The Arabidopsis leucine-rich repeat receptor-like kinase MIK2 is a crucial component of early immune responses to a fungal-derived elicitor

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Summary

- *Fusarium* spp. cause severe economic damage in many crops, exemplified by Panama disease of banana or Fusarium head blight of wheat. Plants sense immunogenic patterns (termed elicitors) at the cell surface to initiate pattern-triggered immunity (PTI). Knowledge of fungal elicitors and corresponding plant immune-signaling is incomplete but could yield valuable sources of resistance.
- We characterized *Arabidopsis thaliana* PTI responses to a peptide elicitor fraction present in several *Fusarium* spp. and employed a forward-genetic screen using plants containing a cytosolic calcium reporter to isolate *fusarium elicitor reduced elicitation* (ferE) mutants.
- We mapped the causal mutation in ferE1 to the leucine-rich repeat receptor-like kinase MIK1-INTERACTING RECEPTOR-LIKE KINASE 2 (MIK2) and confirmed a crucial role of MIK2 in fungal elicitor perception. MIK2-dependent elicitor responses depend on known signaling components and transfer of AtMIK2 is sufficient to confer elicitor sensitivity to *Nicotiana benthamiana*.
- Arabidopsis senses *Fusarium* elicitors by a novel receptor complex at the cell surface that feeds into common PTI pathways. These data increase mechanistic understanding of PTI to *Fusarium* and place MIK2 at a central position in Arabidopsis elicitor responses.

Introduction

*Fusarium* fungi form a large species complex that includes a number of economically important plant pathogens. Current global outbreaks of Panama disease caused by *Fusarium oxysporum* f. sp. *cubense* Tropical Race 4 (*Foc TR4*) critically threaten banana production worldwide and provide a prime example of the devastating properties of the Fusarium wilt pathogen (Dita et al., 2018). Major resistance (*R*) genes have been used widely in agriculture to provide effective and highly specific pathogen resistance, but as the corresponding recognized effectors (avirulence-genes) from the pathogen can be readily lost or altered by mutation, these genes rapidly become ineffective (McDonald & Linde, 2002). For example, various resistance genes of tomato have been overcome by different Fusarium wilt races (de Sain & Rep, 2015). In many crops, genetic sources of resistance against *Fusarium* spp. are scarce, or they provide quantitative resistance such as *Fhb1* in wheat against Fusarium head blight (Hao et al., 2020). Transgenic resistance to Panama disease has been demonstrated successfully, yet no genetic resistance is currently available in commercial banana and no fungicide or biocontrol measure has proven effective (Dale et al., 2017). Targeted fundamental research into plant nonrace-specific basal immunity against *Fusarium* fungi represents one strategy to develop novel sources of quantitative resistance which may be applicable to a broad spectrum of pathosystems and more durable than those mediated by a specific resistance locus.

Plants have evolved diverse pattern-recognition receptors (PRRs) at the cell surface to detect microbial invaders and form multipartite PRR complexes to initiate and regulate intracellular signaling pathways (Couto & Zipfel, 2016). Molecular patterns from diverse origins (modified-self or nonself; collectively termed elicitors) can be perceived by plant PRRs contributing to quantitative disease-resistance via the activation of pattern-triggered immunity (PTI) (Boller & Felix, 2009). Various elicitors from bacteria, fungi and oomycetes are perceived by plants and initiate PTI responses (Saijo et al., 2018). Plants differ substantially in their capacity to recognize and respond to different elicitors, suggesting a rich diversity of PRRs and their cognate microbial ligands, many of which remain unidentified. Much of our current understanding of PTI in plants comes from *Arabidopsis thaliana* where several PRRs and associated signaling components have been identified. A classic example is the PRR FLAGELLIN-SENSITIVE 2 (FLS2), which recognizes bacterial flagellin or its...
conserved peptide derivatives (e.g. flg22) (Felix et al., 1999; Gomez-Gomez & Boller, 2000). A wealth of genetic resources and well-established PTI marker assays make Arabidopsis a highly amenable system towards identifying novel immunity components and determining their role in known signaling pathways.

The most prominent fungal-derived elicitor is the cell wall component chitin and other examples include endopolygalacturonases (PGs), ethylene-inducing xylanase (EIX), and Sclerotinia culture filtrate elicitor 1 (SCFE1) (Ron & Avni, 2004; Zhang et al., 2013; Zhang et al., 2014; Sanchez-Vallet et al., 2015). A conserved 20 amino acid (AA) pattern from NEP1-like proteins (NLPs) – referred to as nlp20 – present in many fungal, oomycete and bacterial species also triggers immune responses in various dicotyledonous plant species (Boehm et al., 2014; Oome et al., 2014). Nonrace-specific elicitors derived from Fusarium spp. cell walls or culture filtrates have been shown to elicit typical PTI responses in diverse plant systems (Ndimba et al., 2003; Chivas et al., 2005; Davies et al., 2006; Dey et al., 2009; Kesten et al., 2019; Li et al., 2019a,b). Nevertheless, specific knowledge of elicitors from Fusarium spp. and corresponding plant immunity components is sparse.

In order to identify novel components involved in nonrace-specific immunity to Fusarium spp., we generated an elicitor-active extract from diverse Fusarium spp. that induces PTI marker responses in Arabidopsis and used this to screen for mutants with compromised immune responses. This revealed a mutant strongly impaired in PTI responses to Fusarium extracts yet fully responsive to other fungal elicitors. We identified a causal mutation in the leucine-rich repeat receptor-like kinase MIK2, which was described previously in the context of abiotic and biotic stress resistance, sexual reproduction and cell-wall integrity sensing (Julkowska et al., 2016; Wang et al., 2016; Van der Does et al., 2017; Engelsdorf et al., 2018). Importantly, MIK2 also contributes to Arabidopsis resistance against Fusarium oxysporum (Van der Does et al., 2017). Here, we describe a new function of MIK2 in early immune responses to extracts from Fusarium spp.

