The tomato receptor CuRe1 senses a cell wall protein to identify Cuscuta as a pathogen

Volker Hegenauer1,2, Peter Slaby1, Max Körner1, Julien-Alexander Bruckmüller3,9, Ronja Burggraf2, Isabell Albert1, Bettina Kaiser2, Birgit Löffelhardt2, Irina Droste-Borel4, Jan Sklenar5, Frank L. H. Menke5, Boris Maček4, Aashish Ranjan6,7, Neelima Sinha6, Thorsten Nürnberger2,8, Georg Felix2, Kirsten Krause3, Mark Stahl2 & Markus Albert1

Parasitic plants of the genus Cuscuta penetrate shoots of host plants with haustoria and build a connection to the host vasculature to exhaust water, solutes and carbohydrates. Such infections usually stay unrecognized by the host and lead to harmful host plant damage. Here, we show a molecular mechanism of how plants can sense parasitic Cuscuta. We isolated an 11 kDa protein of the parasite cell wall and identified it as a glycine-rich protein (GRP). This GRP, as well as its minimal peptide epitope Crip21, serve as a pathogen-associated molecular pattern and specifically bind and activate a membrane-bound immune receptor of tomato, the Cuscuta Receptor 1 (CuRe1), leading to defense responses in resistant hosts. These findings provide the initial steps to understand the resistance mechanisms against parasitic plants and further offer great potential for protecting crops by engineering resistance against parasitic plants.
characteristic molecular patterns uncover pathogens as external invaders and are critical signatures that are detected by the innate immune system of both, animals and plants. This discrimination between self and non-self allows the host organisms to initiate defense reactions and resist pathogen attacks. As part of their innate immune system, plants evolved cell surface receptors to detect molecular patterns. Due to the facts that most plant pathogens are microbes or arthropods and the wider evolutionary distance between plants and those attackers, the presence of “plant pattern recognition receptors” to detect molecular patterns seems a logical consequence of evolution. However, ~4500 higher plant species live as parasites and thus pose an additional threat to plants. Well-known parasitic plants with high agronomical relevance are Striga spp., Orobanche spp., and Cuscuta spp. Most host plants are unable to detect an invasion by parasitic plants and the attackers stay unrecognized due to the limited innate immune system of host plants for detecting dangerous parasitic plants. Yet, a few host exceptions are described, which are able to fend off parasitic plants and stay incompatible. However, the molecular mechanisms behind these are poorly understood, and molecular patterns of parasitic plants, which could mark a plant parasite as a devastating invader, have not yet been described.

Cuscuta spp. are holoparasitic plants which infect a broad spectrum of hosts by connecting to their vasculature via specific feeding structures, called haustoria. Both types of enzymes are known to be present and active in the cell wall, we focused on extracts prepared from the parasite cell wall and tested them for bioactivity in the ethylene bioassay. Cuscuta factor is found in tomato (Solanum lycopersicum) cell walls and tested via CuRe1 (Fig. 1c) as a critical component for the detection of C. reflexa due to a hypothesized Cuscuta factor or pathogen-associated molecular pattern (PAMP) that can be extracted from the parasitic plant and triggers the defense response in a CuRe1-dependent manner. The Cuscuta factor is a heat stable protein and sensitive to treatments with bases (pH > 11), indicating potential secondary modifications present on the peptide backbone. The Cuscuta factor is found in all organs of C. reflexa irrespective of its infectious stage and seems to locate to the parasite’s cell wall. Here, we purified this Cuscuta factor from C. reflexa extracts and identified it as a Glycine-rich protein (GRP). We further characterized its function as a binding ligand for CuRe1 and the specifically triggered plant defense responses.

**Results and discussion**

**Purification and identification of a defense-triggering Cuscuta factor**

Since we knew that the Cuscuta factor originates from the cell wall, we focused on extracts prepared from the parasite cell wall and tested them for bioactivity in the ethylene bioassay specifically induced via CuRe1 (Fig. 1d). Compared to incubation in buffer/water alone, higher amounts of Cuscuta factor were found to be released from cell wall fractions by treatments with pectinases and, with much lower efficiency, by cellulases (Fig. 1d). Both types of enzymes are known to be present and active in penetrating Cuscuta spp. haustoria and can thus lead to an increased release of the Cuscuta factor from the cell walls of invading haustoria during the infection process.

