Title: Lignin-based resistance to *Cuscuta campestris* in tomato

Authors: Min-Yao Jhu[1*], Moran Farhi[1,2*], Li Wang[1,3], Richard N. Philbrook[1], Michael S. Belcher[4,5], Hokuto Nakayama[1], Kristina S. Zumstein[1], Steven D. Rowland[1], Mily Ron[1], Patrick M. Shih[1,4,6,7], Neelima R. Sinha[1@].

Affiliations:

[1] The Department of Plant Biology, University of California, Davis, CA, 95616, United States.

[2] Research, Archer Daniels Midland Company, 1554 Drew Avenue, Davis, California, 95618 USA.

[3] College of Forestry, Beijing Forestry University, Beijing, 100083, PR China.

[4] Feedstocks Division, Joint BioEnergy Institute, Emeryville, CA, United States.

[5] Department of Plant and Microbial Biology, University of California, Berkeley, Berkeley, CA, United States.

[6] Genome Center, University of California, Davis, Davis, CA, United States.

[7] Environmental Genomics and Systems Biology Division, Lawrence Berkeley National.

*These authors contributed equally to the work.

[@]Correspondence to: nrsinha@ucdavis.edu.

Abstract: *Cuscuta* species (dodders) are common agriculturally destructive parasitic angiosperms. However, some tomato cultivars exhibit resistance to dodders. The stem cortex in these lines responds with local lignification upon *C. campestris* attachment, preventing parasite penetration into host. We compared gene expression patterns under *C. campestris* infestation in resistant and

susceptible cultivars and identified *LIF1* (Lignin Induction Factor 1, an *AP2*-like transcription factor), *MYB55*, and *CuRLR1* (*Cuscuta Receptor for Lignin-based Resistance 1*, a *CC-NBS-LRR*) as key factors conferring host resistance. Transient overexpression results suggest that *MYB55* and *LIF1* directly regulate cortical lignification. Moreover, *CuRLR1* functions as a receptor for receiving *C. campestris* signals to regulate lignification-based resistance. We also identified *WRKY16* as a negative regulator of *LIF1* function. These results will aid in developing parasitic plant-resistant crops.

One Sentence Summary: Lignin-based resistance to *Cuscuta* in tomato.

Main Text: Parasitic plants directly attach to hosts using specialized organs known as haustoria. These connections function as physiological bridges to extract nutrients and water from the hosts, making traditional control methods ineffective. Therefore, parasitic angiosperms are among the most devastating pests, reducing the yields of agricultural crops each year by billions of dollars worldwide (*1*, *2*). Members of the *Cuscuta* genus (family Convolvulaceae), also known as dodders, occur worldwide and *Cuscuta* infestations in tomato alone lead to 50–72% yield-reductions (*3*). Despite serious agricultural problems caused by *Cuscuta*, our understanding of the interactions between *Cuscuta* and its hosts is very limited compared to our knowledge of pathogenic fungi, bacteria, and viruses. Only recently, the first receptor (*CuRe1*) for a *Cuscuta* associated molecular pattern and its ligand from *Cuscuta* were identified in tomatoes (*4*). *CuRe1* initiates PAMP (Pathogen-associated molecular pattern)-triggered immunity (PTI) to *Cuscuta reflexa*. However, plants that lack *CuRe1* are still fully resistant to *C. reflexa*; thus, other layers of defense mechanisms, besides *CuRe1*, must also be involved in the responses to these parasites.

Is response to pathogen infection and herbivore feeding, plants often modify their cell walls (*5*). Among different modifications, lignification has been considered a major mechanism for

resistance in plants (*5-8*). Lignified cell walls have higher mechanical strength and are impermeable to water, making them less accessible to cell wall-degrading enzymes (*9, 10*). Thus, cell wall modifications could provide additional layers of resistance to *Cuscuta*.

Cuscuta campestris (*C. campestris*) attacks a wide range of crop species worldwide (*11*). Although tomatoes are usually susceptible (*12*), a few cultivars are resistant to *Cuscuta* (*3, 13*). Hence, these dodder-resistant tomatoes were used to identify genes involved in defense responses. We discovered that the resistance response in these cultivars is based on lignification in the stem cortex upon *C. campestris* infection. Recent work has described the involvement of lignin in the resistance responses to root parasitic plants (*14*), but the regulatory mechanisms remain unknown. Based on comparative transcriptomics and gain-of-function studies in susceptible cultivars, we identified two transcription factors, *MYB55* and *LIF1* (*Lignin induction Factor 1*, an *AP2-like* protein), that regulate biosynthesis of lignin in the cortex. Moreover, *CuRLR1* (a *CC-NBS-LRR*) functions as a receptor for *C. campestris*, leading to lignification-based resistance. Overexpression of *CuRLR1* in susceptible tomato only induced strong lignification upon *C. campestris* attachment or *C. campestris* extract injection. Results of this study may help develop a parasite-resistant system in crops to reduce economic losses.

Response to *C. campestris* in the resistant cultivars

While most tomato cultivars can be parasitized by *C. campestris*, the Heinz hybrid cultivars 9492 and 9553 (H9492 and H9553) exhibit resistance to dodders (*3*). *C. campestris* strands grew well on two susceptible cultivars, H1706 (genome sequenced) and H9775 (Heinz hybrid 9775 – reportedly related to the resistant cultivars) (Fig. 1A). On the other hand, *C. campestris* strands could not form good attachments with H9492 and H9553, and haustoria detached from the host stem, preventing parasite growth (Fig. 1B). Based on biomass measurements, H9492 and H9553

cannot support long-term (over 45 days) growth of *C. campestris*, in contrast to H9775 and H1706 (Fig. 1C).

To identify the basis for resistance, we analyzed dodder attachment on the susceptible and resistant lines using histology and cell wall-specific staining with both Toluidine Blue O (*15*) and Phloroglucinol-HCl (*16*). Upon challenging these different cultivars with *C. campestris* strands, lignin accumulation in the stem cortex was observed in the resistant cultivars H9492 and H9553, but not in the susceptible cultivars H9775 and H1706 (Fig. 1D – 1L). The resistance mechanism involved local lignification in the stem cortex, creating a barrier to haustorium penetration, and dodder attachment on the resistant cultivars (Fig. 1D – 1G). Little to no lignin accumulates in the cortex of both resistant and susceptible cultivars without *Cuscuta* attachment (Fig. 1L). In addition, *Cuscuta* attachment sites usually cause some wounding responses and programmed cell death in both resistant and susceptible cultivars (Fig. 1M).

Identifying the key time point in host-parasite interactions

Changes in the levels of Salicylic Acid and Jasmonic Acid have been reported at 36 to 48 hours after attachment (*17*). To capture the earliest responses to dodder parasitism, we performed a time-course RNA-Seq analysis on 0, 1, 2, 3, and 4 DPA (days post attachment) of *C. campestris* on tomatoes H1706 (susceptible). At these stages, the dodder strands were not embedded in the host and could be removed to collect the attached stem area. Maximal transcriptional changes peaked at 4 DPA (Fig. S1, Table S1), suggesting that the Differentially Expressed (DE) genes include core genes involved in the early response to *C. campestris* infection. Accordingly, we chose 4 DPA for further gene expression analysis of resistant and susceptible cultivars.