Materials and Methods

Plant material and growth conditions

All plant material used for this study is listed in Supporting Information Table S1. For growth in liquid medium, Arabidopsis thaliana seeds were surface-sterilized, stratified for at least 48 h (dark; 4°C) and grown under long-day conditions (16 h : 8 h, light ; dark photoperiod; 20–22°C) in liquid MS medium (½ Murashige & Skoog medium plus vitamins (Duchefa, Haarlem, Holland), 0.25% sucrose, 1 mM MES, pH 5.7). The A. thaliana plants for reactive oxygen species (ROS) measurements were sown on a soil/vermiculite mix (8 : 1), stratified for 2 d (dark; 4°C) and grown under short-day conditions (8 h : 16 h, light ; dark photoperiod; 20–22°C; 55% RH). Nicotiana benthamiana plants for transient expression assays were grown on soil under long-day conditions (16 h : 8 h, light ; dark photoperiod; 21–23°C; 55% RH).

Fusarium elicitor preparation, enrichment and characterization

Fusarium graminearum (isolate Fg006) is part of the TUM Phytopathology collection and Fusarium oxysporum (isolate DSM 62292) was kindly provided by Ludwig Niessen (TU Munich, Germany). Trichoderma atroviride (isolate SZMC 20780) was kindly provided by Laszlo Kredics (University of Szeged, Hungary). Fungal strains were grown in liquid malt medium (3% malt extract, 0.3% peptone) or synthetic liquid medium (Gotthardt et al., 2020) at 25°C on a 70 rpm shaker for 7–10 d. Fungal mycelium was washed thoroughly with distilled water, then lyophilized and ground.

For preparation of FGE (Fusarium graminearum elicitor), FOE (Fusarium oxysporum elicitor) or TAE (Trichoderma atroviride elicitor), mycelial powder was dissolved in water and autoclaved for 20 min at 121°C. The soluble fraction was concentrated and desalted using PD-10 desalting columns (GE Healthcare, Chicago, IL, USA). Crude elicitor fractions (i.e. FGE/FOE) were quantified based on the weight of starting material used (mg lyophilized mycelium ml⁻¹).

For the preparation of enriched elicitor fractions (EnFOE and EnTAE), the soluble fraction obtained above was dialyzed in 122-mm tubing with a 14 kDa cut-off (Biomer, Hamburg, Germany) overnight at 4°C then lyophilized. The powder was dissolved in water, desalted, then incubated 1 : 2 with Macro-Prep High Q anion exchange media (Bio-Rad) on a shaker at RT for 1 h. ‘Qbound’ fractions (supernatant and subsequent water washes) were concentrated, desalted and incubated 1 : 2 with Macro-Prep High S cation exchange media (Bio-Rad). Samples were eluted using 0.5 M NaCl and lyophilized. Finally, the ‘Qbound/Seluate’ powder was dissolved in water and desalted. Protein concentration was determined using a Pierce™ BCA Protein Assay Kit (Thermo Scientific, Waltham, MA, USA).

For F. oxysporum culture filtrate (FOCF) preparation, F. oxysporum was grown in synthetic liquid medium for 10 d and mycelium/spores removed using filter paper followed by centrifugation. One liter of supernatant was autoclaved, lyophilized, resuspended to 15 ml with water, then twice desalted to 60 ml. Preparations were filtered through a membrane with a 5 kDa cut-off (Vivaspin 2; Sartorius Stedim Lab Ltd, Stonehouse, UK) and the > 5 kDa fraction was used for experiments.

For proteinase treatment, elicitor samples were incubated overnight at 50°C ± proteinase K (10 μg ml⁻¹; Carl Roth GmbH, Karlsruhe, Germany) then heat-inactivated for 15 min at 95°C. To determine protein kinase dependency, Col-0AEQ seedlings were pre-treated with K252a (10 μM; Sigma-Aldrich) or DMSO as a solvent-only control 30 min before elicitor application.

Elicitors

Chitin derived from shrimp shell (C9752; Sigma-Aldrich) was ground and re-suspended in water for experiments. Flg22 (QRLSTGSRINSKDDAALQIA), Pep1 (ATKVKAQKRG KEKSSGRPGQH) and the Nep-1-like peptide from
F. oxysporum (FoNLP; AYIMAWYPKPDQPADGNLVSGHR) were synthesized on an Abimed EPS221 system (Abimed, Langenfeld, Germany). RALF17 (NSIGAPREDLPKGCAPGSSAGCKMPPNPYPKPGCEASQRCRGG) was ordered from Pepmic Corp. Ltd (Suzhou, China).

Detection of ROS in A. thaliana leaf discs
Leaf discs (Ø4-mm) from 6–8-wk-old soil-grown plants were floated overnight on 200 µl water in 96-well plates (dark; RT). Before the measurement, water was replaced with 75 µl of a 2 µg ml⁻¹ horseradish peroxidase (type II; Roche) and 5 µM L-012 (WAKO Chemicals, Neuss, Germany) solution. Luminescence was recorded as relative light units (rlu) at 1 min intervals with a Luminoskan Ascent 2.1 (Thermo Scientific) or a Tecan F200 (Tecan, Mannheim, Switzerland) luminometer. Measurements consisted of a 10 min background reading then a 60-min reading after elicitor application to the appropriate final concentration. Recorded values were normalized to average ROS measured over the last 5 min before elicitor treatment followed by subtraction of values for mock-treated controls (included for each genotype on the same plate). Individual measurements over the 60 min (kinetics) after elicitation (time point (T)0) or the highest luminescence achieved (maxima) were used for analyses. To compare elicitor responses between mutant genotypes measured on separate plates, values were normalized to the mean maximum rlu measured in respective wild-type (WT) controls from each plate (set to 1; dashed line in Fig. 2a–c and Fig. S8a–c). Statistical analysis of maximum ROS (unpaired Student’s t-test) to respective WT control with two-tailed p-value) was performed using PRISM 8.0.1 (Graphpad Software, San Diego, CA, USA). Measurement of RALF peptide-induced ROS was performed according to Stegmann et al. (2017) on a Tecan F200 luminometer.

Aequorin luminescence measurements
Eight-day-old liquid-grown apoaequorin-expressing seedlings were placed individually in 96-well plates containing 100 µl of 5 µM coelenterazine-h (PKJ, Kleinblittersdorf, Germany) overnight (dark; RT). Resting luminescence intensities were determined by scanning each well 12 times at 10 s intervals, then a 25 µl elicitor preparation was added at the appropriate concentrations and luminescence measured 180 times at 10 s intervals (Luminoskan Ascent 2.1). The remaining aequorin was discharged using 150 µl discharge solution (2 M CaCl₂, 20% ethanol) per well. Cytosolic elevations of calcium ion concentration ([Ca²⁺]₁cyt) were calculated as \( L/L_{\text{max}} \) (luminescence counts per second (L) relative to total luminescence counts remaining \( (L_{\text{max}}) \) as described previously (Ranf et al., 2012). For [Ca²⁺]₁cyt measurements in different plant tissues, 9-d-old seedlings grown vertically on solid MS medium (as described above with 0.9% agarose; Sigma-Aldrich) were dissected and respective tissues incubated in substrate as indicated above. Individual measurements over 30 min (kinetics) after elicitation (i.e. T0) or the highest luminescence achieved (maxima) were used for analyses. Statistical analysis of maximum [Ca²⁺]₁cyt (\( L/L_{\text{max}} \)) (unpaired Student’s t-test) to respective WT control with two-tailed \( P \) value) was performed using PRISM 8.0.1.