To extract sufficient amounts of the Cuscuta factor from collected plant material, we scaled up the previous protocol and used acidic extraction conditions (0.1 M HCl, pH 1). The analysis was also extended to all of the activities that eluted as distinct peaks from the first cation exchange column (Supplementary Fig. 1). When purifying the extracts by cation exchange or reversed phase chromatography, the Cuscuta factor activity detectable by the CuRe1 receptor eluted in several peaks, indicating presence of activity in structurally different forms (Supplementary Fig. 1). We further purified and enriched the Cuscuta factor(s) from the obtained fractions and performed LC-MS/MS analyses for each sample individually. Several distinct masses correlated with CuRe1-dependent bioactivity and we identified 11 different compounds all of which represented active forms of the Cuscuta factor (Supplementary Fig. 2). MS/MS fragmentation studies of the correlated candidate masses shared similar fragment peaks
(Supplementary Fig. 2) and had the fragment mass of 2077 Da in common. Another characteristic feature of all corresponding MS-spectra was an inconclusive fragmentation pattern with only a few clear but characteristic fragment masses (Supplementary Fig. 2) that have been previously observed\(^\text{15}\). Since the commonly present fragment of 2077 Da is a y-fragment and the spectra of the heavier fragment of 2077 Da is a y-fragment and the spectra of the heavier fragments, we assumed a common origin of all fragments from the same protein. The mass differences of the N-terminal fragmentations could be correlated to those of single amino acid residues, which allowed us to deduce the N-terminal part of the peptide sequence (Supplementary Fig. 2). The information obtained from overlays of seven individual MS/MS fragmentation analyses in four individual LC-MS/MS runs revealed the sequence of the first 15 N-terminal amino acids of the peptide (Fig. 2a and Supplementary Fig. 2). A p-blast search against a translated transcriptome database from \textit{C. reflexa}\(^\text{19}\) resulted in a perfect hit on a glycine-rich protein (GRP) of \textit{C. reflexa} (Fig. 2a). CrGRP consists of 116 amino acids with an n-terminal targeting sequence that predicts an extracellular localization (Fig. 2a). According to the current classification of GRPs\(^\text{20}\), the CrGRP belongs to the class II which comprises a distinguishing c-terminal cysteine-rich region (Fig. 2a). We cloned the corresponding \textit{CrGRP} gene from \textit{C. reflexa} genomic DNA and transiently expressed it in \textit{N. benthamiana} leaves for \texttilde{}72 h with c-terminal GFP or tagRFP fusion tags. We confirmed the predicted localization of \textit{CrGRP} within the cell wall with confocal microscopy of \textit{N. benthamiana} leaves transiently expressing a tagRFP-tagged version of the \textit{CrGRP} (Supplementary Fig. 3). Western blot analyses showed that the protein migrated at the calculated size and does not appear to be secondarily modified in \textit{N. benthamiana} (Fig. 2b). Extracts of these leaves, expressing \textit{CrGRP}, induced ethylene production in a CuRe1-dependent manner like the original \textit{C. reflexa} extract (Fig. 2c). Moreover, when expressing \textit{CrGRP} in \textit{N. benthamiana} leaves for 5–7 days, clear hypersensitive cell death can be observed only when CuRe1 is present but not in control plants (Fig. 2d) lacking the receptor. These findings demonstrate that

![Fig. 2 The \textit{C. reflexa} Glycine-rich protein (CrGRP) triggers CuRe1-dependent defense responses.](https://example.com/fig2.jpg)

\(\text{a} \) C. \textit{reflexa} Glycine-rich Protein (CrGRP), aa-sequence

| Targeting sequence |
|--------------------|
| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| PARELATTTAASFDTNGYGHGGGSHGGWHHGHGG |

