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News and Views

Parasite-resistant ketchup! Lignin-based resistance to parasitic plants in tomato

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Cuscuta (dodder) species are parasitic plants with a wide geographical distribution and host range (Masanga et al., 2021). Among their hosts are several agriculturally important crops, such as potato (*Solanum tuberosum*), alfalfa (*Medicago sativa*), coffee (*Coffea arabica*), tea (*Camelia sinensis*), mango (*Mangifera indica*), and tomato (*Solanum lycopersicum*). Cuscuta plants cannot photosynthesize on their own and are completely dependent on the host for nutrients. The parasite germinates above ground and senses the presence of a potential host via airborne volatiles (Runyon et al., 2006). The thin stem of Cuscuta grows as a vine toward the host, twisting around the host stem before attaching and penetrating it via specialized organs known as haustoria (Yoshida et al., 2016). These connections enter and connect with the host's vasculature to drain water and nutrients (Yoshida et al., 2016).

While the biology of Cuscuta is relatively well understood, there is still a lot to learn about the host plant's defense mechanisms underlying resistance to the parasite. One mechanism employed by plants to protect the vasculature from pathogen entry is the deposition of lignin into its cell walls, which has been shown in response to (e.g.) the root-parasitic plant *Striga hermonthica* (Mutuku et al., 2019). Lignin is a phenolic polymer mainly deposited in secondary cell walls to provide mechanical strength for plant stability and hydrophobicity to water-conducting cells, enhancing water transport efficiency. Lignin deposition is also triggered by biotic and abiotic stresses. Lignin's physico-chemical properties make the wall more difficult to penetrate for pathogens (Mutuku et al., 2019).

Cultivated tomatoes are usually susceptible to *Cuscuta campestris*, but resistant Heinz tomato cultivars have been described (Hembree et al., 1999; Goldwasser et al., 2001). Although these genotypes have been used in the field to control *Cuscuta* spp. infestation, their resistance mechanism remains elusive. In this issue of *Plant Physiology*, Jhu et al. (2022) conduct a thorough comparative study between Cuscuta-resistant and susceptible Heinz tomato cultivars to identify the underlying mechanism and genes responsible for the acquired resistance. The authors worked with four different tomato cultivars, H1706 and H9775 (susceptible to Cuscuta infection) and H9492 and H9553 (Cuscuta-resistant). Because lignification is a major mechanism for resistance against vascular pathogens, the authors first analyzed *C. campestris* attachments on both susceptible and resistant tomatoes using lignin-specific staining in stem cross-sections. Indeed, lignin accumulation in cortex cells was only observed for the resistant genotypes (Figure 1A, B), suggesting that the resistance mechanism involves local lignification to create a physical barrier against the pathogen haustorium.

To identify genes involved in this enhanced barrier formation, the authors performed RNA-seq on resistant and susceptible plants upon infection. Among the 113 differentially expressed genes, the authors identified genes involved in lignin metabolism, such as *LACCASES 4*, *5*, and *17* and *CAFFEOYL-COA 3-O METHYLTRANSFERASE*, several transcription factors (TFs) of unknown function, as well as an N-terminal coiled-coil nucleotide-binding leucine-rich repeat protein (CC-NB-LRR). They focused their subsequent work on the CC-NB-LRR protein, since these proteins typically function as intracellular receptors for avirulence effectors from pathogens, and on the two TFs, an AP2-related protein and MYB55, to characterize upstream regulators of the lignin-based resistance. Because the Heinz tomato cultivars are recalcitrant to stable transformation, functional analysis

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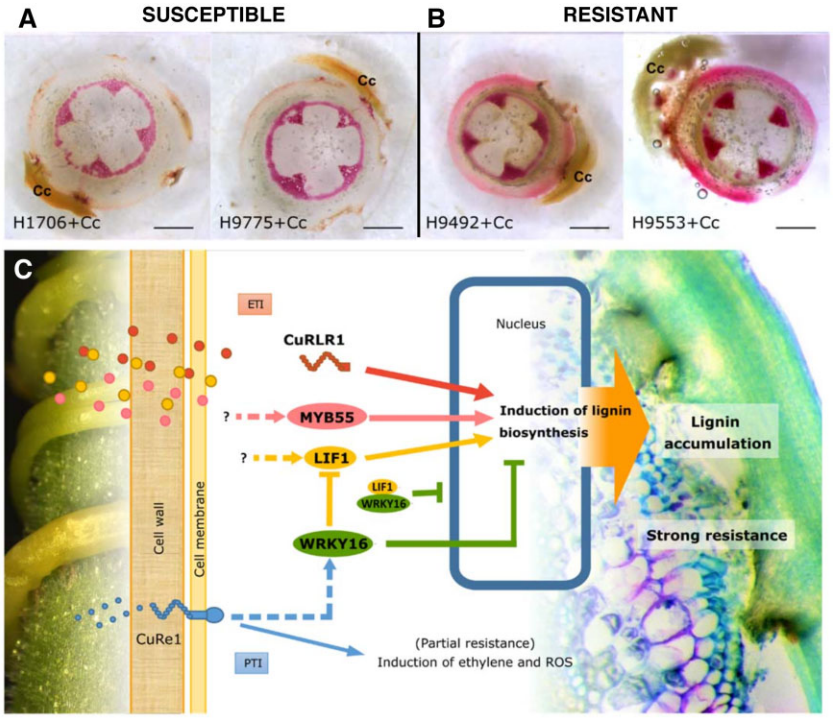