Immunoblot analysis of mitogen-activated protein kinase phosphorylation
Fourteen-day-old liquid-grown seedlings were equilibrated for 24 h in fresh MS medium then elicitor solutions were added to the appropriate concentrations and seedlings were harvested at the stated time points. Proteins were extracted (50 mM Tris-HCl pH 7.5, 100 mM NaCl, 20 mM EDTA, 30 mM glycerophosphate, 30 mM 4-NPP, 20 mM MgCl₂, 4 mM NaF, 4 mM Na₃VO₄, 4 mM Na₂MoO₄, 10 mM DTT, 0.2% Tween-20, 1% protease inhibitor (P9599; Sigma)), quantified, then boiled in SDS sample buffer (50 mM Tris-HCl pH 6.8, 1% β-Mercaptoethanol, 2% SDS, 0.1% bromophenol blue, 10% glycerol). Proteins were separated by SDS-PAGE and transferred to a suitable membrane. Immunoblotting with an antibody against phosphorylated mitogen-activated protein kinases (MAPKs) (p44/p42; 9101; Cell Signaling Technology, Leiden, the Netherlands) was performed. Blots were visualized using SuperSignal West Dura Substrate (Thermo Scientific) in a Fusion SL Imager (Vilber Lourmat, Eberhardzell, Germany) with the accompanying software. Amido Black staining confirmed equal protein loading and blotting (50 µg total protein per sample; a Rubisco band of c. 56 kDa is shown).

Gene-expression analysis
Ten-day-old liquid-grown seedlings were equilibrated in fresh MS medium for 24 h before water or elicitor treatment at the stated final concentrations. Whole seedlings were harvested at 2 h and total RNA was extracted using a conventional Trizol/chloroform method. RNA was treated with DNasel then reverse-transcribed using oligo(dT) and RevertAid reverse transcriptase (Thermo Scientific). Complementary DNA was mixed together with SYBR Green™ (Thermo Scientific) and gene-specific primers (Table S2) then quantitative reverse-transcription (qRT)-PCR performed using an AriaMX Real-Time PCR system (G8830A instrument; Agilent) and the accompanying software (v1.5). Target gene expression was calculated in Excel 2010 (Microsoft, Redwood, WA, USA) using the 2⁻ΔΔCT method described by Livak & Schmittgen (2001) and normalized to the house-keeping gene Ubiquitin 5 (UBQ5). Mean values from two technical replicates included for each sample–primer combination were used for analysis. Data represent relative expression of each target gene compared to the water-treated control for each genotype.

Arabidopsis thaliana mutant screen
Col-0Aeq mutant screening was performed similar to previous studies (Ranf et al., 2012, 2015). Seeds were treated with 0.3% ethyl methane sulfonate (EMS; Sigma-Aldrich), grown on soil and harvested individually. 12–24 M₂ seedlings per M₁ plant were treated.
were measured for aequorin luminescence in response to FGE as described above but without elicitor. FGE was used as we initiated screening before elicitor enrichment. Seedlings with impaired elevations in aequorin luminescence were ‘rescued’ to solid MS media and later transferred to soil for seed production. Mutant phenotypes were verified by quantitative [Ca$^{2+}$]$_{cyt}$ measurements on corresponding M$_3$ offspring. *Fusarium elicitor reduced elicitation 1 (fere1)* was backcrossed (Col-0/ATG) to reduce background mutations and a single F$_2$ offspring homozygous for *fere1* was used for all experiments and subsequent genetic material produced in this study.

Mapping and sequencing of candidate genes

Approximately 200 *fere1* × Landsberg erecta-0 (Ler-0) F$_2$ were grown on soil for seed setting. F$_3$ progeny were phenotyped using quantitative [Ca$^{2+}$]$_{cyt}$ measurements and harvested in pools (25–30 individuals per F$_2$ parent) for gDNA isolation. Pools representing 22 *fere1*-like F$_2$ and five WT-like F$_2$ individuals were used for mapping. The analysis of PCR-based markers localized *FERE1* to a mapping interval of approximately 0.4 Mbp (position: 5372922–5756738) on chromosome 4 containing 121 gene loci (*T AIR v.10;* https://www.arabidopsis.org/) including the *MDIS1-INTERACTING RECEPTOR-LIKE KINASE 2* (*MIK2*) gene locus (position: 5636479–5640952). A W876STOP mutation in *fere1* was confirmed by sequencing and a derived cleaved amplified polymorphic sequence (dCAPS) marker was developed to genotype the mutation (Neff *et al.*, 2002). For genotyping by dCAPS, gDNA samples were amplified by PCR with appropriate primers (Table S2) then digested at 37°C for 2 h with the enzyme *HpyF31* (DdeI) (Thermo Scientific).

Molecular cloning and generation of transgenic lines

In order to generate a MIK2 construct with a C-terminal cMyc fusion (EQKLISEEDL) under the control of its own putative promotor, the full-length genomic fragment of *MIK2* (At4g08850) including a 2-kb upstream region was first amplified from Col-0 gDNA using appropriate primers (Table S2). The pENTR™/D-TOPO™ Cloning Kit was used (Thermo Scientific) to generate a Gateway compatible entry clone then the gene was transferred into pEarleyGate303 for plant expression. To generate a MIK2.1 construct, the MIK2 promotor and cDNA sequences were subcloned into GoldenGate modules using appropriate primers (Table S2) then assembled together into a GoldenGate-modified pCB302 binary vector for plant expression. Consequently, constructs were transferred into *Agrobacterium tumefaciens* (GV3101) and *fere1* plants were transformed by floral-dip transformation. BASTA selection on soil or MS agar plates (glufosinate-ammonium; Bayer CropScience, Langenfeld, Germany) was used to establish independent homozygous T$_3$ lines. The presence of cMyc-tagged MIK2 in *fere1*/MIK2-cMyc seedlings was confirmed via immunoblotting as described below.