Crip29

| Fragment sequence |
|-------------------|
| 61 | 70 | 80 | 90 | 100 | 110 |
| MSSRVFLLLGLSLALSLMIASEV |

Glycine-rich protein (CrGRP) triggers CuRe1-dependent defense responses. \(\text{a} \) Protein sequence of the identified CrGRP; the peptide isolated from \textit{C. reflexa} extracts was sequenced de novo by mass spectrometry and is indicated starting at aa-position 70, dashed line indicates the peptide part which could not be sequenced by MS/MS; Crip21 motif highlighted in yellow. \(\text{b} \) heterologous expression of Grp gene in \textit{N. benthamiana} leaves; WB shows a c-terminally GFP-tagged CrGRP. \(\text{c} \) Ethylene response in leaves of wt or CuRe1-expressing \textit{N. benthamiana}. Plants were treated with extracted \textit{CrGRP} after heterologous expression shown in \(\text{b} \). Bovine serum albumine (BSA; 0.01 mg/ml) buffered in 25 mM MES (pH 5.7) was added as mock control; Penicillium extract (0.05 mg/ml) served as positive control\(^\text{31}\). FW, fresh weight; ethylene measurements show means of three technical replicates; dots indicate single data points; error bars denote SD, asterisks denote student’s t-test, \(p \leq 0.0003\); representative graphs are shown; experiments were repeated more than three times. \(\text{d} \) Expression of CrGRP-GFP in leaves of wildtype (wt) and transgenic CuRe1-expressing \textit{N. benthamiana} plants. GFP alone served as negative control; pictures 5 days after expression.
Crip29 was not out-competed when using structurally unrelated NH₄OH at pH in the C-terminal part with the Crip21 motif of CrGRP (Fig. 2a). Treatment of purified Cuscuta factor with NH₄OH at pH ≥ 11 leads to a total loss of bioactivity and indicated potential secondary modifications on the CrGRP15,16. However, synthesized peptides trigger responses in a picomolar range (Fig. 3b and Supplementary Fig. 4) suggesting that no such secondary modifications are required for the bioactivity of Crip21. Much like the purified Cuscuta factor15, synthesized Crip21 peptide, comprising no secondary modifications, lost all of its activity when treated with NH₄OH (Supplementary Fig. 5a). We analyzed the NH₄OH-treated peptide by MS/MS and observed multiple reaction products of Crip21 among which the most prominent ones were 82 or 99 Da smaller (Supplementary Fig. 5b), clearly showing that the treatment of Crip21 with NH₄OH is modifying the peptide itself.

We infiltrated Crip21 into the leaves of resistant S. lycopersicum, susceptible Solanum pennellii and the introgression line IL-1-1 lacking CuRe115, to check whether Crip21 also induces visible HR in tomato. After 7 days, only the cultivated tomato or an introgression line (IL) with functional CuRe1 showed Crip21-dependent cell death while S. pennellii and the IL lacking CuRe1 did not (Fig. 3c).

**Binding of CrGRP and Crip to the receptor CuRe1.** To test for a direct interaction of the peptide epitope Crip with the receptor CuRe1, we n-terminally labelled a 29-aa-long Crip peptide with biotin (bio-Crip29). The bio-Crip29 peptide is an N-terminally (+4 aa) and C-terminally (+4 aa) prolonged Crip21 (Fig. 2a) and was as active as Crip21 (Fig. 2b). The Crip21 minimal epitope has been prolonged to introduce a higher number of Lysine residues to increase the chance for a successful chemical crosslinking of NH₂ groups on the ligand with NH₂ groups on the receptor. We then examined the interaction of CuRe1 proteins with bio-Crip29 in affinity-crosslinking experiments in planta. N. benthamiana leaves expressing the myc-tagged receptor CuRe1 were first incubated with the bio-Crip29 derivative, either alone or together with an excess of non-modified Crip as competitor, and the leaves were subsequently treated with a chemical cross-linker. When analyzed for the presence of biotin, immunoprecipitates of CuRe1 showed clear labeling which was absent in samples treated with an excess of non-modified Crip as competitor (Fig. 3d). In turn, binding of bio-Crip29 was not out-competed when using structurally unrelated peptides such as flg22 (Fig. 3d). These findings demonstrate the Crip21 derived peptide epitope Crip as the specific ligand for the CuRe1 receptor. To corroborate direct protein–protein interaction of the full-length CrGRP with CuRe1 as it may occur under physiological conditions, both proteins were co-expressed with different c-terminal tags and the interaction of both could be demonstrated in co-immunoprecipitation assays (Fig. 3e).

