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License to not kill: How a biotrophic pathogen keeps the host alive

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Plants and microbes coexist in nature by establishing a variety of interactions ranging from the most beneficial, such as symbiotic (Kawaguchi and Minamisawa, 2010), where both the microbe and the plant show enhanced ability to thrive and survive, to the most destructive, such as pathogenic, where the microbe can cause infection or even plant death (Dodds and Rathjen, 2010).

The pathogenic interaction between plants and microbes resembles a battle whereby both sides use "ammunition" to attack the other. In the case of the plant, upon pathogen invasion, an array of responses, such as reactive oxygen species (ROS) production, occurs that restrict or prevent pathogen growth (Marcec et al., 2019). In the case of the pathogen, a group of small proteins, called effectors, are directed toward specific targets inside the host to manipulate host immune responses (Jones and Dangl, 2006). Therefore, effectors constitute a key part of the arsenal of the pathogen. Understanding effector functions and targets is crucial for protecting crops from pathogens.

In this issue of *Plant Physiology*, Wan et al. (2022) focus on one of the most destructive pathogens, *Puccinia striiformis*, which causes the stripe rust disease in wheat (*Triticum aestivum*). *Puccinia striiformis* (*Pst*) is a fungus that infects wheat spikes, stems, and leaves and currently represents a global threat to food security. Understanding how effectors operate inside the host plant cell and their targets is pivotal to design strategies to combat the disease.

In this study, the authors investigated the role of the effector Pst27791, which is serine-rich and contains a signal peptide sequence of 17 amino acids that allows the secretion of the effector into the host. The authors first tested whether the effector signal peptide was functional using a signal sequence trap assay in yeast (*Saccharomyces cerevisiae*) where the Pst27791 signal peptide was fused with invertase, a secreted enzyme involved in sucrose metabolism (Jacobs et al., 1997). The authors found that the invertase fused with the Pst27791 signal peptide was active and concluded that indeed this effector was secreted.

However, how this effector contributes toward infection once inside the host was unclear. *Puccinia striiformis* is a biotrophic pathogen that keeps the host alive during infection and therefore must suppress programmed cell death (PCD) of the host cell (Zheng et al., 2013). To test whether the Pst27791 effector interferes with PCD, the authors expressed Pst27791 in *Nicotiana benthamiana* leaves along with various other effectors and reporters. They showed Pst27791 can stop PCD when triggered by Pst322 effector (Wang et al., 2012). Pst27791 also suppresses the ROS burst and expression of defense genes in response to flg22, a trigger of pathogen-triggered immunity.

The authors next examined Pst27791 in wheat using overexpression and RNA interference (RNAi) in transgenic wheat lines. When Pst27791 was stably overexpressed, ROS accumulation and expression of the salicylic acid-related defense genes pathogenesis-related proteins 1 and 2 (TaPR1/2) was reduced; however, when the effector was silenced in RNAi lines, *P. striiformis* infection was compromised. These results suggest that Pst27791 plays an important role in pathogenicity by suppressing plant immunity, but how is this orchestrated at the molecular level?

To investigate this, the authors conducted a high-throughput yeast-two hybrid (Y2H) assay by using the effector as a bait against a cDNA library of *Pst*-infected wheat leaves (Yang et al., 2020) with the aim to identify targets from the host. They identified a Raf-like mitogen-activated protein kinase called TaRaf46 with unknown function. By using two approaches, a Y2H assay and constructs expressing TaRaf46 fragments of different domains, the authors determined that

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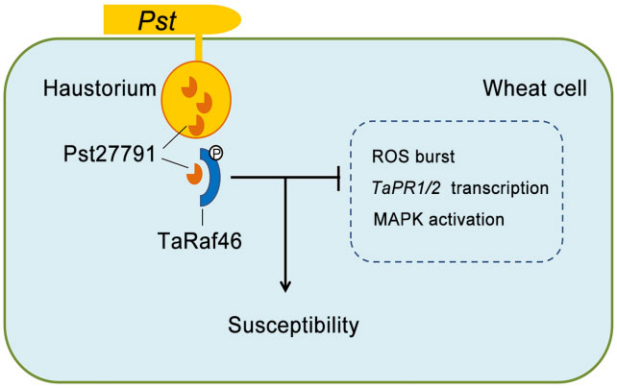

Figure 1 Operational mechanism of the effector Pst27791 during wheat infection. During infection, Pst27791 enters wheat host cells where it binds to TaRaf46. This interaction stabilizes TaRaf46, which suppresses plant immunity by affecting MAPK activation, expression of *TaPR1/2*, and ROS burst (Wan et al., 2022).

the interaction between TaRaf46 and Pst27791 occurred via the kinase domain and N-terminal region of TaRaf46 (Figure 1). Co-immunoprecipitation assays conducted in planta verified this interaction in vivo.

In general, the role of Raf-kinases is largely unknown (Colcombet and Hirt, 2008). In this specific example, although the authors determined that the Pst27791 effector interacts with TaRaf46, it was unclear why this interaction would be beneficial for the pathogen. The authors first silenced the three TaRaf46 copies in wheat to test whether infection was compromised in those lines. They showed that *TaRaf46*-silenced plants accumulated ROS and had increased expression of TaPR1/2, suggesting that plant immune responses were enhanced. When challenged with a virulent strain called CYR31, *TaRaf46*-silenced plants showed reduced infection compared to non-silenced plants, strongly indicating that TaRaf46 targeting by the pathogen is necessary for infection.

Next, the authors generated overexpressed lines of TaRaf46 and found that infection was enhanced. However, enhanced infection was not observed when they overexpressed the kinase-inactive form $TaRaf46^{K95M}$. These results suggested that kinase-active TaRaf26 functions to suppress immune responses. How does the pathogen take advantage of this at the molecular level? To answer that question, the authors first investigated whether TaRaf46 kinase activity was somehow modified by Pst27791. The in vitro kinase assay and a co-expression assay in *N. benthamiana* plants showed that the effector did not affect TaRaf46 kinase activity or that kinase activity was necessary for the interaction between TaRaf46 and Pst27791. The authors proceeded to test whether TaRaf46 accumulation was affected by Pst27791. When authors measured TaRaf46-GFP protein accumulation in the presence and absence of Pst27791 in co-infiltrated leaves of *N. benthamiana*, they found a significant increase of TaRaf46 in the presence of the effector, indicating that the Pst27791 effector stabilizes the kinase.

In conclusion, this study characterized one of the molecular components used by *P. striiformis* during wheat infection. The authors showed how a single *P. striiformis* effector can have an important positive contribution toward disease by targeting and stabilizing a defense-suppressing kinase. Although this study helps us understand a bit more about stripe rust disease, *P. striiformis* contains thousands of effectors. Therefore, the mechanisms by which other effectors operate and how *P. striiformis* orchestrates its ammunition to win the battle with the plant remain to be determined.

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