**Transient expression in *Nicotiana benthamiana* and immunoblotting**

MIK2 variants with C-terminal cMyc fusion (CaMV35S promotor) were generated by GoldenGate fusions using appropriate primers (Table S2). The kinase-dead MIK2 variant (MIK2^km-cMyc) was generated by inserting a K802A mutation into the conserved ATP-binding site of the kinase domain via site-directed mutagenesis. The truncated MIK2 variant present in *fere1* (AA 1-875) was also generated (FERE1-cMyc). The *A. tumefaciens* (GV3101) cultures carrying the appropriate constructs were grown overnight in medium containing 100 μM acetosyringone then harvested, washed and re-suspended in infiltration medium (10 mM MgCl$_2$, 10 mM MES, pH 5.7) supplemented with 150 μM acetosyringone to an absorbance of 0.5 at 600 nm. Cell suspensions were incubated for 2 h at RT, mixed 1:1 with GV3101 carrying the p19 silencing suppressor, then infiltrated via a needle-less syringe into leaves of 6–8-wk-old *N. benthamiana* plants. ROS measurements were performed using leaf discs from infiltrated sites 48 h post-infiltration. Additional leaf discs (20 per construct and repeat) were used to generate total protein extracts for visualization of recombinant MIK2-cMyc variants. Proteins were separated by SDS-PAGE (8% gel; 75 μg total protein per sample) and transferred to a suitable membrane. Immunoblotting with an antibody against cMyc (1:500; 13-2500; Life Technologies, Carlsbad, CA, USA) was performed overnight followed by a 2 h incubation with anti-mouse antibody conjugated to horseradish peroxidase (1:5000; sc-516102; Santa Cruz, Dallas, TX, USA). Blots were visualized on Fuji Medical X-Ray Films (100NIF; 13x18; FujiFilm, Tokyo, Japan) using SuperSignal West Femto Substrate (Thermo Scientific) then stained with Coomassie Brilliant Blue (Sigma-Aldrich) to verify equal protein loading.

**Co-immunoprecipitation**

BRI1-ASSOCIATED RECEPTOR KINASE 1 (BAK1) with a C-terminal green fluorescent protein (GFP) fusion (CaMV35S promotor) was generated by GoldenGate cloning using appropriate primers (Table S2). GV3101 strains carrying BAK1-GFP, MIK2-cMyc or p19 were co-infiltrated 1:1:1 into *N. benthamiana* leaves as described, followed by infiltration with EnFOE or water (mock) solutions after 3 d. Total protein was extracted (25 mM Tris-HCl pH 7.5, 150 mM NaCl, 10% glycerol, 0.5% NP-40, 1 mM EDTA, 1% PVPP, 10 mM DTT, 1 mM PMSF, 1% protease inhibitor; 30 min incubation) from infiltrated tissues 20 min after elicitation then incubated with GFP-Trap Magnetic Agarose Beads (gtma-10; Chromotek, Munich, Germany) for 2 h. Samples were washed three times (buffer as previous without addition of NP-40, PVPP and DTT) then boiled in SDS sample buffer. Immunoblotting was performed as described for cMyc, or with anti-GFP (1:500; 3H9; Chromotek) then anti-rat (1:5000; A9542; Sigma-Aldrich) antibodies.
Fusarium oxysporum infection assays

Two-week-old Arabidopsis seedlings were transferred to finely sieved soil containing 10K F. oxysporum spores per cm² (isolate Fe5176). Plants were grown under 11 h:13 h, light:dark photoperiod (28°C; 80% RH) and scored after 2 wk into three categories: Unaffected (not visibly different from mock-treated plants), affected (visibly stunted/wilted with chlorotic leaves) or decayed (no green leaf material remaining).

Results

A crude elicitor fraction from Fusarium species elicits typical pattern-triggered immunity (PTI) responses in Arabidopsis

In order to determine the feasibility of identifying Fusarium-relevant PTI components by forward genetics, we characterized the hallmark PTI response of cytosolic elevation of calcium ion concentration ([Ca²⁺]ₙ) in Arabidopsis to crude Fusarium graminearum elicitor (FGE) or Fusarium oxysporum elicitor (FOE) fractions derived from fungal mycelium. Application of FGE or FOE to apoaequorin-expressing reporter plants (Knight et al., 1991; Ranf et al., 2011) induced [Ca²⁺]ₙ elevations, implicating the presence of elicitor activity in the extracts (Fig. 1a). As chitin oligomers derived from fungal cell walls are potent elicitors of immune responses in plants, we tested whether the induced responses were triggered by chitin. The CHITIN ELICITOR RECEPTOR KINASE 1 mutant (cerk1) lacks an essential component of chitin-mediated PTI in Arabidopsis (Miya et al., 2007) yet showed a WT response to crude Fusarium elicitor preparations such as FOE, indicating that chitin is not a major contributor of the responses we observed (Fig. 1b).

As crude mycelial fractions likely contain multiple elicitors that might initiate variable PTI responses, we enriched an elicitor fraction from F. oxysporum (EnFOE) by ion exchange resins. EnFOE gave robust and consistent responses with multiple PTI marker assays in Arabidopsis, including [Ca²⁺]ₙ elevations, ROS production, MAPK-phosphorylation and defense-marker gene activation (Fig. 1c–f). EnFOE was furthermore capable of eliciting ROS production in Brassicaceae such as Brassica rapa and Capsella rubella, as well as in barley (Hordeum vulgare) and in soybean (Glycine max), but not in the Solanaceae member N. benthamiana (Fig. S1). EnFOE-induced ROS responses could be abolished using a kinase inhibitor and initial biochemical characterization of EnFOE revealed that its PTI-triggering activity is sensitive to proteinase K treatment and shows a typical dose-response profile (Fig. S2a–d). EnFOE enriched from F. oxysporum grown in synthetic liquid medium (free from plant...
peptides) also gave comparable PTI responses in Arabidopsis including ROS production, confirming that the immunogenic activity derives from the fungus and not the plant-based growth medium (Fig. S2c).