**To identify aa-residues of Crip21 which are critical for CuRe1 activation,** the 21 aa-residues were individually substituted by alanine or serine (substitutions for the cysteines), respectively (Supplementary Table 1). Replacement of the cysteine residues at positions 7, 17, 20, and 21 by Serine led to a reduced functionality or in case of C7 to a complete loss of function. In contrast, the other aa residues seemed less important and had no measurable effects on activity (Supplementary Table 1).

**Crip21 peptide motifs in other plants.** When p-blasting the Grp and Crip21 aa-sequences against a database of the translated C. campestris genome21 and transcriptome22, we found a Grp homolog (Supplementary Fig. 6), which also contains a peptide motif (CcCrip21) with a sequence similarity of ~70% to the C. reflexa Crip21. Especially the glycine residues and the six cysteines are highly conserved (Supplementary Fig. 6). A comparable Grp sequence has been also found in the sequence database of C. australis23, with the CaCrip21 peptide showing exactly the same 21 aa sequence long peptide as CcCrip21 (Supplementary Fig. 6a). The C. campestris CcCrip21, or C. australis CaCrip21, respectively, showed full activity at similar concentrations in CuRe1-dependent ethylene induction (Supplementary Fig. 6b). This corroborates previous findings in which we could show that the defense-triggering Cuscuta factor is also present in other Cuscuta species15. In general, GRPs are widely distributed all over the plant kingdom. Even in cultivated tomato (S. lycopersicum) we found a homolog with an aa-sequence similarity of 57% to CrGRP and we thus assumed the corresponding peptide motif to Crip21, SlCrip21 could serve as an endogenous trigger for tomato CuRe1. We therefore tested the synthesized peptide SlCrip21 in our bioassays where SlCrip21 exhibited only residual activity when applied at concentrations ≥1000 nM (Supplementary Figs. 6c and 7).

By substitution of single aa-residues within Crip21 using SlCrip21 as a template (Supplementary Fig. 7), and testing those peptides for bioactivity via CuRe1, we discovered that replacement of the Alanine at position 11 in Crip21 by a Tyrosine residue (as is the case in SlCrip21) abolished its CuRe1-dependent activity (Supplementary Fig. 7), which is possibly important to avoid autoimmune responses in tomato. However, substituting the tyrosine (Y11) of SlCrip21 by Alanine did not restore activity, indicating that additional changes in the peptide sequence of SlCrip contribute to avoiding self-recognition in tomato (Supplementary Fig. 7). The biological function of the full-length protein SIGR is unclear and SIGR may probably play other roles in tomato not related to cellular defense responses and independent of tomato CuRe1.