Gene expression in resistant and susceptible host response to *C. campestris*

We challenged the resistant H9492 and H9553, and susceptible H9775 and H1706 cultivars with *C. campestris* strands. We collected stem tissues at 4 DPA for RNA-seq and differential gene analysis in dodder infested versus uninfested plants. In principal component analysis (PCA) on the transcriptomes of resistant and susceptible cultivars (Fig. S2) PC1 accounted for 57% of the variation and significantly clustered the data into two separate sets: infested and non-infested samples. However, PCA did not separate different cultivars into distinct genotypic groups. Thus the transcriptional differences in response to *C. campestris* likely involve a small number of genes.

Next, we conducted differential gene expression (DGE) analyses by comparing *C. campestris* infested and uninfested host plants using an interaction design model (design model = infested or uninfested condition + genotype + condition: genotype) (Data S2). Based on our communication with the Kraft Heinz Company, both H9492 and H9553 were developed in the same breeding program. However, H9553 is more resistant to *C. campestris* than H9492 (Fig. 1C). We suspected that enhanced resistance to *C. campestris* is due to the alterations in key regulatory genes, and focused on the 94 differentially expressed genes (Table S2) that have different expression patterns in the H9553 resistant cultivar under dodder infestation compared to the susceptible cultivars, H1706 and H9775. Consistent with our observations of lignin accumulation in resistant tomato cultivars upon *C. campestris* infestation (Fig. 1), many of these genes are known to be involved in the lignin biosynthetic pathway, including Laccase genes and Caffeoyl CoA 3-O-methyltransferase (CCOAOMT) (Fig. S3). To narrow down the potential candidates regulating resistance, we focused on transcription factors (TF) as possible key regulators of lignin biosynthesis pathways, and membrane located or cytosolic receptors which may receive signals from *C. campestris*. Using these criteria, we selected three candidate genes for further study,

including a TF related to *AP2*, a *MYB55* TF, and a gene encoding an N-terminal coiled-coil nucleotide-binding site leucine-rich repeat receptor (*CC-NBS-LRR*) (Fig. S4).

Transient overexpression of candidate genes using Virus-based Gene Expression (VGE)

To evaluate the role of the candidate genes in lignification-based resistance in tomato, we cloned *GUS*, *AP2*, *MYB55* and *CC-NBS-LRR* genes into Virus-based Gene Expression (VGE) vectors (map in Fig. S5; sequence in Data S3) for transient overexpression in the susceptible H1706. We saw significant GUS expression in the stem around the injection site (Fig. S6A – C), and lack of lignification due to the process of injection itself (Fig. 2A and D). Therefore, we used *GUS*-injected plants as our mock controls for VGE experiments. We sectioned and stained injected stems with lignin-specific Phloroglucinol-HCl for lignin detection. VGE with *MYB55* and *AP2-like* successfully overexpressed *MYB55* and *AP2-like* in the first internode near the injection site and induced stem lignification in the susceptible H1706 (Fig. 2B – 2C and 2G). We named this *AP2-like* protein *LIF1* (*Lignin Induction Factor* 1) based on the ability of *LIF1* (and *MYB55*) to induce lignin biosynthesis in the cortex.

In contrast, the H1706 plants with VGE of *CC-NBS-LRR* were very similar to those with GUS VGE (Fig. 2E). Previous studies indicated that many genes in the *NBS-LRR* family encode intracellular receptors that detect pathogens and trigger defense signaling (*18*). We suspected that this *CC-NBS-LRR* might also function as a receptor for signals from *Cuscuta* that are needed to initiate subsequent defense responses, such as lignin accumulation in the resistant cultivars. Hence, we compared the response differences between *Cuscusta* infested and uninfested susceptible H1706 with *CC-NBS-LRR* VGE (Fig. 2E – 2F and 2H). Our results showed the overexpression of *CC-NBS-LRR* only induced lignification upon *C. campestris* attachment (Fig. 2H), and suggest that perception of *C. campestris* signals by the *CC-NBS-LRR* receptor leads to lignification-based

resistance. Thus, we named this receptor *CuRLR1* (*Cuscuta Receptor for Lignin-based Resistance 1*). Furthermore, VGE of these selected genes induced lignin accumulation in the cortex and made H1706 more resistant to *C. campestris* (Fig. 2I – 2L).

Lignin is a complex polymer and phloroglucinol-HCl staining is a fast and efficient lignin detection method but it only detects the cinnamaldehyde end groups of lignin, preferentially staining the G and S-type aldehyde form monolignols (*19, 20*). Therefore, we also conducted an acetyl bromide assay to determine total lignin content. Based on our results, the overexpression of *MYB55* and *LIF1* both increased total lignin content. Surprisingly, the overexpression of *CuRLR1* slightly increased the total lignin content even without *Cuscuta* signals. With *Cuscuta* signals, the total lignin content was much higher in *CuRLR1* overexpressing plants (Fig. 2M). We used HPLC to show that p-coumarate and trans-ferulate are both increased in *CuRLR1* overexpressed plants, but the samples with *Cuscuta* signals have much higher levels of these two precursors than the samples without *Cuscuta* signals (Fig. S7). PYRO-GC analysis showed that samples from *CuRLR1* overexpressing plants without *Cuscuta* signals have the largest percentage of H-lignin and the largest concentration of coumarate derivatives (Fig. 2N). H-lignin has been correlated with both stress response as well as defense from pathogen intrusion because this is a form of "defense" lignin that can be generated and deposited more rapidly than G or S lignin (*5, 21, 22*). Our results show that *CuRLR1* overexpression alone leads to an increase in the upstream steps of the lignin biosynthesis pathway producing H-type monolignols, while *Cuscuta* signals may actually up-regulate the final biosynthesis pathways leading to G-type and S-type monolignols (Fig. 2M and 2N). Since H-lignin and coumarate are not incorporated into lignin as aldehydes they are not detected by phloroglucinol staining.

Regulatory mechanisms and networks leading to resistance responses

Since both H9492 and H9553 cultivars arose in the same breeding program, enhanced resistance to dodders observed in these two cultivars is likely due to the presence of some unique sequence polymorphisms in these cultivars. The resistance-specific nucleotide polymorphisms could contribute to the regulation of our candidate genes, so we specifically identified SNPs (single nucleotide polymorphisms) that are common in H9553 and H9492 but different from H9775 (Table S3). We also specifically focus on these resistance-specific SNPs in the promoter regions of our candidate genes (Table S4). Our SNP analysis detected resistance specific SNPs only the *LIF1* promoter region, and one of these SNPs is located at a WRKY binding site (Fig. S8). This SNP could interrupt WRKY binding affinity, leading to *LIF1* expression differences between resistant and susceptible cultivars upon *C. campestris* attachment. Surprisingly, we noticed that WRKY16 was highly upregulated at 4 DPA in all four Heinz cultivars (Fig. S9). Host tissues surrounding haustoria from the tomato M82 cultivar also show upregulated expression of WRKY16 at 4DPA (Fig. S9). Thus WRKY16 is a commonly upregulated host response gene across different cultivars and may play an important role in transduction of *C. campestris* signals upon host attachment.

