RESEARCH HIGHLIGHT

Flooding plant apoplast through water and solute channels

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Inducing water-soaking symptoms is a key early step during the pathogenesis of many fungal, oomycete, and bacterial pathogens. A recent publication in *Nature* by Nomura et al. showed that the conserved AvrE-family type III effectors from several Gram-negative plant bacterial pathogens function as water and solute channels in the plant cell membrane, creating an aqueous nutrient-rich apoplastic space for the multiplication of these bacterial pathogens.

Plant fungal, oomycete, and bacterial pathogens, such as the rice blast fungal pathogen *Magnaporthe oryzae* and tomato and *Arabidopsis* bacterial speck disease pathogen *Pseudomonas syringae* pv. tomato, are known to induce water-soaking symptoms during their early stage of infection.[1] The scientific community has been aware for quite a long time that water in plant apoplast makes plants more vulnerable to pathogen attacks,[2] but the precise mechanism by which plant pathogens induce water-soaking symptoms remained enigmatic until fairly recently.

Plant fungal, oomycete, and bacterial pathogens share a common trait of relying on effectors that are delivered into plant cells to cause diseases.[3] Type III effectors, which are injected into plant cells by the type III secretion system, are required for the pathogenicity of Gram-negative plant bacterial pathogens. Recent studies have demonstrated that the type III effectors from plant bacterial pathogens function to produce an aqueous apoplast.[4,5] For example, HopM1 from *P. syringae* targets AtMIN7 for degradation, which probably disrupts host plasma membrane to create an aqueous apoplast.[5] Another type III effector AvrHah1 from *Xanthomonas gardneri* activates the expression of a pectate lyase gene to promote water-soaking symptoms.[6]

Even though the type III effector AvrE from *P. syringae* has also been shown to cause water-soaking symptoms, the underlying mechanism is not fully understood.[4,5] Due to AvrE's extremely large size, high toxicity to plant and yeast cells, and no functional domain or obvious homology to proteins with known functions, it was challenging for scientists to figure out the exact structure and biochemical function of AvrE.[4,7] Recently, a paper published in *Nature* by Nomura et al. provided compelling evidence that AvrE-family effectors fold into water and solute channels in the plant cell membrane, allowing the passage of water and nutrient into the apoplastic space.[7]

The conserved AvrE/DspE-family effectors, which comprise AvrE from *P. syringae*, DspA/E from *Erwinia amylovora*, WtsE from *Pantoea stewartii*, and DspE from *Pectobacterium carotovorum*, are major virulence factors in these bacterial pathogens.[7] To find clues on the tertiary structures of AvrE-family effectors, Nomura et al.[7] constructed their three-dimensional models based on AlphaFold2 predictions. Their predicted structures all resemble a mushroom, with a striking β-barrel structure in the middle forming the stem (Fig. 1a). Considering their similarities to bacterial porins, Nomura et al. reasoned that the β-barrel stem structure of AvrE-family effectors probably inserts into the plant cell membrane to form channels.

Nomura et al. noticed that after *dspE* or *avrE* cRNA was injected into *Xenopus* oocytes, these cells showed a swelling phenotype, suggesting that water is taken up by these oocytes.[7] Consistent with these observations, *Arabidopsis* protoplast expressing AvrE swelled to a greater extent compared to controls. These data strongly support that AvrE-family effectors function as aquaporin channels to increase water permeability (Fig. 1b, left). The diameter of the predicted AvrE/DspE channels was estimated to be 15–20 Å, much larger than the size of a water molecule or a single ion. Nomura et al. found that the 332 Da fluorescein dye with an estimated maximum molecular diameter of 7 Å entered oocytes expressing DspE or AvrE, while the 27 kDa eGFP with an estimated minimum diameter of 30 Å did not. This data is further confirmed by the release of fluorescein or eGFP from liposomes in the presence of the DspE effector.

The data presented by Nomura et al. not only solved a long-standing puzzle, but also pointed a new direction for plant disease control.[7] Since the AvrE-family effectors function as water- and solute-permeable channels to favor the pathogenicity of these pathogens, Nomura et al. speculated that chemical blockers of these channels could be developed for controlling plant diseases. To identify compounds that fit the pore size of these channels, the authors focused on a class of synthetic PAMAM dendrimers with programmable diameters. PAMAM G1 with a diameter of 22 Å was demonstrated to be a potent inhibitor by blocking AvrE-family channels in oocytes and dye release from DspE-dependent liposome (Fig. 1b, right). A key question is whether this PAMAM G1 can block AvrE-family effectors' virulence function in plants. The authors showed that PAMAM G1 adequately suppressed *P. syringae* pv. tomato DC3000 infection in an AvrE-dependent manner. In contrast, PAMAM G1 did not inhibit another type III effector HopM1-induced water-soaking symptoms. Strikingly, PAMAM G1 completely blocked the fire blight disease on pears caused by *E. amylovora*. These data indicate that PAMAM G1 can effectively combat plant pathogens that are dependent on an AvrE-family effector. Given that PAMAM G1 behaves differently from antibiotics and does not inhibit bacterial growth in vitro, it should not induce drug resistance of bacterial pathogens.