In general, assigned functions of plant GRPs are multifaceted and range from the stabilization of cell walls to hypothesized regulating functions during abiotic and biotic stress reactions24,25, which makes it difficult to speculate about the role of the respective Grp in tomato or Cuscuta. Future work will have to reveal what the in vivo function of CrGRP for C. reflexa could be. By BLAST searching for Crip21 peptide homologs, we got hits for this peptide motif related to GRPs of many plant species. Peptides giving the best hits and showing the highest sequence identity to Crip21 were synthesized and tested for their capability to trigger ethylene in samples of CuRe1-expressing N. benthamiana as well as in cultivated tomato (S. lycopersicum;
Fig. 3 The peptide epitope crip21 of CrGRP triggers the tomato receptor CuRe1. a Synthesized peptides deriving from CrGRP induce ethylene production in transgenic CuRe1-expressing N. benthamiana. Peptides were applied at concentrations of 1 µM each. b Dose-dependent induction of ethylene by the CrGRP derived, synthesized peptides in CuRe1-expressing N. benthamiana; numbers on x-axis indicate peptide concentrations in nM; bio-crip29 is the N-terminally biotinylated peptide used in binding studies; for a and b: Bovine serum albumin (BSA; 0.01 mg/ml) buffered in 25 mM MES (pH 5.7) was added as mock control; Penicillium extract (0.05 mg/ml) served as positive control31. FW, fresh weight; ethylene measurements show means of three technical replicates; dots indicate single data points; error bars denote SD, representative graphs are shown, experiments were repeated independently more than three times. c crip21 peptide induces HR-type of cell death in a CuRe1-dependent manner. Leaves of S. lycopersicum, an introgression line (IL 12-2) with functional CuRe1 and leaves of IL 8-1-1 and S. pennellii (right; both lacking CuRe1) were infiltrated with 100 nM crip21 and photographs were taken 7 days later; painted lines indicate infiltrated leaf area. The effects shown are representative for ten infiltrated leaves per tomato IL or species, respectively; experiments have been independently repeated three times. d Affinity-crosslinking of crip29-biotin with CuRe1 in planta. Solubilized proteins were immune-precipitated and analyzed for myc-tagged (bottom) and biotinylated proteins (top); CrGRP82-106 served as competitor; e Co-immunoprecipitation experiments demonstrate interaction of CuRe1 with CrGRP full length protein after co-expression for ~48 h in planta.
DNA extraction and cloning of CrGRP. C. refulsa plants were grown under long day conditions (16 h day/8 h night) at 22°C in a greenhouse. Genomic DNA was extracted from frozen tissue using the Plant DNA Preparation Kit (Jena Biosciences, Germany), and PCR was performed with gene-specific primers for the candidate gene (C_rfla_35S:CuRe1:myc) and Cr-GRP: Forward (ATGAGTCTAAGGGTTCTTCTTCCCT, REV: AGCGCTTGGCGCATCAATTGCG). The PCR products were cloned into the pCRBlu/GW/TOP (TA-cloning vector (Invitrogen®), Thermal Diverser). Reverse primers without stop codon allowed for C-terminal GFP tag after recombination via LR-reaction (LR-clonase II Plus enzyme mix, Invitrogen®) into respective vectors (pB7FW2.0, pKF7FW2.0, both with C-terminal GFP tag: plant systems biology, university of Gent). For cloning of a CrGRP cDNA, total RNA was extracted from tomato plants (N. Benthamiana, Chro-maTest, Germany), and cDNA was synthesized by reverse transcription (First-Strand cDNA Synthesis Kit, GE Healthcare Life Sciences). PCR was performed with primers above. For subcellular localization, CrGRP has been cloned via LR-reaction into a modified version of pGWB660, including a tagRFP™.

CrGRP expression and protein isolation. The 35S:CrGRP-GFP construct (in vector pB7FW2.0) was performed similarly and served as mock control for treatments. For monitoring hypersensitive responses (HR) in leaves, 35S:CrGRP-GFP or 35S:GFP constructs were expressed in either transgenic, CuRe1-expressing or wt. N. Benthamiana plants. Leaves from these plants were harvested, ground in liquid nitrogen to fine powder, supplemented with buffer (~3x volume), and centrifuged (45 min, 100,000 g, 4°C). The supernatant was then collected for further testing. An extraction of total RNA was performed against the myc tag present at the c-terminus of CuRe1 using myc-trap agarose beads (ChromoTek, IZB Martinsried, Germany) as described above. All samples were further analyzed by MS/MS fragmentation studies using an Easy nano-LC Synthesis Kit, GE Healthcare Life Sciences); PCR was performed with primers above. For subcellular localization, CrGRP has been cloned via LR-reaction into a modified version of pGWB660, including a tagRFP™.