Based on our hypothesis, the genes that are differentially expressed upon *C. campestris* attachment in both resistant cultivars may be regulated by the three candidate genes that we selected. Therefore, in order to understand the relationships between the candidate genes and their targets, we conducted DGE analysis with ANOVA and selected 9776 DEGs with FDR less than 0.1 (Table S6). Next, we used Barnes-Hut t-distributed stochastic neighbor embedding (BH-SNE) to generate gene clusters based on gene expression patterns (Data S4) (*23*). Among the 85 gene clusters generated (Table S7), four clusters were selected based on their GO (Gene Ontology) enrichment terms (Fig. S10 and Table S7). We focused on genes included in these four clusters,

and our candidate genes, to construct gene co-expression networks (GCNs) for different treatments and cultivars to identify central hub genes (Data S5). Surprisingly, *CuRLR1*, *CuRe1* and *WRKY16* had few connections or almost no connection with other genes in the GCN in both susceptible and resistant cultivars without *Cuscuta* attachments (Fig. 3A-D). However, *CuRe1* and *WRKY16* became central hub genes in resistant cultivars upon *C. campestris* attachments and connected with *CuRLR1* (Fig. 3D). Therefore, we propose that all tomato cultivars have *Cuscuta* receptors, like CuRe1 (*4*) and CuRLR1, and WRKY16 is a key factor in the transduction of *C. campestris* signals upon attachment of the parasite to the host.

Functional characterization of *WRKY16* by CRISPR/Cas9 knockouts and VGE

To validate the function of WRKY16 and its role in lignification-based resistance, we produced stable *WRKY16* edited M82 lines using the CRISPR/Cas9 targeted gene knockout system (*24*). Our homozygous null mutants were generally smaller than M82 wild type (Fig. 3E and 3F) even though *wrky16* and M82 wild type are at the same developmental stage (Fig. 3K). However, *wrky16* plants are more resistant to *C. campestris* than M82 wild type (Fig. 3E – 3J). Homozygous *wrky16* lines continuously produce cortical lignin and have stronger resistance to *C. campestris* attachment (Fig. 3I – 3J and 3L).

To evaluate the interaction between WRKY16 and the other three candidate genes, we transiently overexpressed *LIF1*, *MYB55*, *CuRLR1*, and *GUS* controls in the susceptible M82 wild type and *wrky16* tomatoes (M82 background). All stem samples were sectioned and stained with Phloroglucinol-HCl. *MYB55* and *LIF1* successfully induced stem lignification in both susceptible M82 and *wrky16* plants (Fig. S11). Thus *WRKY16* does not influence *LIF1* and *MYB55*-induced lignification responses, and acts either upstream of *LIF1* and *MYB*55, or in another pathway that is independent of the *LIF1* and *MYB55* signaling pathway.

On the other hand, overexpression of *CuRLR1* with *C. campestris* infection was able to induce lignification in M82 and also enhance the lignification in *wrky16* tomatoes (Fig. S12). While *wrky16* lines produced more lignin and had stronger resistance responses upon *C. campestris* attachments compared to wild type, injecting with the *CuRLR1* expression vector induced an even stronger resistance response and even more lignin accumulation (Fig. S12), suggesting that the CuRe1-WRKY16 pathway and the *CuRLR1* downstream pathway may be independent, with these two mechanisms having additive effects.

Sub-cellular localization and interactions between the candidate genes

One described mechanism for triggering innate immunity following TMV infection in tobacco involved interaction and subsequent nuclear localization of the SPL6 TF with the TIR-NBS-LRR receptor (*18, 25*). Therefore, we investigated the potential interactions between our candidate genes and their sub-cellular localization to uncover potential regulatory mechanisms. Based on our results using translational GFP fusions, *LIF1* and *WRKY16* are located mainly in the nucleus (Fig. S13), while *CuRLR1* is located in both the nucleus and the cytosol. Split-YFP experiments using transient infiltration in *N. benthamiana* leaves show that the LIF1 and WRKY16 proteins interact and get localized to the cytoplasm (Fig. S13). Interactions between other combinations, CuRLR1-LIF1, CuRLR1-WRKY16, or CuRLR1-CuRe1, were not detected in our experiments.

Analysis of the *Cuscuta* signal using *Cuscuta* extract injections

In order to further discern the nature of the major signals that trigger lignification-based resistance, we injected the first internode of the resistant H9553 with *Cuscuta* extracts subjected to different treatments (Fig. 4). Untreated or filtered *Cuscuta* extract injections induced the accumulation of lignin in the cortex region (Fig. 4B-C). On the other hand, alteration of *Cuscuta*

extract pH from 5.8 to 9 abolished lignin accumulation (Fig. 4D-E), suggesting either instability or sequestration of the *Cuscuta* signaling molecules in alkaline conditions. In addition, heat-treated extract and proteases-treated extract could not trigger the lignification response (Fig. 4F-J). Furthermore, filtration of extracts through devices with different molecular weight cutoffs indicates that fractions smaller than 30KD cannot trigger strong lignification response (Fig. S14). Thus, the active *Cuscuta* signal for induction of lignin-based resistance is larger than 30KD but smaller than 100KD, and distinct from the previously identified *Cuscuta* signal that binds *CuRe1* (*4*).

Discussion

Cuscuta spp. cause massive loss in infested tomato fields in the United States. Our study reveals the underlying molecular genetic mechanisms for lignin-based resistance responses in resistant tomato cultivars. Lignin is a complex phenolic polymer, which is generated from three major monolignols, paracoumaryl alcohol, coniferyl alcohol, and sinapyl alcohol, using covalent crosslinks formed via free radical polymerization (*26*). Accumulation of lignin in plant stems or roots has been shown to reinforce plant resistance to invading herbivores, parasites and pathogens (*27-33*). Lignification at the host-parasite interface in roots has been reported in plants that are resistant to root parasitic plants (*34-37*). Tomato plants have incompatible reactions to the stem parasitic *Cuscuta reflexa,* and have lignified and suberized cell walls at infection sites (*38*). In contrast, tomato is susceptible to *C. campestris*. We identified a strong lignin based resistance response toward *C. campestris* attack in certain tomato cultivars.