Because *F. oxysporum* infects Arabidopsis via the roots and plant organs or tissues can vary in their responsiveness to different elicitors (Ranf et al., 2011), we tested organ-specific responses to EnFOE and compared these with responses to other fungal elicitors. We included chitin as a potent elicitor of root PTI responses, as well as the 23-AA Nep1-like peptide sequence from *F. oxysporum* (FoNLP) (Boehm et al., 2014). Although both roots and shoots are responsive, EnFOE-triggered [Ca\(^{2+}\)]\(_{cyt}\) elevations were higher in roots than in shoots. This was similar to the chitin response and in contrast to the FoNLP response, which was hardly detectable in roots (Fig. S3a–f). Weak PTI responses of roots also were reported for bacterial peptide elicitors elf18 and flg22, which are detected predominantly in aboveground tissues (Ranf et al., 2011). [Ca\(^{2+}\)]\(_{cyt}\) elevations in response to nlp20 were exclusively detected in the meristematic zone of the root (Wan et al., 2018), which could explain the low responsiveness to FoNLP observed in root tissues. Taken together, these data confirm that PTI responses to EnFOE are strongest in the root and may therefore be spatially relevant for basal resistance against *F. oxysporum*.

Arabidopsis responses to EnFOE are partially dependent on known PTI signaling components and are suppressed by an effector from *Fusarium oxysporum*

In order to determine whether known PTI components are necessary for recognition of EnFOE, we monitored EnFOE-induced PTI responses in a range of PTI-signaling mutants. PTI responses to EnFOE were found to be independent of SUPPRESSOR OF BIR1 (SOBIR1); a core component of receptor complexes containing receptor-like proteins (Liebrand et al., 2014) and of CERK1, as shown previously for FOE (Figs 1b, 2a), BOTRYTIS-INDUCED KINASE 1 (BIK1) and PBS1-LIKE 1 (PBL1), cytoplasmic kinases involved in many PTI pathways (Ranf et al., 2014), and the ROS-producing NADPH oxidase RESPIRATORY BURST OXIDASE HOMOLOG PROTEIN D (RBOHD) are required for full ROS responses to EnFOE (Fig. 2a). Hence, there is overlap in signaling components required for responses to EnFOE with those described for other elicitors.

The receptor-like kinase SOMATIC EMBRYOGENESIS RECEPTOR KINASE 3 (SERK3), also known as BAK1, is a central regulator of plant immune responses to immunogenic peptides and associates with multiple PRRs to initiate signal transduction in a partially redundant manner with other SERK-family proteins (Chinchilla et al., 2007; Liebrand et al., 2014). EnFOE-induced ROS production was reduced in bak1-4 compared to WT plants suggesting that BAK1 is involved in EnFOE-triggered immune signaling (Fig. 2b,d). Mutants representing additional SERK proteins (a family of five leucine-rich repeat receptor-like protein kinases (LRR-RLKs) in Arabidopsis) with documented roles in cell-surface signaling pathways did not show a significant reduction in EnFOE responsiveness (Fig. 2b). To further test for recognition of EnFOE by known PRRs, we measured EnFOE responses in additional mutant lines representing RECEPTOR-LIKE PROTEIN (RLP) 23, RLP30 and RLP42, each involved in detection of fungal elicitors (Zhang et al., 2013, 2014; Albert et al., 2015), or RÉSISTANCE TO FUSARIUM OXYSPORUM (RFO) 1, RFO2 and RFO3, known to be involved in quantitative resistance to *F. oxysporum* in Arabidopsis (de Sain & Rep, 2015). In each of these mutant lines, WT-like ROS responses were observed (Fig. 2c). Together, this suggested that our *Fusarium* elicitor preparations contain an unidentified effector that triggers canonical PTI responses in Arabidopsis.

In order to determine the potential biological relevance of EnFOE-mediated PTI in Arabidopsis–*Fusarium* interactions, we tested whether an effector from an adapted *F. oxysporum* strain is able to target EnFOE-mediated PTI responses. The *F. oxysporum* effector protein Avr2 functions as a virulence factor by suppressing PTI including flg22-induced responses (Di et al., 2017). Two independent Arabidopsis lines stably expressing Avr2 lacking its signal peptide (Avr2-D) were severely impaired in ROS responses to EnFOE (Fig. 2e). These lines show a mild growth phenotype (Di et al., 2017). *F. oxysporum* therefore produces an effector protein that can partially suppress PTI responses to an elicitor fraction derived from itself and subverting these responses might be relevant for establishing pathogenesis.

Forward genetics identified the *fusarium elicitor reduced elicitation 1* mutant

As forward genetic approaches do not require the molecular identity of the elicitor, we generated and screened Arabidopsis (Col-0) EMS populations for attenuated [Ca\(^{2+}\)]\(_{cyt}\) responses to FGE following protocols successfully used for isolating PTI-associated mutants (Ranf et al., 2015). We focused on mutants with strongly reduced responses to FGE but largely unaltered responses to chitin and flg22. This identified a mutant, which we termed *fere1*, with partially reduced responses to crude FGE and FOE fractions, genetically supporting the presence of similar elicitor activity in different *Fusarium* species (Fig. S4a,b). This activity also was present in an elicitor preparation from *F. oxysporum* culture filtrate (Fig. S4c). EnFOE-induced PTI responses are abolished in *fere1* showing that we successfully enriched the relevant immunogenic fraction of FOE (Fig. 3a,b). We further confirmed that *fere1* is able to respond to the fungal elicitors chitin and FoNLP, to which WT-like or slightly enhanced responses were observed, suggesting specificity for EnFOE-induced PTI (Fig. 3c).

The gene underlying the *fere1* phenotype encodes the receptor-like kinase MIK2

In order to identify the causal mutation in *fere1*, we generated a *fere1* outcross population with ecotype Ler-0 and confirmed phenotypic segregation of a single recessive locus which we mapped subsequently using insertion/deletion markers (Salathia et al., 2007). The mapping interval
contained a gene for an LRR-RLK called MIK2 (MALE DISCOVERER 1 (MDIS1)-INTERACTING RECEPTOR LIKE KINASE 2; also known as LRR-KISS). Because MIK2 was the only gene in the mapping interval that encodes an obvious candidate PRR complex component by protein domain architecture, we sequenced MIK2 in fere1 and identified a single-nucleotide polymorphism (SNP) resulting in a premature W876STOP codon within the cytoplasmic kinase domain. A gene model representing the fere1 SNP and various mik2 T-DNA insertion alleles used in this study is shown in Fig. 4(a). MIK2 is a class XIIIb LRR-RLK with described roles in cell-wall integrity sensing, root growth, sexual reproduction, and response to abiotic and biotic stress (Julkowska et al., 2016; Wang et al., 2016; Van der Does et al., 2017). MIK2 has one intron and the pre-mRNA is spliced into two forms (both of which are affected by the
mutation in fere1), with splice variant MIK2.1 being ≤50-fold more abundant than MIK2.2 (Van der Does et al., 2017).

