Thaumatin-like protein as a virulence factor of the pine wood nematode, Bursaphelenchus xylophilus

Haru KIRINO¹, Kohki YOSHIMOTO¹, Ken-ichi KONAGAYA², Ryoji SHINYA¹ (¹Meiji University, Japan, ²FFPRI, Japan)

Symptoms of cell death

100(%)

ine wilt disease, which is caused by Bursaphelenchus xylophilus, is a serious threat to pine forests worldwide. Pine wilt disease is thought to be initiated by sequential excessive hypersensitive responses (e.g. inducing a series of plant cell death, high expression of pathogenesis-related (PR) genes) to B. xylophilus. However, no molecule has yet been

DISCUSSION

identified as a pathogenic molecule.

Two-step screening discovered plant thaumatin-like 2 of *B. xylophilus* as a strong candidate pathogenic protein.

(Kirino et al., 2020; 2022; Shinva et al., 2021)

This protein may have a role in molecular mimicry and induce hypersensitive responses

in host pines because thaumatin-like proteins are important PR proteins for plants usually.

(Kirino et al., 2020)

2nd screening using pine seed embryos is the novel functional analytic method

that uses recombinant pathogenic candidate molecules of *B. xylophilus* in host pines.

(Kirino et al., 2022)

This method has potential as a powerful tool screening pathogenic molecu

for screening pathogenic molecules of *B. xylophilus* in their native host.

(Kirino et al., 2022)

PURPOSE Narrowing the secreted proteins that induce cell death in model tobacco plants as pathogenic candidate proteins **MATERIALS & METHODS** Pathogenic candidate proteins Nematode protein secreted by B. xylophilus Construction of vectors Infiltration of the vectors into plant leaves to expressed nfection pathogenic candidate proteins . Observation of cell death in plants Model tobacco plants Agrobacterium tumefaciens N. benthamiana **RESULTS** Rates of cell death induced by the pathogenic candidate proteins GFP control plant thaumatin-like 1 plant thaumatin-like 2 plant cysteine protease inhibitor-like o-glycosyl hydrolase family 30 catheosin 1 Symptoms of no cell death cathepsin 2 glutathione s-transferase 1 glutathione s-transferase 2 cysteine protease 1 cysteine protease 2

■Cell death ■No cell death

(Fisher's exact test; p < 0.01 **, p < 0.05 *)

5 proteins induced significant cell death in model plants.

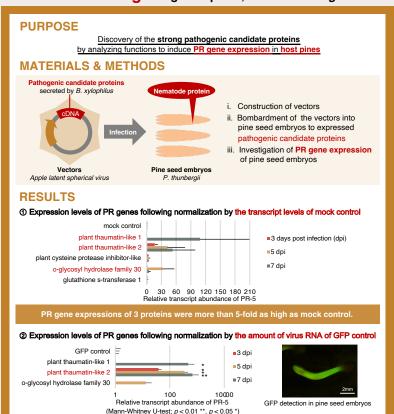
unknown protein 1

unknown protein 2

aspartic protease

1st screening using model plant, Nicotiana benthamiana

2nd screening using host pines, Pinus thunbergii



Plant thaumatin-like 2 induced high expression of PR genes at all time points.