Figure 1 Resistance of Heinz tomatoes against Cuscuta relies on a lignin-based response. A and B, Lignin-specific staining of stem cross-sections of (A) susceptible (H1706, H9775) and (B) resistant (H9492, H9553) cultivars with *C. campestris* attached (+ Cc). Additional lignin depositions are visible in the resistant cultivars as pink staining. C, Model of *C. campestris* resistance response in Heinz tomato cultivars. Pathway in red: cytosolic CuRLR1 signaling pathway. Pathways in pink and yellow: MYB55 and LIF1 as positive regulators of lignin deposition. Pathways in yellow and green: lignin-based-resistant responses mediated by WRKY16 and LIF1, with a potential connection to CuRe1. Pathway in blue: PAMP/MAMP-triggered immunity mediated by CuRe1. This figure was assembled using Figures 1 and 7 from Jhu et al. (2022).

for the selected genes was performed using virus-based gene expression (VGE) and virus-induced gene silencing (VIGS).

Transiently overexpressing the two TFs in the susceptible tomato cultivars resulted in additional lignin deposition in the cortex, similar to the phenotype observed in infected resistant cultivars, and enhanced resistance to infection by Cuscuta. The authors hence named the AP2-related TF "LIGNIN INDUCTION FACTOR 1" (LIF1) (Figure 1C). Expression of the CC-NB-LRR gene in susceptible cultivars initially did not show any effects but resulted in excessive lignin deposition and enhanced resistance once the plant was challenged with the parasite. The authors therefore named this gene *Cuscuta R-GENE FOR LIGNIN-BASED RESISTANCE 1* (*CuRLR1*) (Figure 1C). Importantly, Cuscuta plants growing on VIGS knockdown tomato plants for all three genes had higher survival rates when compared with mock control plants. These results demonstrated that the chosen candidate genes are indeed involved in the observed lignin-based resistance of the tomato cultivars.

Next, the authors focused on a single-nucleotide polymorphism (SNP) analysis of the tomato cultivars to evaluate whether resistance-specific SNPs contribute to the regulation or function of their candidate genes. Since the resistant cultivars H9492 and H9553, as well as the susceptible cultivar H9775, are from the same breeding program and therefore have the same genetic background, SNPs shared between H9492 and H9553 but different from H9775 could account for the observed differences in parasite susceptibility. While the authors did not find any obvious differences in the coding region of their candidate genes, they found a SNP in the promoter region of *LIF1* that could impact binding of WRKY-type TFs. Hence, the authors reanalyzed their RNA-seq data and identified *WRKY16* as a potential candidate involved in the transcriptional regulation of *LIF1*. Knocking out *WRKY16* in a related and transformable tomato cultivar resulted in continuous production of cortical lignin and, consequently, in improved Cuscuta resistance, indicating that WRKY16 may indeed be involved in this resistance pathway, albeit as a negative regulator. Furthermore, transient overexpression of both *MYB55* and *LIF1* in the *wrky16* mutant resulted in more lignification, depicting additive effects of these genes in lignin-related responses. Conversely, transient overexpression of *CuRLR1* in *wrky16* plants no longer resulted in additional lignin-deposition and resistance in response to Cuscuta infection, making RLR1 epistatic to WRKY16 (Figure 1C).

On a protein level, the authors showed that WRKY16 interacted with LIF1, with this interaction resulting in relocation of the proteins from the nucleus to the cytoplasm, potentially preventing the LIF1 TF from activating its target defense genes. Accordingly, WRKY16 may be a negative regulator of this lignin-based immune pathway

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that functions upstream of RLR1, LIF1, and MYB55. As such, WRKY16 could either prevent an autoimmune response in the absence of the parasite or serve to dampen the immune response following successful activation to prevent an over-activation and potential developmental penalties.

Finally, the authors used different Cuscuta extracts to test for any effector proteins that may induce lignification and could act as a substrate for the CC-NB-LRR receptor RLR1. They found that proteins with a size between 30 and 100 kDa are responsible for triggering this lignin-based defense reaction and are hence a different signal than the 11-kDa PAMP molecule that is sensed by the known Cuscuta RECEPTOR 1 (CuRe1) (Hegenauer et al., 2020).

In summary, the work presented here showed that resistance to Cuscuta infection in these Heinz tomato cultivars relies on a lignin-based response. The authors proposed a multilayered model for Cuscuta resistance response in tomato (Figure 1C). CuRLR1 is a cytosolic factor that may receive signaling molecules from the pathogen or play a role in signal transduction upon pathogen perception, triggering downstream signals that ultimately induce lignification as a resistance response. MYB55 and LIF1 function as positive regulators of lignin deposition, whereas WRKY16 is a negative regulator of the same pathway. WRKY16 and LIF1 physically interact to mediate lignin-based-resistant responses, potentially involving CuRe1, which in turn mediates PAMP/MAMP-triggered immunity. Finally, in addition to contributing insights into plant–pathogen interactions, this work provides potential implications for enhancing crop resistance to parasitic plants. For example, by introducing the CuRLR1 protein into crops, resistance to *C. campestris* could be provided without triggering ectopic lignification, which is often associated with stunted growth and, consequently, yield loss.

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