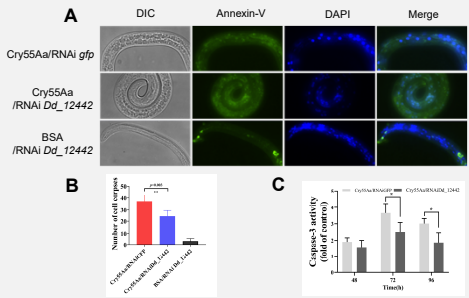
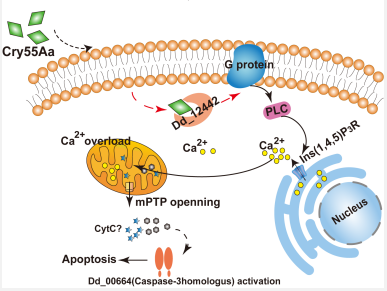


Fig.5 Dd_12442 is required for Cry55Aa-mediated apoptotic cell death(A)Toxin treated animals were stained by Annexin-V and DAPI,Cry55Aa/RNAi *gfp* (first line), Cry55Aa/RNAi *Dd_12442* (second line) and BSA/RNAi *Dd_12442* (third line). (B) Numbers of cell corpses per nematode were counted.(C) Caspase-3 activity were detected in RNAi *Dd_12442* and *gfp* animals.



The schematic diagram of Cry55Aa against *D. destructor*

CONCLUSION

Cry55Aa is a pore-forming toxin that could disrupt intestinal cells, and once inside, it interacts with Dd 12442. The interaction with Dd_12442 causes an increases in cytoplasmic Ca^{2+} concentrations. Then, elevated Ca^{2+} levels promote mitochondrial damage which activates apoptotic cell death in *D. destructor*.