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A war on the cell wall

Plant diseases caused by a wide variety of pathogens have had tremendous negative impacts on human beings. For example, the oomycete pathogen *Phytophthora infestans*, which causes severe late blight disease on potatoes, triggered the infamous Irish Potato Famine. To prevent such tragedies from happening again and to reduce crop losses, it is imperative to implement effective disease control and management strategies. A deeper and more comprehensive understanding of how plant pathogens cause disease is critical for developing novel and effective strategies for controlling plant diseases.

CELL WALL: A KEY BATTLEGROUND BETWEEN PLANTS AND PATHOGENS

Throughout human history, defensive walls were frequently built around cities, making them impenetrable by enemies. A different kind of wall, the plant cell wall, represents the first protective barrier against invasive pathogens. The plant cell wall is mainly composed of cellulose, pectin, and hemicellulose. To penetrate the plant cell wall, plant pathogens secrete cell-wall-degrading enzymes (CWDEs), which could disrupt all three major components of the plant cell wall. Among these CWDEs, cellulases and beta-glycosidases participate in cellulose depolymerization; polygalacturonase and pectate lyase depolymerize the pectin backbone homogalacturonan; xylanase degrades the linear backbone of hemicellulose xylan to xylose (Kubicek et al., 2014). After the plant cell walls are disrupted, pathogens enter the plant cells and secrete effector proteins to suppress the host immune system. On the other side, plants rely on plasma-membrane-localized pattern recognition receptors to detect conserved molecules in plant pathogens called pathogen-associated molecular patterns (PAMPs) to activate PAMP-triggered immunity (Malinovsky et al., 2014). Cleavage products of the plant cell wall produced by pathogen CWDEs could be perceived by plants as damage-associated molecular patterns (DAMPs) to induce DAMP-triggered immunity (DTI).

A BRUTAL ATTACK BY LPMOs

A recent *Science* paper revealed novel functions of copper-bound lytic polysaccharide monooxygenases (LPMOs) from the potato late blight pathogen *Phytophthora infestans* in plant-cell-wall penetration (Sabbadin et al., 2021).

Through transcriptomic analysis, Sabbadin et al. found that the AA17 family of LPMOs are the most induced carbohydrate-active enzymes during the early infection of tomato plants by *P. infestans*. Among them, *PiAA17C* was the most upregulated gene, while *PiAA17A* and *PiAA17B* were also induced during infection (Sabbadin et al., 2021). Based on the peak masses from MALDI-TOF and electrospray ionization mass spectrometry analysis, the authors postulated that *Pi*AA17A, -B, and -C catalyze a C4-oxidative cleavage of polygalacturonic acid in the presence of ascorbic acid, producing a C4-ketone in a beta position. The resulting beta-keto acid is unstable and spontaneously decarboxylates (Figure 1). The C4-enol generated from the decarboxylation reaction undergoes tautomerization, producing a more stable ketone form (Figure 1). Furthermore, the authors found that *Pi*AA17C is not active on esterified pectin, but after preincubation with pectin methylesterase, the substrate can be degraded, indicating that *Pi*AA17C can recognize the exposed carboxylic groups after de-esterification (Sabbadin et al., 2021).

Is *PiAA17C* required for the successful infection of potato plants by *P. infestans*? To test this, *in vitro*-synthesized double-stranded RNA targeting *PiAA17C* was delivered into protoplasts of *P. infestans* followed by colony regeneration (Sabbadin et al., 2021). As expected, the authors found that the introduction of double-stranded RNA targeting *PiAA17C* reduced the virulence of *P. infestans* on potato leaves compared with control lines (Figure 1). The authors also confirmed this result using stable gene silencing. *P. infestans* lines that exhibited silencing of *PiAA17C* showed a significant reduction in lesion size on inoculated potato leaves compared with the wild-type strain (Figure 1) (Sabbadin et al., 2021).

WIDE DISTRIBUTION OF LPMOs IN PATHOGENS AND PESTS

LPMOs have been found in fungi, oomycetes, bacteria, and viruses (Jagadeeswaran et al., 2021). Sabbadin et al. revealed the essential roles of the AA17 family of LPMOs in host penetration (Sabbadin et al., 2021). In addition to AA17, other families of LPMOs, such as AA9 and AA16, have been identified in major plant fungal pathogens, including the rice blast pathogen *Magnaporthe oryzae,* the wheat head blight pathogen *Fusarium graminearum*, and the gray mold pathogen *Botrytis cinerea* (Jagadeeswaran et al., 2021). The AA16 family of LPMOs has been found in plant oomycete pathogens. Importantly, the LPMO CbpD from the human bacterial pathogen *Pseudomonas aeruginosa* has been shown to promote systemic infection (Askarian et al., 2021). Hence, it is highly likely that more LPMOs will be shown to be involved in infections by plant fungal, oomycete, bacterial, and perhaps viral pathogens in the near future. In addition to pathogens, the AA15 family of LPMOs has been found in sucking insects such as aphids and thrips and in chewing insects such as leafhoppers (Jagadeeswaran et al., 2021).