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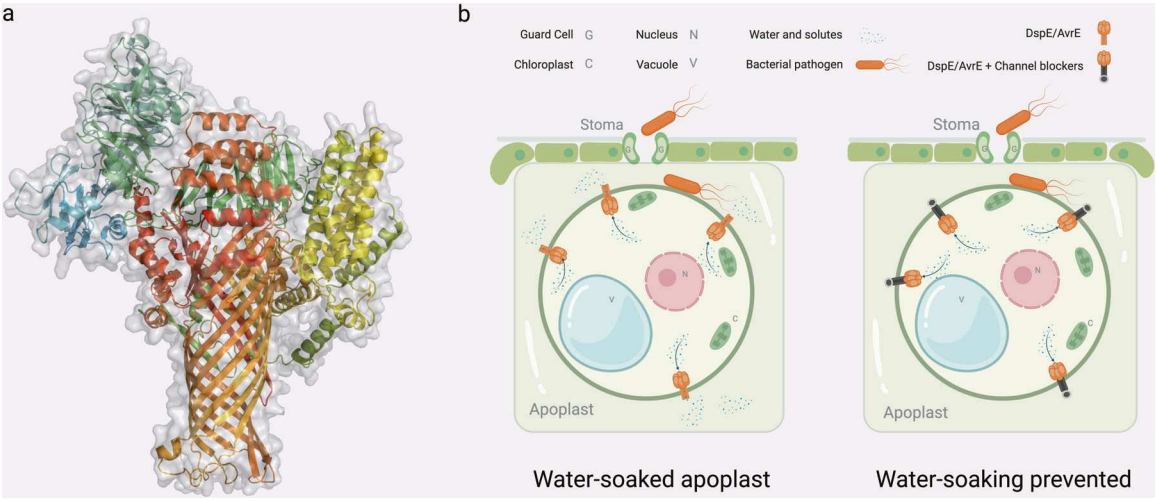

Fig. 1 The predicted stucture of DspE resembles a mushroom and AvrE-family type III effectors function as water- and solute-permeable channels in plant cell membrane to promote water-soaking symptoms. a Three-dimensional model of *E. amylovora* DspE (residues 298–1838) generated by AlphaFold2 using MMseq2 (ColdlabFold). The N-terminus and C-terminus are colored blue and red, respectively. Surface representation of DspE is shown on a transparent white background. The stem of the mushroom-like shape was demonstrated to serve as a water- and solute-permeable channel. **b** Schematic comparison between the functional and blocked water and solute channels formed by the DspE/AvrE-family effectors. Bacterial pathogens deliver the DspE/AvrE-family effector proteins into the plant cells and subsequently these effectors fold into water- and solute-permeable channels in the plant cell membrane (left), creating a water-soaked apoplast. In the presence of water and solute channel blockers, the water-soaking symptoms are prevented (right). Created with BioRender.com.

So far, the vast majority of the published papers on pathogen effectors almost exclusively focus on targeting host molecules such as proteins, RNAs, or DNAs to manipulate host resistance or susceptibility. The data presented by Nomura et al. demonstrated that AvrE-family effectors could directly function as water- and solute-permeable channels in eukaryotic cell membranes.[7] Further studies will be necessary to find out how the water and solute channel function of AvrE-family effectors facilitates the outflow of water and solutes from plant cells to apoplasts. It will also be important to connect water and solute channel function of AvrE-family effectors with their biological activities in suppressing plant defense responses and eliciting cell death. Water-soaking symptoms can also be induced by plant pathogens without AvrE-family effectors. Hence, it is worthwhile to investigate whether similar mechanisms have been developed by other pathogens using effectors outside the AvrE-family to cause diseases. Along this line, five RxLR proteins from plant oomycete pathogens were found to share putative structural similarities with AvrE.[8,9] Among them, HaRxL23, a conserved and validated RxLR effector, has been shown to have a similar function to AvrE and partially complement *avrE* loss-of-function mutants.

The exact function of AvrE-family effectors has remained obscure since it was reported more than two decades ago.[10] This breakthrough study greatly benefited from the Artificial Intelligence deep learning system AlphaFold2 and cryo-electron microscopy (cryo-EM).[7] In the foreseeable future, the biochemical and molecular functions of many other pathogen effectors could be uncovered with the help from AlphaFold2 and cryo-EM as functions of proteins are determined by their tertiary structures.

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ADDITIONAL INFORMATION

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