Three key genes, *LIF1*, *MYB55*, and *CURLR1* regulate lignin accumulation in the cortex. Of these, *CuRLR1* responded to *Cuscuta* signals and further reinforced lignin deposition in the resistant cultivars. *CuRe1*, an LRR receptor-like serine/threonine-protein kinase (RLP), recognizes

a small modified peptide from *Cuscuta* spp. extracts (*4*). The *Cuscuta* signals that trigger the lignin-based defense responses appear to be larger heat-sensitive protein/s (Fig. 4). In conclusion, we propose a model for *Cuscuta* resistance response in tomato (Fig. 5). *CuRLR1* is a newly discovered cytosolic receptor, which receives large signaling molecules from *C. campestris*. This triggers downstream signal transduction and induces a lignin-based resistance response (Fig. 5, red labeled pathway). Transient overexpression of *MYB55* and *LIF1* induced lignin accumulation in the cortex, placing *MYB55* and *LIF1* as positive regulators in the lignin biosynthesis pathway (Fig. 5, pink and yellow labeled pathway). Other yet undiscovered *Cuscuta* receptors or factors may induce *MYB55* and *LIF1* expression upon *Cuscuta* attachment. On the other hand, *wrky16* plants showed lignin accumulation and stronger resistance to *Cuscuta*, suggesting that *WRKY16* is a negative regulator of this lignin-based resistance pathway (Fig. 5, green labeled pathway). Based on our DNA-Seq and BiFC data, we propose that *WRKY16* regulates the function of *LIF1* by a combination of inhibition of *LIF1* transcription and physical capture of LIF1 proteins to block their entry into the nucleus (Fig. 5, yellow and green labeled pathway). *CuRe1* is reported to mediates PAMP/MAMP-triggered immunity (PTI/MTI) (*4*) (Fig. 5, blue labeled pathway). GCN analysis indicates a coexpression connection between *CuRe1* and *WRKY16* (Fig. 3A-D). *CuRe1* and *WRKY16* both became central hub genes in resistant cultivars upon *Cuscuta* attachments (Fig. 3D), suggesting the hypothesis that *WRKY16* may act downstream of *CuRe1* (Fig. 5, blue labeled pathway). Thus, we envision crosstalk between different resistance pathways that may be triggered together to enhance host defense responses.

Our work has implications for enhancing crop resistance to parasitic plants. Notably, overexpression of the *CuRLR1* protein induced upregulation of lignin precursors, but extensive lignin accumulation was only be triggered by *Cuscuta* signals. The identification of *CuRLR1*

provides a path forward to introduce resistance into other important agricultural crops like potato, sugar beet, carrot, pea, soybean, chili and sesame that are attacked by *Cuscuta* (*39*, *40*).

Acknowledgments

We are grateful to S. Dinesh-Kumar, A. B. Britt, S. Brady, and D. Runcie for their input on this research, and S. Dinesh-Kumar, A. B. Britt, S. Brady, and E. D. Marable for editing suggestions. We thank C. Wong and J. Lu for image analysis, and N. Johnson and M. Axtell for help with verifying *Cuscuta* species. We also thank R. Ozminkowski, Manager of Agricultural Research from HeinzSeed for providing seeds of three Heinz cultivars the UC Davis Plant Transformation Facility for generating transgenic plants, and CyVerse for online data storage. **Funding:** This work was funded by USDA-NIFA (2013-02345). M. J. was supported by Yen Chuang Taiwan Fellowship, Taiwan Government Scholarship (GSSA), Elsie Taylor Stocking Memorial Fellowship, and Katherine Esau Summer Graduate Fellowship program; M.F. was supported by United States-Israel Binational Agricultural Research and Development Fund (postdoctoral fellowship no. FI–463–12), M.S.B. and P.M.S. were supported by the DOE Joint BioEnergy Institute (http://www.jbei.org) supported by the U.S. Department of Energy, Office of Science, Office of Biological and Environmental Research through contract DE-AC02-05CH11231 between Lawrence Berkeley National Laboratory and the U.S. Department of Energy. **Author contributions:** M.F. initiated the project with supervision from N. S. and established the connection with Heinz. M.J. verified *Cuscuta* species. M.J., M.F., and R.N.P. conducted histochemical and biomass analysis. M.R. modified the VGE vector and M.F. cloned candidate genes into VGE vectors. M.J., R.N.P., and L.W. conducted VGE overexpression experiments and analysis for all candidate genes. S.D.R. and M.J. developed lignin quantification methods. M.J. produced VGE overexpressed samples and M.S.B. performed acetyl bromide, HPLC and Pryo-GC-MS analysis with P.M.S.'s supervision. M.F. made all DNA-Seq and RNA-Seq libraries. M.F. conducted SNP analyses and DEG analyses to pick primary candidate genes. M.J. performed SNP

analyses and DEG analyses, and BH-SNE clustering to select candidate genes for GCNs. S.D.R. developed the RSMod R package. H.N., S.D.R., and M.J. modified the GCN analysis script. M.J. and L.W. conducted *wrky16* plant phenotyping and VGE overexpression experiments on *wrky16*. M.J. and L.W. performed subcellular localization and BIFC experiments. M.J., R.N.P., and L.W. conducted *Cuscuta* extract injection experiments. M.J. wrote the initial manuscript draft for the main text with primary editing from N. S.. M.J., R.N.P., L.W., M.S.B., M. R. and H.N. wrote the initial draft for materials and methods. M.J., M.F., R.N.P., L.W., M.S.B., H.N., K.Z., P.M.S., and N.R.S. contributed to the editing of this manuscript. N.R.S. supervised the whole project.

Competing interests: The authors declare that they have no competing interests.

Data and materials availability: All data is available in the main text or the supplementary materials. All DNA-Seq and RNA-Seq raw data are deposited on NCBI SRA PRJNA550259. All R scripts and package for analysis are deposited on GitHub.

Supplementary Materials:

Materials and Methods

Figures S1-S14

Tables S1 to S7

References (*41-59*)

