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Parasitic *Cuscuta* factor(s) and the detection by tomato initiates plant defense

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ABSTRACT

Dodders (*Cuscuta* spp.) are holoparasitic plants that enwind stems of host plants and penetrate those by haustoria to connect to the vascular bundles. Having a broad host plant spectrum, *Cuscuta* spp infect nearly all dicot plants – only cultivated tomato as one exception is mounting an active defense specifically against *C. reflexa*. In a recent work we identified a pattern recognition receptor of tomato, “Cuscuta Receptor 1” (CuRe1), which is critical to detect a “Cuscuta factor” (CuF) and initiate defense responses such as the production of ethylene or the generation of reactive oxygen species. CuRe1 also contributes to the tomato resistance against *C. reflexa*. Here we point to the fact that CuRe1 is not the only relevant component for full tomato resistance but it requires additional defense mechanisms, or receptors, respectively, to totally fend off the parasite.

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Metazoans and plants possess an innate immune system to mount active defense against pathogen attacks. Most plant pathogens are microbes or herbivorous arthropods that the immune systems of plants are able to detect by sensing microbe- or herbivore-associated molecular patterns (MAMPs/HAMPs).^{1,2} These molecular patterns, indicative for “non-self,” serve as molecular signals that trigger specific plant pattern recognition receptors (PRRs) and initiate plant defense signaling to fend off the pathogen.^{3,4} Besides the pathogens mentioned, there exist ~4,500 plant species that live parasitic on other plants and genera such as *Striga*, *Orobanche* or *Cuscuta* are known to cause tremendous crop loss.

The plant genus *Cuscuta* (dodder) comprises about 200 species distributed in all moderate climate zones. All *Cuscuta* species live as stem holoparasites with a broad host spectrum, preferentially for dicotyledonous plants. The different *Cuscuta* species grow as yellowish, orange or slightly greenish vines that wind around the stems of their host plants.⁵ Most dodder species have no or only marginal amounts of chlorophyll and their photosynthesis is insufficient for surviving.^{6–9} All *Cuscuta* species possess neither roots nor expanded leaves and penetrate host plants with haustoria that directly connect to the vascular bundles. Right after germination, *Cuscuta* seedlings sense host plant volatiles which support the finding of an appropriate host.¹⁰ In the parasite, initial physical contact induces the formation of

haustoria,¹¹ specific organs which are generally important for parasitic plants to penetrate the host tissue.¹² The penetration phase is accompanied by the expression of cell-wall modifying enzymes leading to structural rearrangements within the cell-walls of the parasite¹³ and the loosening of the host tissues.^{14,15} After reaching the vascular bundles, the parasitic haustorium connects to the host xylem and phloem. This allows the parasite to withdraw water, nutrients, and carbohydrates to grow and complete its lifecycle.^{8,16,17} *Cuscuta* parasites also take up macromolecules such as proteins, viruses or RNAs.^{18–22} Recently, RNAs were shown to move between host plant and parasite in a bidirectional manner and to a much higher extent than previously expected.²³

Not much is known about how host plants can sense parasitic *Cuscuta* spp. and how they initiate cellular programs to fend off plant parasites. In our recent study,²⁴ we made use of the special case *Cuscuta reflexa* and its resistant host plant *Solanum lycopersicum* (cultivated tomato) to get insights in the early steps occurring in the plant-plant dialog. Tomato displays an active and clearly visible resistance reaction directly at the penetration sites of the parasitic haustoria a few days after the initial contact with the parasite and successfully fends off *C. reflexa*.^{25–27}

In this study we show that extracts of *C. reflexa* induce the production of reactive oxygen species (ROS) and the

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biosynthesis of the stress related phytohormone ethylene, plant defense responses usually known to occur during plant–microbe interaction and typically induced by pathogen-associated molecular patterns (PAMPs).^{2,28} We could isolate and characterize the trigger of these responses from *C. reflexa*, a 2 kDa peptide with an o-esterified modification, and we further screened an introgression library of *S. lycopersicum* x *S. pennellii*²⁹ to map responsiveness to this parasitic factor, since *S. pennellii* is insensitive to parasitic extracts and susceptible for a *C. reflexa* infestation.¹³ We identified a gene encoding a plasma membrane-bound receptor, the Leucine-rich repeat receptor like protein (LRR-RLP) “Cuscuta receptor 1’ (CuRe1) which senses the parasitic “Cuscuta factor” (CuF). CuF initiates defense responses in the formerly insensitive host plant *Nicotiana benthamiana* after transient expression of *CuRe1*. Stable transformation of a *CuRe1* construct into *N. benthamiana* lead to a drastically reduced *C. reflexa* growth and to an increased resistance.