In order to gain evidence that the fere1 SNP caused the loss of Fusarium elicitor responses, we tested T-DNA insertion lines with impaired MIK2 function (Alonso et al., 2003; Rosso et al., 2003). Three independent mik2 alleles showed largely abolished PTI responses to EnFOE, but not to chitin in which a slightly enhanced ROS response was seen (Fig. 4b). In addition, we generated mik2-3 mutants carrying the apoaequorin reporter (mik2-3AEQ) and observed a similar loss-of-function phenotype in response to EnFOE as shown for fere1 (Fig. 4c). Finally, the PTI loss-of-function phenotype in fere1 to EnFOE was fully complemented by stable MIK2 expression under the control of its own putative promoter as shown by ROS and [Ca^{2+}]_cyt response measurements. Likewise this was achieved by using the predominant MIK2.1 splice form amplified from cDNA in its native form (i.e. untagged), as well as the full-length genomic region of MIK2 with a C-terminal cMyc peptide fusion (Figs 4d,e, S5). Bioassays performed with these lines indicated that fere1 is more susceptible to F. oxysporum whereas WT-level resistance was restored in MIK2-complemented lines (Fig. S6). Together these data demonstrate that a MIK2 loss-of-function underlies the fere1 phenotype and that MIK2 is essential for EnFOE-induced PTI responses.

MIK2-dependent elicitor activity is not restricted to the Fusarium genus

The strong PTI loss-of-function phenotype of mik2 mutants to EnFOE can be used to genetically verify whether MIK2-dependent elicitor activity is present in other fungal species. To determine whether a conserved elicitor activity is present outside of the Fusarium genus, a comparable elicitor enrichment procedure was performed for Trichoderma atroviride (EnTAE). Trichoderma is a fungal genus from the same order (Hypocreales within the class of Sordariomycetes) as Fusarium (Crous et al., 2004). EnTAE gave a strong ROS response in WT plants, which was severely impaired in the three independent mik2 T-DNA mutants (Fig. S7a). Similar to EnFOE, ROS responses to EnTAE were independent of CERK1, yet partially or fully dependent on BAK1, BIK1, PBL1 and RBOHD (Figs S7a, 2a). The EnTAE-induced ROS response was reduced in fere1 plants yet fully restored in fere1/MIK2 complementation lines, confirming that MIK2 also is essential for PTI responses to EnTAE (Fig. S7b,c). Together, these data show that MIK2-dependent elicitor activity also is present in fungi closely related to Fusarium.

FERONIA is required for the full response to EnFOE

So far, MIK2 has not been implicated in PTI responses, but its expression is affected by pathogen infection or elicitor treatment (based on the Arabidopsis eFP Browser (Winter et al., 2007); Expression sets 1007966202 and 1008080727). Notably, MIK2 is important for resistance against F. oxysporum which previously was linked to its role in cell-wall integrity (CWI) monitoring (Van der Does et al., 2017). Our data suggest that the additional role of MIK2 in EnFOE-triggered PTI also could contribute to this resistance. PTI and CWI sensing are intimately interconnected (Hamann, 2012; Wolf, 2017; Engelsdorf et al., 2018), and it has been reported that mik2 shows reduced defence activation following isoxaben-induced inhibition of cellulose biosynthesis (Van der Does et al., 2017; Engelsdorf et al., 2018). To determine whether CWI sensing generally interferes with EnFOE sensing, we tested EnFOE responsiveness in mutant genotypes representing cell-surface RLKs with known roles in CWI sensing (e.g. HERCULES 1 (herk1), WALL-ASSOCIATED KINASE 2 (wak2), and...
THESEUS 1 (the1) (Engelsdorf et al., 2018). A WT-like ROS response was observed in herk1, wak2, and two independent the1 alleles (the1-1 [null mutant] and the1-4 [reported to be hypermorphic (Merz et al., 2017)]) to EnFOE (Fig. S8a), showing that impairment in CWI sensing generally does not interfere with EnFOE responses.

Fig. 4 Pattern-triggered immunity (PTI) marker responses in various mik2 alleles and MDIS1-INTERACTING RECEPTOR-LIKE KINASE 2 (MIK2)-complemented Arabidopsis thaliana plants to fungal elicitors. (a) MIK2 gene model showing predicted splice forms (MIK2.1, MIK2.2) and locations of the fusarium elicitor reduced elicitation1 (fere1) single nucleotide polymorphism (SNP) or relevant T-DNA insertions. (b) Maximum reactive oxygen species (ROS) accumulation (in relative light units; rlu) measured in individual leaf discs (each plotted point) from Col-0 or three independent mik2 T-DNA mutants over 60 min after elicitation with enriched Fusarium oxysporum elicitor (EnFOE; 0.5 µg ml⁻¹) or chitin (75 µg ml⁻¹) ± SD (n = 12, 12, 12, 12, 20, 20, 20, left to right). (c) Cytosolic elevation of calcium ion concentration ([Ca²⁺]ₗ) kinetics in Col-0 or mik2-3 seedlings after elicitation with EnFOE (0.5 µg ml⁻¹). Data represent mean values measured in individual seedlings ± SD at each time point (n = 24). (d) Maximum ROS accumulation (rlu) measured in individual leaf discs (each plotted point) from Col-0, fere1, fere1/MIK2.1 or fere1/MIK2-cMyc over 60 min after elicitation with EnFOE (0.5 µg ml⁻¹) or chitin (75 µg ml⁻¹) ± SD (n = 12, 12, 12, 12, 12, 12, 12, left to right). (e) Maximum [Ca²⁺]ₗ measured in individual Col-0, fere1, fere1/MIK2.1 or fere1/MIK2-cMyc seedlings (each plotted point) over 30 min after elicitation with EnFOE (0.5 µg ml⁻¹; left y-axis) or chitin (75 µg ml⁻¹; right y-axis) ± SD (n = 28, 28, 28, 20, 20, 20, 20, left to right). Data shown are pooled from multiple independent experiments (b: 3 for EnFOE, 5 for chitin; c; d: 3; e: 5 for EnFOE, 4 for chitin). Asterisks indicate significant difference (unpaired Student’s t-test: *P < 0.05; ***, P < 0.001) compared to the respective wild-type (WT) control (Col-0 or Col-0-AEQ).
Interestingly, a markedly reduced EnFOE-induced ROS response was observed in FERONIA fer-4 mutants and llg1-2 mutants (LORELEI-LIKE GPI ANCHORED PROTEIN 1; a chaperone of FERONIA involved in perception of RAPID ALKALINIZATION FACTOR (RALF) peptides and receptor complex assembly) (Fig. S8c–e), indicating a role of FERONIA in EnFOE-induced PTI signaling consistent with its role in controlling various immune signaling processes (Li et al., 2015; Shen et al., 2017; Stegmann et al., 2017; Xiao et al., 2019). WT-like responses to Pep1, an endogenous 23-AA damage-associated peptide recognized by LRR-RFks PEPR1 and PEPR2 (PERCEPTION OF THE ARABIDOPSIS DANGER SIGNAL PEPTIDE 1/2) in Arabidopsis (Yamaguchi et al., 2010), were observed in fer-4 and llg1-2 mutants however. As FERONIA and LLG1 are required for full EnFOE-induced PTI responses and Fusarium spp. such as F. oxysporum are known to secrete functional plant RALF homologs as pathogenicity factors (Masachis et al., 2016), we speculated whether MIK2 was involved in responses to RALF peptides. ROS production upon treatment with a synthetic RALF17 peptide was not impaired in mik2-3 whereas llg1-2 and fer-4 were completely insensitive, suggesting that MIK2 is not directly involved in responses to a PTI-inducing RALF peptide (Fig. S8f).