Confocal microscopy. Images of transiently transformed N. benthamiana were taken 5 days after A. tumefaciens infiltration with a Zeiss confocal laser scanning microscope (LSM880, Carl Zeiss Microscopy GmbH, Carl-Zeiss-Promenade 10, Jena, Germany) and the attached C-Apochromat ×10/0.45 M27 objective. The tag-RFP fluorescence was excited with 561 nm and emission was detected at 563-607 nm. Autofluorescence of plant cell walls (lignin) was excited at 405 nm and emission was detected at 410-470 nm. Pinhole, detector gain and digital gain settings were adjusted to provide an optimal balance between fluorescence intensity and background signal. Data were processed with the ZEN 2.3 software.

Binding assays and immunoprecipitation assays. Direct interaction of CrGRP with CuR1 was tested by co-immunoprecipitation. For immunoprecipitation, leaves of N. benthamiana were transiently transfected with 35S:CuRe1:myc or 35S:CrGRP-GFP alone each, or co-expressed in combination for ~48 h. Leaf material was harvested, frozen in liquid nitrogen and ground to fine powder. Samples of 300 mg were solubilized and used for immunoprecipitation as reported29 using a G-Trap agarose beads (Chromotek, JBI Martinsried, Germany). Samples were separated by SDS-PAGE (8% Acrylamide gels) and transferred to nitrocellulose membrane. Western blots were probed using the a-GFP (Acris (now OriGene) Polyclonal Antibody to GFP, Cat. No.: R1091P; dilution 1:5000 in 5% BSA; goat; UniProt: P42212) or a-myc (Sigma Polyclonal anti-c-Myc Antibody; Cat. No.: C9356; dilution: 1:5000 in 5% BSA; from rabbit; UniProt: P01106) antibodies, diluted according to the instructions of the suppliers, and developed with secondary antibodies conjugated to alkaline phosphatase as described29. Anti-Goat IgG (whole molecule) - Alkaline Phosphatase antibody produced in goat, Cat. No.: A1418, dilution: 1:50,000 in 5% BSA; OR: Anti-Rabbit IgG (whole molecule) - Alkaline Phosphatase antibody produced in goat, Cat. No.: A3687, dilution 1:50,000 in 5% BSA). In two cross-linking of biotinylated Crip29 (Crip29 aa-sequence: GKGKNCGNSGC CRAYNQQKCRKCAYKG) to CuRe1 was performed as described30,31 leaves of N. benthamiana expressing 35S:CuRe1:myc, or control plants (N. benthamiana expressing 35S:RLP23:myc) were infiltrated with biotinylated bio-Crip29 (10 nM in ddH2O) with or without unlabelled Crip21 (or unlabelled Crip82-106) (10 µM) as competitor or with flg22 peptide as competition control. Five minutes after peptide treatment 2 ml M9 (cethylenglycol bismuccinimidyldicarbonate) in 25 mM HEPES buffer (pH 7.5) was infiltrated into the same leaves for cross-linking of peptides to the receptor proteins. Twenty minutes after cross-linking, leaf samples were harvested and frozen in liquid nitrogen; immunoprecipitations were performed against the myc tag present at the c-terminus of CuRe1 using myc-trap agarose beads (Chromotek, JBI Martinsried, Germany) as described above. All peptides, including biotinylated bio-Crip29, were synthesized by GenScript® and ordered with a purity of >95%. Biotinylated Crip29 was detected on blots by
Streptavidine-conjugated Alkaline Phosphatase (Strep-AP, Roche diagnostics; Streptavidin-AP conjugate, Cat. No.: 11089161001, dilution: 1:1000 in 5% Albumin Fraction V, biotin-free).

**Reporting summary.** Further information on research design is available in the Nature Research Reporting Summary linked to this article.

**Data availability** Source data are provided with this paper. Any other supporting data are available from the corresponding author upon request.