Besides CuRe1, there are 3 genes encoding for CuRe1 homologs (CuRe1-likes; Solyc04g0014400; Solyc08g016210; Solyc08g016310) within the tomato genome, sharing 64 – 81 % amino acid sequence identity (Fig. 1A). Receptors with up to 80 % aa-sequence identity to CuRe1 seem exclusively present in Solanaceae plants. Only receptors with less than 45 % aa-sequence can be found outside the Solanaceae. We cloned all CuRe1-like genes from tomato²⁴ and expressed them heterologously in *N. benthamiana*. However, in contrast to CuRe1 none of these receptors was able to trigger defense-related responses like ethylene induction when treated with the CuF or crude *C. reflexa* extract (Fig. 1B).

The recognition of the parasitic cell wall associated CuF or related other *Cuscuta* factors by these receptors could be supposable. Nonetheless, the initiated cellular signaling program must be distinct from the defense related responses induced by CuRe1 as we could not measure the emission of ethylene (Fig. 1) after treatment with CuF.

During a susceptible interaction the parasite has to hook up the host plant’s developmental processes to establish a connection to the vascular system. Therefore, the parasite has to (ab-)use existing host mechanisms including the signals and perception systems to succeed in infecting other plants. If the CuRe1-like receptors are critical to recognize and process any molecular cues of *Cuscuta* spp is possible but remains to be demonstrated. The roles of CuRe1-likes for the harbouring host plant e.g. as receptors for endogenous signals involved in developmental processes or as receptors to detect MAMPs is still unclear and up to date no function could be assigned to any receptor of this clade.

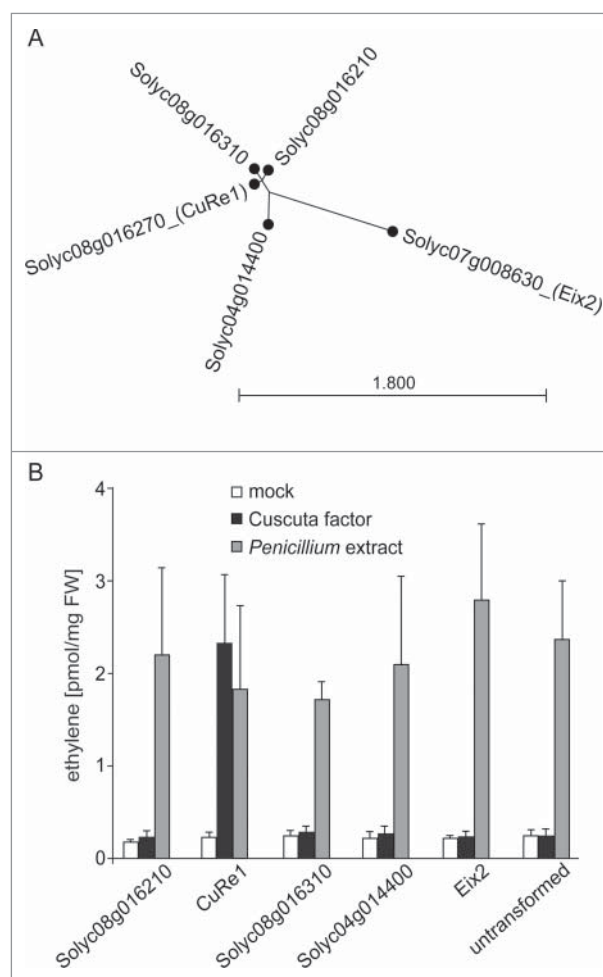


Figure 1. Functionality of CuRe1-like receptors. (A) Tree shows relationship of CuRe1 and CuRe1-like genes; Eix2: receptor for fungal Xylanase³³ served as reference. (B) Ethylene response of *N. benthamiana* leaves expressing receptor CuRe1-like constructs and treated with *C. reflexa* extract or controls (mock = 0.01 mg/ml BSA in water; *Penicillium* extract = positive control); values represent means of n = 3 replicates plus stdv.