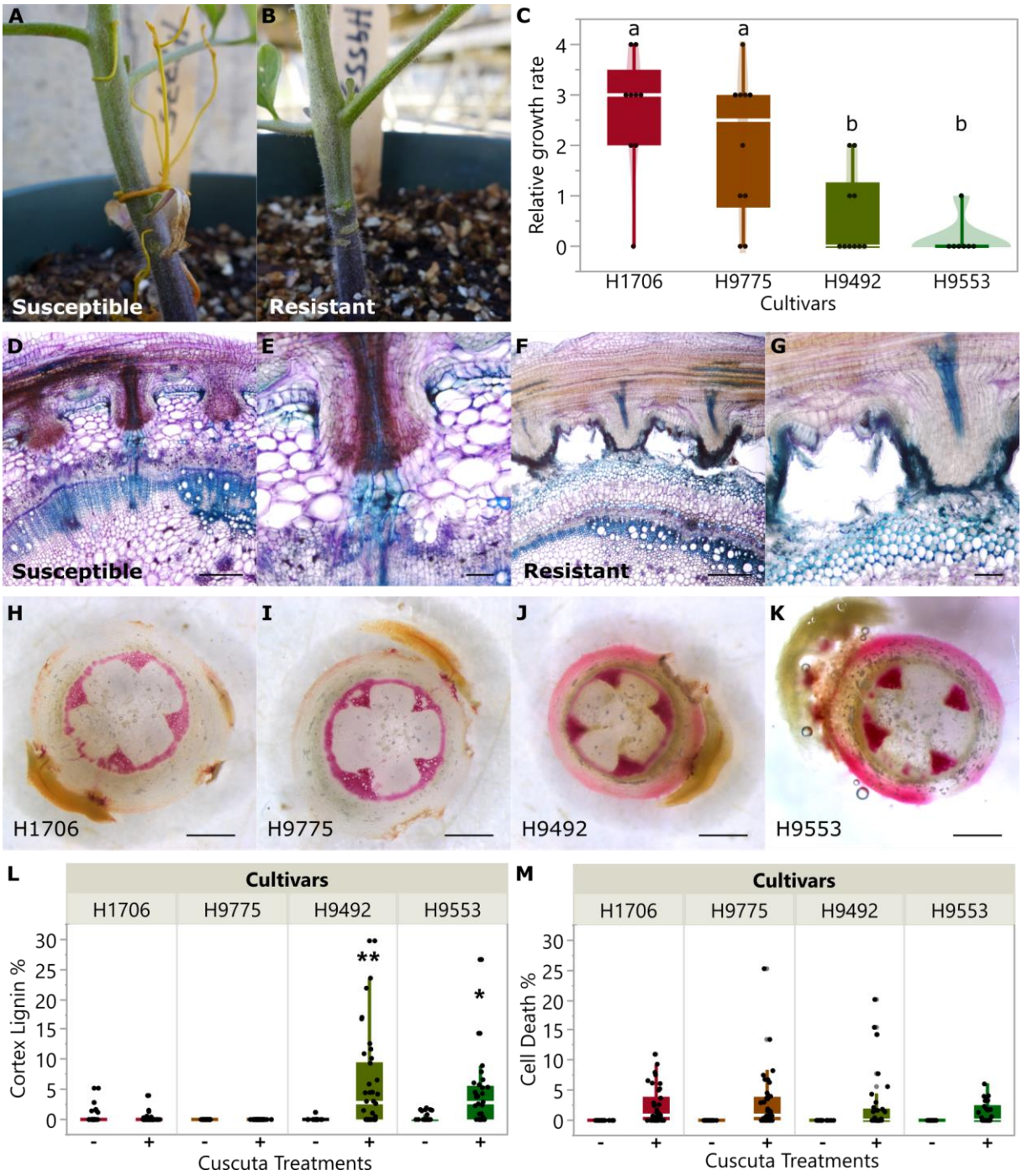

Fig. 1. The comparison of resistant responses to *C. campestris* in tomato cultivars. (A) *C. campestris* grows on the susceptible H9775, (B) and cannot attach on the resistant H9553. (C) The biomass ratio of host and *C. campestris* (*Cuscuta* weight/ tomato weight) on different cultivars. Data was assessed using pair-wise comparisons with Tukey test. P-values between "a" and "b" are

< 0.05. (D-G) 100 μm vibratome longitudinal sections of *C. campestris* haustoria attaching to H1706 (D-E) and H9553 (F-G), and stained with Toluidine Blue O. Lignin is stained as blue. (D and F) Scale bar, 40 μm. (E and G) Scale bar, 10 μm. (H-K) are ~300 μm sections of the haustoria attachment sites stained with Phloroglucinol-HCl. Scale bar, 1 mm. Lignin is stained as red. Stem cross sections of H1706 (H), H9775 (I), H9492 (J), and H9553 (K) with *C. campestris* attached. (L) Cortex lignin area percentage in different cultivars. Data presented are assessed using multiple comparisons with Dunnett's test. "*": p-values < 0.05, "**": p-values < 0.01. (M) Cell death area percentage in different cultivars.