Received: 20 February 2020; Accepted: 28 September 2020; Published online: 20 October 2020

**References**

1. Bohm, H., Albert, I., Fan, L., Reinhard, A. & Nurnberger, T. Immune receptor complexes at the plant cell surface. *Curr. Opin. Plant Biol.* 20C, 47–54 (2014).
2. Macho, A. P. & Zipfel, C. Plant PRRs and the activation of innate immune signaling. *Mol. Cell* 54, 263–272 (2014).
3. Zipfel, C. & Oldroyd, G. E. Plant signalling in symbiosis and immunity. *Nature* 543, 328–336 (2017).
4. Kaiser, B., Vogg, G., Fürst, U. B. & Albert, M. Parasitic plants of the genus Cuscuta and their interaction with susceptible and resistant host plants. *Front. Plant Sci.* 6, 45 (2015).
5. Westwood, J. H., Yoder, J. I., Timko, M. P. & dePamphilis, C. W. The evolution of parasitism in plants. *Trends Plant Sci.* 15, 227–235 (2010).
6. Spallek, T., Mutuku, M. & Shirasu, K. The genus Striga: a witch’s herb. *Nature* 543, 47–54 (2014).
7. Li, J., Lis, K. E. & Timko, M. P. Molecular genetics of race-specific resistance in cowpea to Striga gesnerioides (Willd.). *Pest Manag. Sci.* 65, 520–527 (2009).
8. Li, J. & Timko, M. P. Gene-for-gene resistance in Striga-cowpea associations. *Science* 325, 1094 (2009).
9. Duriez, P. et al. A receptor-like kinase enhances sunflower resistance to Orobanche cumana. *Nat. Plants* 5, 1211–1215 (2019).
10. Yoshida, S., Cui, S., Ichihashi, Y. & Shirasu, K. The haustorium, a specialized invasive organ in parasitic plants. *Annu. Rev. Plant Biol.* 67, 643–667 (2016).
11. Dörr, I. Fine structure of intracellular growing cuscuta-hyphae. *Protoplasma* 67, 123–& (1969).
12. Dörr, I. Contact of cuscuta-hyphae with sieve tubes of its host plants. *Protoplasma* 75, 167–& (1972).
13. Ihl, B., Tutakhil, N., Hagen, A. & Jacob, F. Studies on cuscuta-reactions of Orobanche cumana. *scientia* 353, 478–481 (2016).
14. Johnsen, H. R. et al. Cell wall composition profiling of parasitic giant dodder (Cuscuta reflexa) and its host: a priori differences and induced changes. *New Phytol.* 207, 805–816 (2015).
15. Hegensauer, V. et al. Detection of the plant parasite Cuscuta reflexa by a tomato cell surface receptor. *Science* 353, 478–481 (2016).
16. Hanisch, F. G., Jovanovic, M. & Peter-Katalinic, J. Glycprotein identification and localization of O-glycosylation sites by mass spectrometric analysis of deglycosylated/alkylaminylated peptide fragments. *Anal. Biochem.* 290, 47–59 (2001).
17. Vaughan, K. C. Attachment of the parasitic weed dodder to the host. *Protoplasma* 219, 227–237 (2002).
18. Vaughan, K. C. Dodder hyphae invade the host: a structural and immunocytochemical characterization. *Protoplasma* 220, 189–200 (2003).
19. Olsen, S. et al. Getting ready for host invasion: elevated expression and action of xyloglucan endotransglucosylases/hydrolases in developing haustoria of the holoparasitic angiosperm *Cuscuta*. *J. Exp. Bot.* 67, 695–708 (2016).
20. Czolpynska, M. & Burek, M. Plant glycine-rich proteins in stress response: an emerging, still prospective story. *Front. Plant Sci.* 9, 302 (2018).
21. Vogel, A. et al. Footprints of parasitism in the genome of the parasitic flowering plant Cuscuta campestris. *Nat. Commun.* 9, 2515 (2018).
22. Ranjan, A. et al. De novo assembly and characterization of the transcriptome of the parasitic weed Cuscuta pentagona identifies genes associated with plant parasitism. *Plant Physiol.* 166, 1186–1199 (2014).
23. Sun, G. L. et al. Large-scale gene losses underlie the genome evolution of parasitic plant Cuscuta australis. *Nat. Commun.* 9, 2683 (2018).
24. Mangeon, A., Junqueira, R. M. & Sachetto-Martins, G. Functional diversity of the plant glycine-rich proteins superfamily. *Plant Signal. Behav.* 5, 99–104 (2010).
25. Mangeon, A. et al. The tissue expression pattern of the AtGTP5 regulatory region is controlled by a combination of positive and negative elements. *Plant Cell Rep.* 29, 461–471 (2010).
26. Franz-Wachtel, M. et al. Global detection of protein kinase D-dependent phosphorylation events in nodocazole-treated human cells. *Mol. Cell Proteomics* 11, 160–170 (2012).
27. Strohhalm, M., Kavan, D., Novak, P., Volny, M. & Havlicek, V. mMass 3: a cross-platform software environment for precise analysis of mass spectrometric data. *Anal. Chem.* 82, 4648–4651 (2010).
28. Nakamura, S. et al. Gateway binary vectors with the bialaphos resistance gene, aap, as a selection marker for plant transformation. *Biosci. Biotechnol. Biochem.* 74, 1315–1319 (2010).
29. Albert, M. et al. A two-hybrid-receptor assay demonstrates heteromer formation as switch-on for plant immune receptors. *Plant Physiol.* 163, 1504–1509 (2013).
30. Albert, I. et al. An RLP23–SOBR1–BAK1 complex mediates NLP-triggered immunity. *Nat. Plants* 1, 15140 (2015).
31. Thueirig, B., Felix, G., Binder, A., Roller, T. & Tamm, L. An extract of Penicillium chrysogenum elicits early defense-related responses and induces resistance in Arabidopsis thaliana independently of known signalling pathways. *Physiol. Mol. Plant Pathol.* 67, 180–193 (2005).