HIGS AS A CONTROL MEASURE

The authors demonstrated that RNA-based silencing approaches, including host-induced gene silencing (HIGS), could provide an effective strategy to control plant diseases

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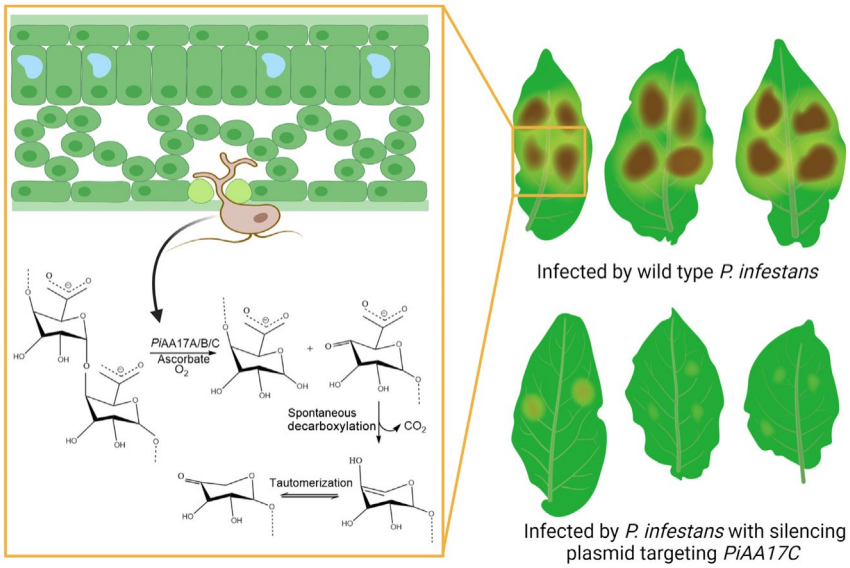

Figure 1. A schematic model illustrating the molecular mechanism by which LPMO degrades the plant cell wall to facilitate infection by Phytophthora infestans.

Once plant leaves are infected by the fungal-like oomycete pathogen *P. infestans*, *Pi*AA17A, -B, and -C from *P. infestans* oxidatively cleave polygalacturonic acid at the C4 position in the presence of ascorbic acid, leading to the formation of a C4-ketone in a beta position. The resulting beta-keto acid (the product on the right side) is not stable and undergoes spontaneous decarboxylation, leading to the formation of a C4-enol, which goes through tautomerization to produce a more stable ketone form. As a result, plant cell walls are degraded, causing a severe disease symptom. Potato leaves treated *P. infestans* with stably transformed silencing plasmid targeting *PiAA17C* showed a marked reduction in lesion size. The figure was created with the software BioRender (BioRender.com).

(Sabbadin et al., 2021). HIGS is RNAi-based technology in which small interfering RNAs are produced in the host plants and subsequently moved to pathogens or pests to silence pathogen or pest genes (Koch and Wassenegger, 2021). In this case, one plausible solution is to engineer small interfering RNAs into potato or tomato plants to silence *LPMO* genes in *P. infestans*, providing an effective tactic to control late-bight diseases on these plants. HIGS could be explored to target *LPMO* genes in other plant pathogens and pests as well.

PLANT DEFENSE MECHANISMS AGAINST LPMOs WARRANT EXTENSIVE INVESTIGATION

Despite the essential roles of LPMOs in plant pathogenesis and the wide distributions of LPMOs in pathogens and pests, plant defense mechanisms against these LPMOs have not been extensively investigated yet. Studies have shown that plants have already developed several defense mechanisms against other types of CWDEs from pathogens, which could give us some clues.

Scientists have identified different types of CWDE inhibitors, which mitigate the detrimental effects caused by these CWDEs from plant pathogens (Juge, 2006). As the best characterized CWDE inhibitors, polygalacturonase-inhibiting proteins belong to leucine-rich-repeat proteins, while both *Triticum aestivum* xylanase inhibitor (TAXI) and xyloglucan endoglucanase-inhibiting protein (XEGIP) show some structural similarity to pepsin-like aspartic proteases (Juge, 2006). One of these CWDE inhibitors, XIP-I, a wheat xylanase inhibitor protein, structurally resembles pathogenesis-related protein 8. Plants could also have developed inhibitors for different families of LPMOs. In fact, the polyphenol cinnamtannin B1 was identified as an inhibitor of several microbial LPMOs in the AA9 and AA10 families (Tokin et al., 2021).

During coevolution with pathogens, plants have acquired the ability to detect cleavage products of CWDEs to activate DTI. Cell-wall-associated kinases were reported to function as DAMP sensors to detect the oligogalacturonides produced through cleavage of unesterified pectic homogalacturonan by endopolygalacturonases (Malinovsky et al., 2014). Related to this, one recent study has already shown that oxidized and native cellulose- or cello-oligosaccharides, as cleavage products of the AA9 family of LPMOs, induce DTI responses, including reactive oxygen species production, callose deposition, and transcriptional reprogramming, in *Arabidopsis* plants against the necrotrophic fungal pathogen *B. cinerea* (Zarattini et al., 2021). Several leucine-rich-repeat receptor-like kinases are required for this defense response.

In addition, CWDEs themselves can be sensed by cell-wall-localized receptor-like proteins as apoplastic effectors to induce an effector-triggered defense (ETD) (Stotz et al., 2014; Wan et al., 2021). For instance, the apoplastic xyloglucan-specific endoglucanase PsXEG1 from *Phytophthora sojae* is detected by the receptor-like protein RXEG1 in *Nicotiana benthamiana* to activate an effector-triggered defense. In addition to cell-wall-localized pattern recognition receptors, a cell-wall-localized nucleotide-binding site–leucine-rich-repeat receptor-type resistance protein, Rsc4-3, has recently been shown to recognize *Soybean mosaic virus* cylindrical inclusion protein in the apoplast to induce a hypersensitive response, a hallmark of effector-triggered immunity (Yin et al., 2021). Last but not least, PAMP-triggered immunity and effector-triggered immunity have been demonstrated to be interconnected, and they promote each other to produce more robust defense responses through mutual potentiation (Ngou et al., 2021).

Clearly, more studies are needed to decipher plant resistance mechanisms against LPMOs, so we can develop knowledge-based strategies to help plants win this battle on the cell wall and minimize the damage to plants caused by LPMOs.

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