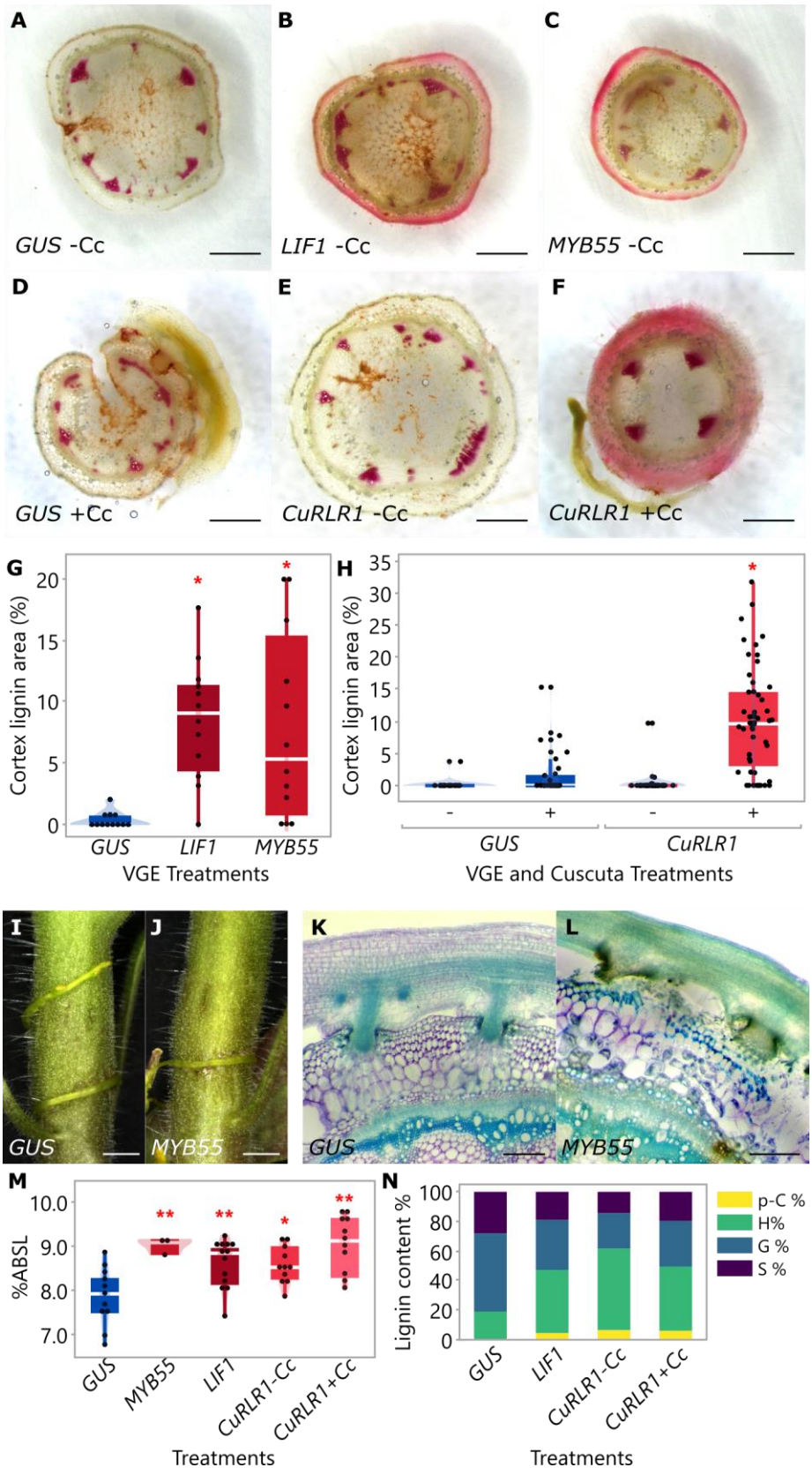

Fig. 2. Virus-based Gene Expression (VGE) of candidate genes in tomato H1706. (A-F) ~300 µm hand sections of stems near injection sites stained with Phloroglucinol-HCl. Lignin is stained red. VGE overexpressed *GUS* (A), *LIF1* (B), *MYB55* (C), and *CuRLR1* (E) in H1706 stem without *C. campestris* infestation. Stem cross section of VGE overexpressing *GUS* (D) and *CuRLR1* (F) in H1706 with *C. campestris* infestation. (G) Cortex lignin area percentage in *LIF1* and *MYB55* overexpressed stems and (H) in *CuRLR1* overexpressed stems with and without *C. campestris* infestation. (G-H) Data presented are assessed using Dunnett's test. "*": p-values < 0.01. (I-L) Transient overexpression of candidate genes in susceptible H1706 induces lignin accumulation in the cortex and makes H1706 became more resistant to *C. campestris*. (I-J) Scale bar, 2 mm. (K-L) Scale bar, 30 µm. (M) Acetyl bromide assay for total lignin content in candidate gene VGE overexpressed stems. Data presented are assessed using Dunnett's test. "*": p-values < 0.05, "**": p-values < 0.01. (N) PYRO-GC assay for monolignol composition in the samples from *CuRLR1* overexpressed plants with and without *C. campestris* infestation.

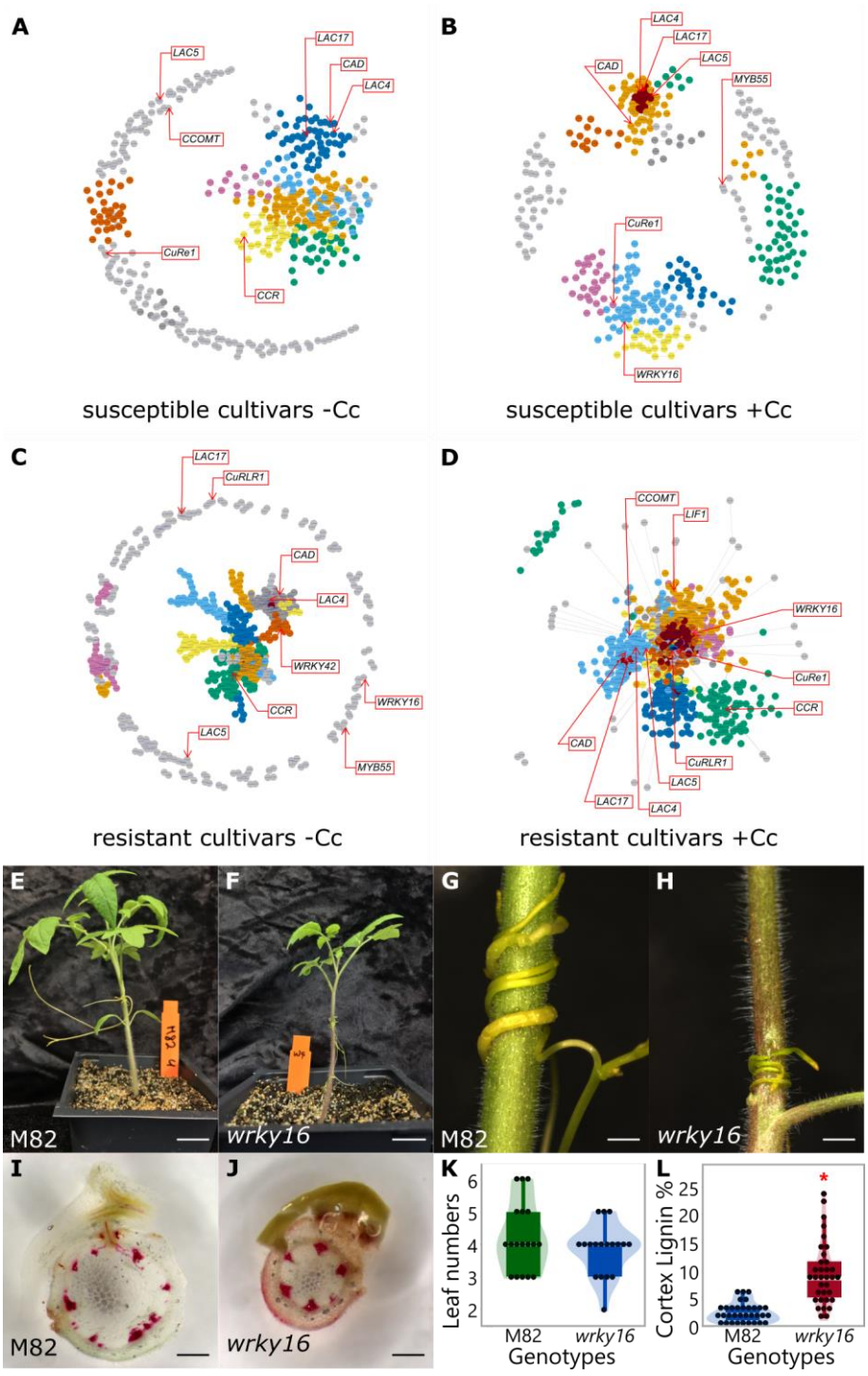

Fig. 3. Gene co-expression network (GCN) analysis to identify key regulators and assess their role in resistance using CRISPR/Cas9 gene knockouts. (A-D) Gene co-expression networks (GCNs) of different *C. campestris* treatments in susceptable and resitant cultivars. –Cc and +Cc

indicates without or with *C. campestris* infection treatments respectively. (E-F) overall phenotype comparsion between M82 and homozygous *WRKY16* CRISPR lines (*wrky16*). Scale bar, 2 cm. (G-H) *C. campestris* growing on M82 and *wrky16*. (I-J) ~300 μm hand sections of M82 and *wrky16* stems near *Cuscuta* attachment site stained with Phloroglucinol-HCl. Lignin is stained red. (K) Leaf number of *wrky16* and M82. (L) Cortex lignin area percentage in M82 and *wrky16* stems. Data presented are assessed using student's t test. "*" indicates p-value is less than 0.01.