**Acknowledgements**

The work of M.A. was funded by the German Research Foundation (DFG AL 1426/1-2 and 1-3; AL 1426/4-1). K.K. and J.-A.B. were supported by grant 16-TF-KK from the Tromsø Research Foundation. The work of N.S. was supported by USDA-NIFA (2013-02345). We thank Farid El Kasm from the ZMBP Tübingen for kindly providing us the modified pGWB660 including the tagRFP. We would further like to thank Rory Pruitt for constructive criticism and critical reading of the manuscript.

**Author contributions**

V.H. isolated and identified the *C. reflexa* GRP as defense trigger. V.H., M.K., B.K., and B.L. prepared *Cuscuta* extracts and purified the GRP. M.K. did microscope work and photography. J.A.B., A.R., N.S., and K.K. helped with bioinformatics, gave access to unpublished *Cuscuta* sequencing data and helped with the identification of the GRP gene/RNA. V.H. and P.S. helped with primer design, GRP cloning, expression, and minimal peptide motif identification. I.D.B., J.S., F.L.H.M., B.M., V.G.E., and M.S. did mass spec analyses and helped with MS-data interpretation. I.A. and R.B. performed binding studies; B.L. tested peptides for activity in bio-assays. V.H., K.K., T.N., G.F., M.S., and M.A. designed and discussed the experiments. All authors discussed the data referring to their respected experience and helped with interpretations and data analyses. V.H., G.F., K.K., N.S., M.S., and M.A. wrote the manuscript.

**Funding**

Open Access funding enabled and organized by Projekt DEAL.

**Competing interests**

The authors declare no competing interests.

**Additional information**

Supplementary information is available for this paper at https://doi.org/10.1038/s41467-020-19147-4.

**Correspondence and requests for materials should be addressed to M.A.**

**Peer review information** *Nature Communications* thanks the anonymous reviewers for their contribution to the peer review of this work. Peer review reports are available.

Reprints and permission information is available at http://www.nature.com/reprints

**Publisher’s note** Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

© The Author(s) 2020