MIK2 has a close homolog (60% AA identity) termed MIK2-LIKE which may have genetic relatedness to MIK2 in the context of CWI monitoring via control of root growth angle, yet seemingly does not fulfill the same function as MIK2 in responses to cellulose-biosynthesis inhibition (Van der Does et al., 2017). Although the strong loss-of-function phenotype of fer1 and other mik2 alleles in response to EnFOE argues against redundancy of this response, we tested two independent mik2-like T-DNA insertion mutants for EnFOE-induced PTI responses. The mik2-like mutant plants did not show a reduced ROS response to EnFOE (Fig. S8b), whereas mik2/mik2-like double mutants showed a loss-of-function phenotype consistent with loss-of-function MIK2. Taken together, these data suggest that failure in CWI sensing does not generally interfere with Arabidopsis responses to EnFOE, but FERONIA and LLG1 contribute to full sensitivity.

Ectopic expression of AtMIK2 in Nicotiana benthamiana confers sensitivity to EnFOE

As EnFOE did not trigger ROS production in leaf discs from solanaceous N. benthamiana plants (Fig. S1), we investigated whether transient expression of Arabidopsis MIK2 could confer sensitivity to EnFOE. Indeed, a rapid production of ROS in N. benthamiana leaf discs after elicitation with EnFOE was observed upon heterologous expression of functional AtMIK2, but not a kinase-dead variant or the truncated FERE1 variant (Figs 5a,b, S9). This indicates that the AtMIK2 protein is sufficient to confer sensitivity to EnFOE and its kinase domain is essential for EnFOE-induced signaling in N. benthamiana. Notably, we found that BAK1 weakly associates with MIK2 when co-expressed in N. benthamiana and that this association is enforced upon EnFOE application (Fig. S10).

Discussion

Considering that fungi represent ubiquitous phytopathogens, it is likely that plants are capable of recognizing many immunogenic patterns from these organisms via a diverse assortment of cell-surface receptors. The Arabidopsis genome alone encodes over 400 receptor-like kinases (RLKs) with a predicted extracellular domain and only a small fraction of these have a demonstrated biological role (Shiu & Bleecker, 2001). We therefore sought potential pattern-triggered immunity (PTI) components to Fusarium extracts that could be underlying quantitative resistance and identified MDIS1-INTERACTING RECEPTOR-LIKE KINASE 2 (MIK2) as a key module in Fusarium sensing. Although we have not yet identified the elicitor, the finding that MIK2-dependent elicitor activity is present in diverse Fusarium spp. and the related Trichoderma atroviride suggests that MIK2 is key to detection of a conserved molecular pattern.

Fig. 5 Transient expression of Arabidopsis thaliana MDIS1-INTERACTING RECEPTOR-LIKE KINASE 2 (MIK2) protein variants in Nicotiana benthamiana. (a) Protein model of MIK2 variants showing relevant domains (LRR, leucine-rich repeat; TM, transmembrane). (b) Reactive oxygen species (ROS) accumulation kinetics in leaf discs from N. benthamiana either untransformed or transiently transformed with Arabidopsis wild-type (WT) MIK2-cMyc, the truncated FUSARIUM ELICITOR REDUCED ELICITATION 1 (FERE1)-cMyc variant, or a kinase dead variant (MIK2^km-cMyc) after elicitation with EnFOE (2 µg ml⁻¹). Data represent mean relative light units (rlu) measured from individual leaf discs ± SD at each measurement point (n = 20). ROS kinetics indicated with a dashed line are shown without SD for clarity. Data shown are pooled from multiple independent experiments (b: 3).
Enriched *Fusarium oxysporum* elictor (EnFOE)-mediated responses in Arabidopsis are dependent on several components previously implicated in PTI pathways and are suppressed by the *F. oxysporum* effector protein Avr2. Although the precise target of Avr2 is not yet known, the general PTI-suppressive capacity of Avr2 (Di et al., 2017) provides further evidence that EnFOE-mediated signaling converges into common PTI pathways and could therefore contribute to basal resistance. We could confirm that *fusarium elictor reduced elicitation 1 (fer1)* is more susceptible to *F. oxysporum* as shown previously for mik2 T-DNA mutant lines (Van der Does et al., 2017) and further demonstrated that the reduced resistance could be complemented by MIK2 expression. The relative contribution of EnFOE-mediated PTI in quantitative resistance against *Fusarium* spp., or to other fungi with MIK2-dependent elicitor activity, remains a topic for future studies. However, the current lack of a purified elicitor and confounding effects of MIK2 function in biological processes other than susceptibility might complicate these studies.