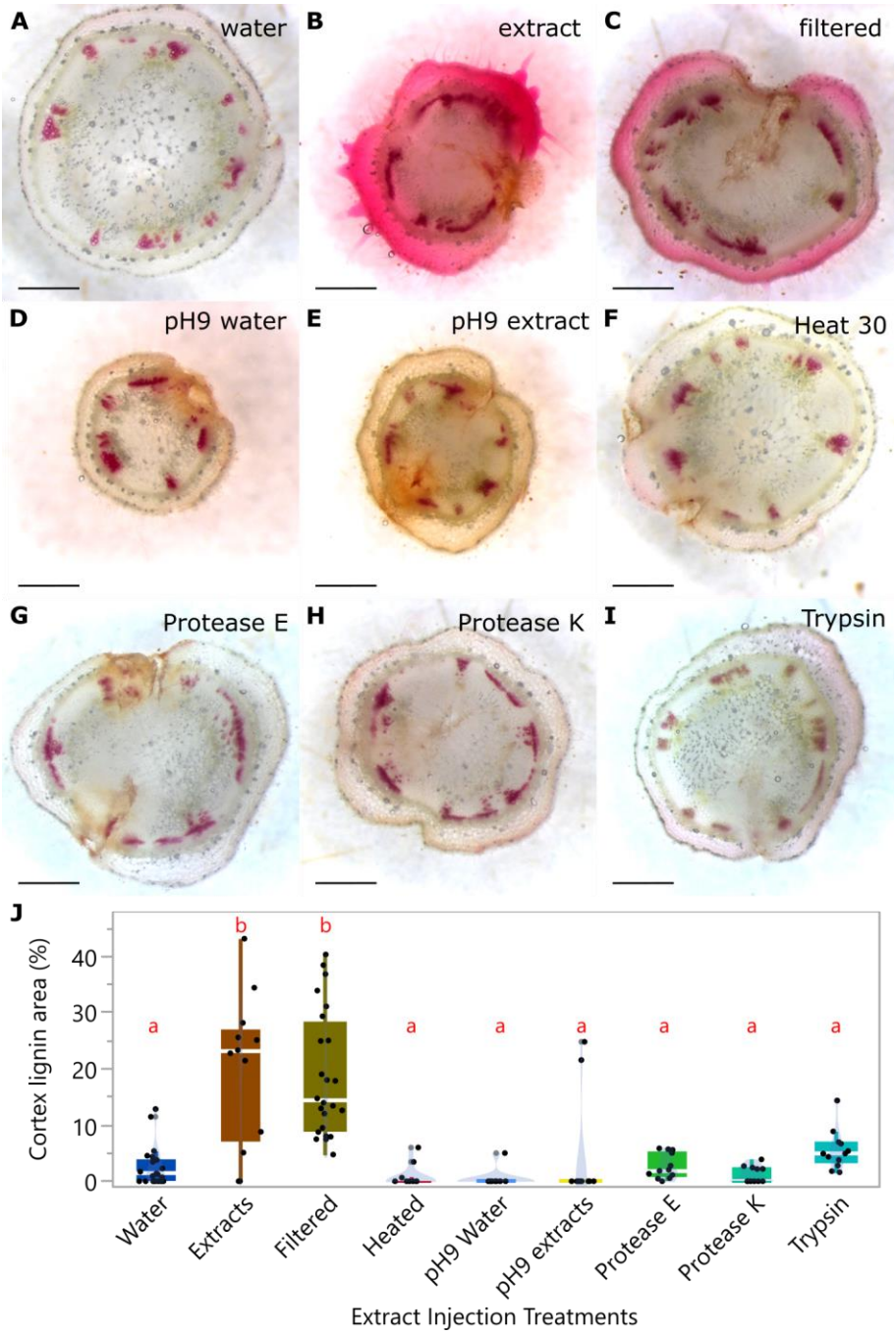

Fig. 4. *C. campestris* extract injections to detect *Cuscuta* signals. (A-I) ~300 µm hand sections of resistant H9553 stems near injection sites stained with Phloroglucinol-HCl. Lignin is stained red. The H9553 plants are injected with (A) water, (B) untreated *C. campestris* extract (pH 5.8), (C) *C. campestris* extract filtered with 0.2 µm filter, (D) pH 9 water, and (E) pH 9 *C. campestris*

extract, (F) heat-treated *C. campestris* extract (95°C for 30 minutes), (G) Protease E-treated *C. campestris* extract, (H) Protease K-treated *C. campestris* extract, and (I) Trypsin-treated *C. campestris* extract. (J) Percentage of lignified cortex area in total stem area. The samples injected with water serve as negative controls. Different treated or untreated *C. campestris* extracts are compared to negative controls. Data presented are assessed using pair-wise comparisons with Tukey test. P-value of the contrasts between “a” and “b” are less than 0.01.