In fact, the specific recognition of the *Cuscuta* factor by tomato CuRe1²⁴ and the induction of the plant defense system seems unique and has probably evolved by incident exclusively in tomato. As far as tested, the *Cuscuta* factor seems present in other *Cuscuta* species as well but seems absent from plant species outside this genus.²⁴ The full resistance toward parasitic *C. reflexa*, however, seems not to depend on CuRe1 alone but requires additional mechanisms maybe related to those known for Effector triggered immunity (ETI) occurring during plant–microbe interaction (overview in Fig. 2).^{24,30,31} An nucleotide binding site leucine-rich repeat (NBS-LRR) protein, as part of a second layer of immunity and as a potential element of ETI, has been found to be relevant for resistance during the plant–plant interaction of cowpea against witch-weed (*Striga* spp.).³² In case of the *C. reflexa* interaction with tomato

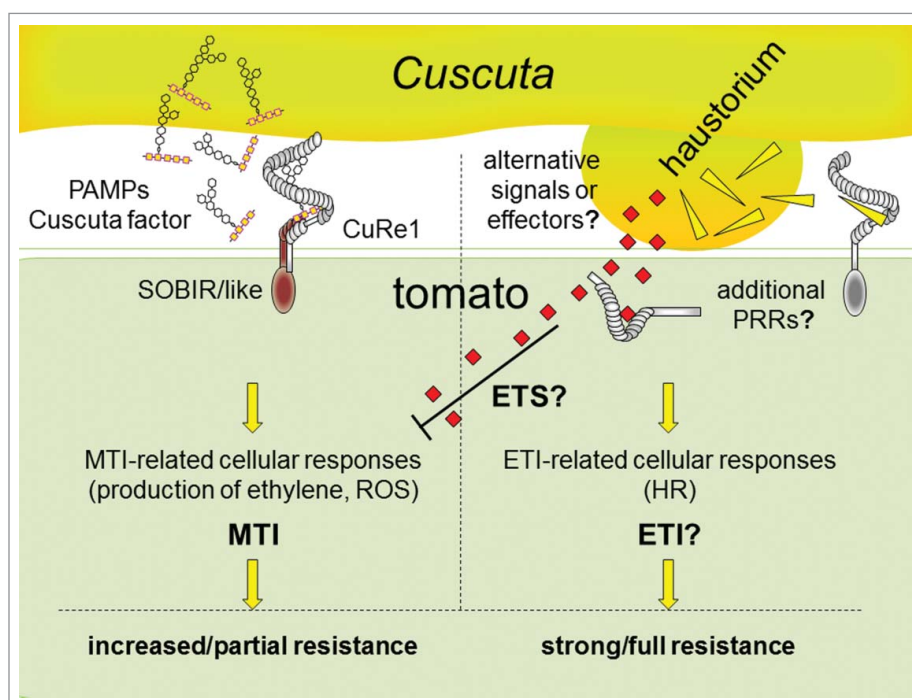


Figure 2. Model for defense and resistance of tomato to *Cuscuta* spp infestation. (Left): The *Cuscuta* factor is detected as a parasite-associated molecular pattern (PAMP) by the plasma membrane-bound PRR CuRe1 and initiates MTI-type responses in tomato, including the production of ethylene and ROS. MTI, apart from increasing resistance against various microbial pathogens, leads to increased resistance of tomato to *Cuscuta* attacks. (Right): Hypothesized ETS (effector triggered susceptibility), ETI (effector-triggered immunity) or alternative principles in tomato might, synergistically with or independently from MTI, confer full resistance of tomato to *Cuscuta* infestation.

additional components of resistance still have to be identified. If the CuRe1-like or other receptors are involved in such tomato-specific defense—maybe in a long term process—has to be further studied.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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