The full extent of MIK2 function in PTI and how this relates to its other described roles remains an open question. MIK2 domain architecture is similar to class XIIa LRR-RLK members FLAGELLIN-SENSITIVE 2 (FLS2) and EF-Tu RECEPTOR (EFR), which are bona fide PRRs, yet the involvement of MIK2 in cell wall integrity (CWI) sensing and salt tolerance (Julkowska et al., 2016; Van der Does et al., 2017; Engelsdorf et al., 2018) suggests that it may be involved in perception of both endogenous and exogenous signals. This does not exclude that MIK2 also could function as a direct peptide receptor. Indeed, the almost complete loss-of-function phenotype in diverse mik2 alleles to EnFOE indicates a key role in elicitor perception. Comparably, the RLK FERONIA, which has multiple functions in plants, also was shown to be a receptor for RALF peptides (Stegmann et al., 2017). The plant response to EnFOE is rapid and within the time frame of directly-perceived elicitors such as flg22 (Chinchilla et al., 2007). Therefore, direct binding of the elicitor is a likely explanation yet we cannot fully exclude something is released from the plant upon elicitor treatment (i.e. a damage-associated molecular pattern (DAMP)), which then triggers the observed responses. Residual enzymatic activity in EnFOE appears unlikely, however, because the extracts have been autoclaved before elicitor enrichment. Additionally, *Nicotiana benthamiana* would have to release a similar plant signal without having the corresponding MIK2-dependent response. Together with the ability of MIK2 to confer EnFOE sensitivity to *N. benthamiana*, this argues for MIK2 being a key part of plant recognition machinery that directly interacts with EnFOE. This hypothesis is further supported by the enhanced association of BAK1 with MIK2 in *N. benthamiana* after application of EnFOE, which is reminiscent of ligand–dependent receptor–complex formation (Chinchilla et al., 2007). Considering its involvement in responses to internal and external stimuli, MIK2 might therefore be an integrator of diverse signals for coordinated plant response to the environment.

Determining the presence of MIK2-dependent elicitor activity in diverse fungal species may greatly widen the role of MIK2 not only in resistance against fungal pathogens, but also to interactions with endophytic and beneficial fungi. *Fusarium* and *Trichoderma* spp. are common in soil and root ecosystems, and also can act as opportunistic plant symbionts that penetrate into the root epidermis and outer cortex to establish a long-term, robust colonization (Harman et al., 2004; Bacon & Yates, 2006). MIK2 could therefore modulate root colonization via perception of EnFOE-like elicitor activity from these fungi. Conserved elicitors from both pathogenic and commensal fungi can be equally perceived by plants and trigger PTI responses. However, mechanisms by which root systems initiate localized PTI responses at sites of pathogen attack without endangering commensal microbes have been demonstrated recently (Zhao et al., 2020). It will therefore be interesting to examine the role of MIK2 and MIK2-dependent PTI at local sites of root colonization by both pathogenic and commensal fungi.

Molecular identification of EnFOE is an important target for future work. However, elicitor identification has proven challenging in some cases (e.g. for *Sclerotinia* culture filtrate elicitor 1 or enigmatic MAMP of Xanthomonas) (Jehle et al., 2013; Zhang et al., 2013). Genetic resources can greatly assist elicitor characterization. For example, the LIPOOLIGOSACCHARIDE-SPECIFIC REDUCED ELICITATION (LORE) receptor mutant was crucial for further chemical dissection of the plant PTI response to enriched lipopolysaccharide fractions from *Pseudomonas* species and guided the identification of medium chain length 3-hydroxy fatty acids as the actual bacterial elicitors (Kutschera et al., 2019). Similar approaches with MIK2 could be used to guide EnFOE identification. Moreover, physical and/or genetic interaction studies with MIK2 may disclose the nature of the PRR complex responsible for PTI responses to EnFOE.

Alongside MIK2, several other LRR-containing cell-surface receptors have been recognized to contribute towards resistance against *Fusarium* spp. in plants, including homologous LRR-RLK genes from barley (*HvLRRK-I7*) and wheat (*TaLRRK-6D*) which contribute to basal defense against *Fusarium* head blight (Thapa et al., 2018), and Arabidopsis *RFO2* and tomato *I-7* genes which encode receptor-like proteins involved in *Fusarium* wilt resistance (Shen & Diener, 2013; Gonzalez-Cendales et al., 2016). Fundamental research in the model Arabidopsis will accelerate the process of identifying additional cell-surface receptors and downstream PTI components in crop plants, where *Fusarium* species cause relevant yield losses but comparable genetic resources are currently unavailable. MIK2 has putative orthologs of unknown function in several crop species, which may allow for comparative genomics between model and crop towards improved *Fusarium*-resistance breeding. Future genetic studies can be used to validate whether the PTI responses that we observe in soybean and barley to EnFOE correspond to a conserved recognition mechanism or to species-specific sensing of additional elicitors present in our enrichments. Finally, there are several examples of successful transfer of PRRs to enhance disease resistance (Boutron & Zipfel, 2017; Ranf, 2018). PRRs or other components found in Arabidopsis might be transferred to crops or recombined with crop genes to link up with endogenous immune components and thereby engineer durable and broad-spectrum resistance.
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Author contributions

RH and SR designed the study; SR, RH, FLWT, ADC and JM planned the experiments and interpreted results; ADC, JM and LR optimized methodology and performed experiments; ADC and JM prepared figures; and ADC and RH wrote and revised the manuscript. All authors discussed and approved the final manuscript.

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Supporting Information

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.

Fig. S1 *Fusarium* elicitor responses in additional plant species.
Fig. S2 Characterization of *Fusarium* elicitor responses.

Fig. S3 Root versus shoot responses to fungal elicitors.

Fig. S4 *Fusarium* crude elicitor responses in WT and *fere1* mutants.

Fig. S5 Visualization of recombinant MIK2-cMyc protein in *fere1/MIK2-cMyc*.

Fig. S6 *Fusarium oxysporum* infection assays.

Fig. S7 ROS response measurements in basal immunity-relevant mutant genotypes and MIK2-complemented plants to an enriched *T. atroviride* elicitor fraction.

Fig. S8 Elicitor responses of CWI-sensing and related mutants.

Fig. S9 Visualization of recombinant MIK2-cMyc protein variants from *N. benthamiana* protein extracts.

Fig. S10 EnFOE-induced BAK1-MIK2 association.

Table S1 Plant lines used in this study.

Table S2 Primers used in this study.

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