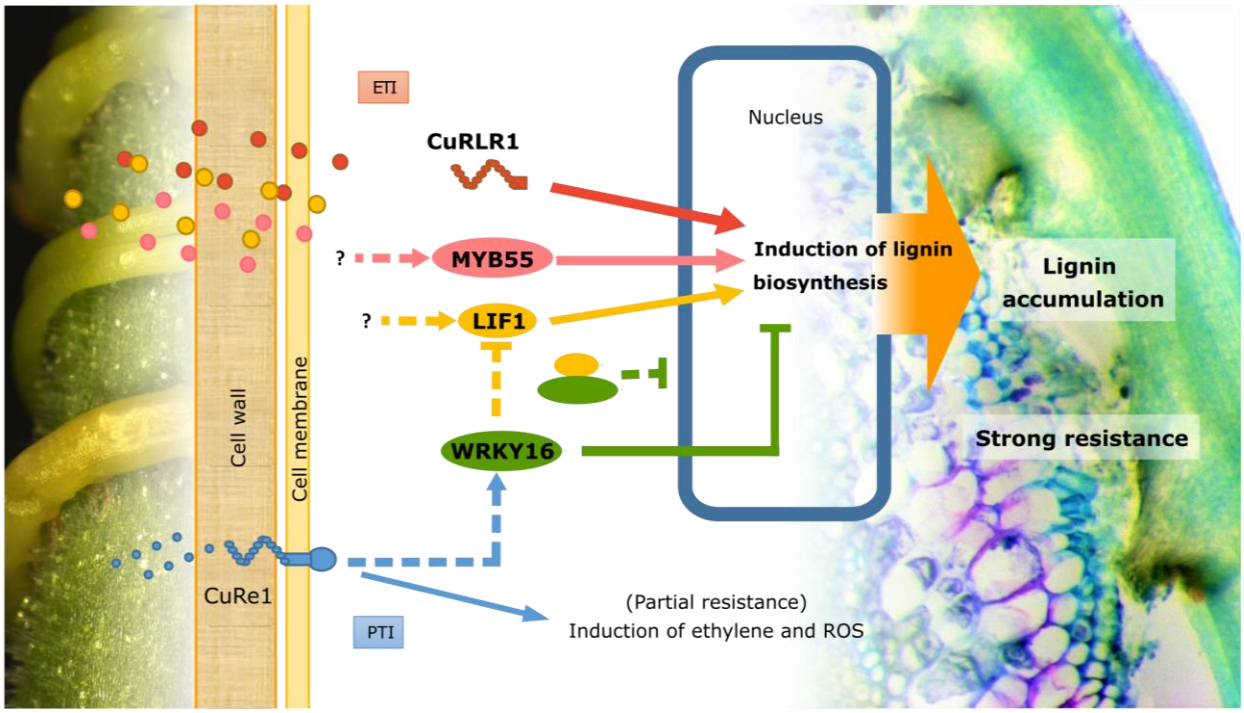

Fig. 5. Model of *C. campestris* resistance response in tomato cultivars. Red labeled pathway: newly identified cytosolic *CuRLR1* receptor, which receives large signaling molecules from *C. campestris*. This triggers downstream signal transduction and induces a lignin-based resistance response. This resistant response may be an effector-triggered immunity (ETI). Pink and yellow labeled pathway: *MYB55* and *LIF1* function as positive regulators in the lignin biosynthesis pathway. Yellow and green labeled pathway: *WRKY16* and *LIF1* mediated lignin-based resistant responses and with a potential connection to *CuRe1*. Blue labeled pathway: previously identified *CuRe1* mediated PAMP/MAMP-triggered immunity (PTI/MTI) pathway.

References

1. G. N. Agrios, in *Plant Pathology (Fifth Edition)*. (Academic Press, San Diego, 2005), pp. 705-722.
2. J. I. Yoder, J. D. Scholes, *Current Opinion in Plant Biology* **13**, 478-484 (2010).
3. G. Yaakov, W. T. Lanini, R. L. Wrobel, *Weed Science* **49**, 520-523 (2001).
4. V. Hegenauer *et al.*, *Science* **353**, 478-481 (2016).
5. J. C. M. S. Moura, C. A. V. Bonine, J. De Oliveira Fernandes Viana, M. C. Dornelas, P. Mazzafera, *Journal of Integrative Plant Biology* **52**, 360-376 (2010).
6. C. P. Vance, T. K. Kirk, R. T. Sherwood, *Annual Review of Phytopathology* **18**, 259-288 (1980).
7. F. G. Malinovsky, J. U. Fangel, W. G. T. Willats, *Frontiers in Plant Science* **5**, (2014).
8. D. Bellincampi, F. Cervone, V. Lionetti, *Frontiers in Plant Science* **5**, (2014).
9. N. H. Bhuiyan, G. Selvaraj, Y. Wei, J. King, *Plant signaling & behavior* **4**, 158-159 (2009).
10. J. Barros, H. Serk, I. Granlund, E. Pesquet, *Annals of botany* **115**, 1053-1074 (2015).
11. W. T. Lanini, M. Kogan, *Biology and Management of Cuscuta in Crops*. (2005), vol. 32.
12. F. M. Ashton, *Cuscuta Spp. (Dodder): A Literature Review of Its Biology and Control*. (Division of Agricultural Sciences, University of California, 1976).
13. K. J. Hembree, W. Lanini, N. Va, in *Proc. Calif. Weed Sci. Soc*. (1999), vol. 51, pp. 205-206.
14. S. Cui *et al.*, *New Phytologist* **218**, 710-723 (2018).
15. T. P. O'Brien, N. Feder, M. E. McCully, *Protoplasma* **59**, 368-373 (1964).
16. S. Liljegren, *Cold Spring Harbor Protocols* **2010**, pdb.prot4954 (2010).
17. J. B. Runyon, M. C. Mescher, G. W. Felton, C. M. De Moraes, *Plant, Cell & Environment* **33**, 290-303 (2010).
18. M. S. Padmanabhan *et al.*, *PLOS Pathogens* **9**, e1003235 (2013).
19. C. L. Cass *et al.*, *Journal of Experimental Botany* **66**, 4317-4335 (2015).
20. F. Pomar, F. Merino, A. R. Barceló, *Protoplasma* **220**, 0017-0028 (2002).
21. S.-H. Zhang, Q. Yang, R.-C. Ma, *Journal of Integrative Plant Biology* **49**, 993-1002 (2007).
22. Q. Liu, L. Luo, L. Zheng, *International journal of molecular sciences* **19**, 335 (2018).
23. A. Ranjan *et al.*, *Plant Physiology* **172**, 328-340 (2016).
24. C. Pan *et al.*, **6**, 24765 (2016).
25. M. S. Padmanabhan, S. P. Dinesh-Kumar, *Current Opinion in Microbiology* **20**, 55-61 (2014).
26. J. L. Ferrer, M. B. Austin, C. Stewart, J. P. Noel, *Plant Physiology and Biochemistry* **46**, 356-370 (2008).
27. C. Gayoso, F. Pomar, E. Novo-Uzal, F. Merino, Ó. Martínez de Ilárduya, *BMC Plant Biology* **10**, 232 (2010).
28. P. Taheri, S. Tarighi, *Journal of Botany* **2012**, 6 (2012).
29. C. Kumari, T. K. Dutta, P. Banakar, U. Rao, *Scientific Reports* **6**, 22846 (2016).
30. A. R. War *et al.*, *Plant Signaling & Behavior* **7**, 1306-1320 (2012).
31. P. J. Reimers, J. E. Leach, *Physiological and Molecular Plant Pathology* **38**, 39-55 (1991).

32. S. Dhakshinamoorthy, K. Mariama, A. Elsen, D. De Waele, *Nematology* **16**, 565-576 (2014).
33. Y. Zhang *et al.*, *Molecular Plant Pathology* **20**, 309-322 (2019).
34. D. D. Cameron, A. M. Coats, W. E. Seel, *Annals of Botany* **98**, 1289-1299 (2006).
35. Y. Goldwasser, J. Hershenhorn, D. Plakhine, Y. Kleifeld, B. Rubin, *Physiological and Molecular Plant Pathology* **54**, 87-96 (1999).
36. M. D. Lozano-Baena, E. Prats, M. T. Moreno, D. Rubiales, A. Pérez-de-Luque, *Plant Physiology* **145**, 437 (2007).
37. A. PÉRez-De-Luque *et al.*, *Annals of Botany* **95**, 935-942 (2005).
38. A. Sahm, H. Pfanz, M. Grünsfelder, F. C. Czygan, P. Proksch, *Botanica Acta* **108**, 358-364 (1995).
39. E. E. Gaertner, *Memoirs. Cornell Agricultural Experiment Station* **294**, 56 pp. (1950).
40. D. A. Cooke, I. D. Black. (1987), pp. 33.