ELR is a true pattern recognition receptor that associates with elicitors from diverse *Phytophthora* species

Emmanouil Domazakis¹, Doret Wouters¹, Jan Lochman², Richard G. F. Visser¹, Matthieu H. A. J. Joosten³ and Vivianne G. A. A. Vleeshouwers¹

¹Plant Breeding, Wageningen University and Research, Droevendaalsesteeg 1, 6708 PB, Wageningen, The Netherlands.

²Department of Biochemistry, Faculty of Science, Masaryk University, Kotlářská 2, 61137 Brno, Czech Republic

³Laboratory of Phytopathology, Wageningen University & Research, Droevendaalsesteeg 1, 6708 PB Wageningen, The Netherlands.

Corresponding author: Vivianne G. A. A. Vleeshouwers, Plant Breeding, Wageningen University and Research, Droevendaalsesteeg 1, 6708 PB, Wageningen, The Netherlands, vivianne.vleeshouwers@wur.nl

Summary

The first layer of plant immunity against pathogens is mediated by cell surface pattern recognition receptors (PRRs) that recognize pathogen molecules in the apoplast. Several pairs of PRRs and their matching extracellular ligands have been described but, in many cases, actual evidence for ligand binding by the PRR is lacking. The receptor-like protein ELR from *Solanum microdontum*, which triggers cell death upon co-expression with elicitors of various *Phytophthora* species and enhances resistance to late blight caused by *Phytophthora infestans*, was previously identified as the elicitor receptor by forward genetic screenings employing the INF1 elicitor of *P. infestans*. In this study, we investigated whether ELR associates with INF1 and other elicitors that are secreted by diverse *Phytophthora* spp. We performed *in planta* and *in vitro* co-immunoprecipitation of ELR with several affinity-tagged elicitors, as well as *in planta* transient co-expression assays. We found that ELR physically interacts with the class I elicitors INF1 and ParA1, from *P. infestans* and *Phytophthora parasitica*, respectively, which is in line with their ability to cause cell death when co-expressed with ELR in potato. Together, we demonstrate that ELR is a genuine PRR that binds elicitors of *Phytophthora* species.

Introduction

A major part of a plant's defense response against microbes depends on the effective detection of immunogenic patterns, such as microbe-associated molecular patterns (MAMPs) (Couto and Zipfel, 2016; Gust and Felix, 2014; Jones and Dangl, 2006; Ranf, 2017; van der Burgh and Joosten, 2019). MAMPs are recognized in the plant apoplast, typically by membrane-localized pattern recognition receptors (PRRs). All plant PRRs that have been identified so far contain a variable number of leucine-rich repeats (LRR), lysine motifs or lectin domains in their ecto-domain, a transmembrane domain and may contain a kinase domain. LRR-type receptor-like proteins (RLPs) and receptor-like kinases (RLKs) predominantly recognize proteinaceous MAMPs or effectors, of which the latter are compounds produced by pathogens with the aim to perturb plant defense responses. Despite the high amount of PRRs encoded by plant genomes, and the increasing number of PRRs discovered over the past 20 years, for most of them it has not been determined whether they are actually indeed true receptors of their matching ligands, or just involved in the complex that mediates resistance to pathogens (Boutrot and Zipfel, 2017; Dodds and Rathjen, 2010; Tang et al., 2017; Zipfel, 2014). RLPs are relatively understudied when compared to RLKs, and providing evidence for binding of MAMPs or effectors has been challenging and is rather limited. Successful examples of RLPs for which ligand binding has been shown are the *Arabidopsis* RLP42/RBPG1 and RLP23, which associate with a fungal endo-polygalacturonase and the widely conserved MAMP nlp20, respectively (Albert et al., 2015; Zhang et al., 2014). From Solanaceous plants, LeEIX2 from tomato was found to associate with an ethylene-induced xylanase (Ron and Avni, 2004) and the LRR-RLP RXEG1 from *Nicotiana benthamiana* binds the glycoside hydrolase PsXEG1 from *Phytophthora sojae* and provides resistance to this oomycete pathogen (Wang et al., 2018).

Elicitins are a family of structurally conserved extracellular oomycete proteins, specific to the genera *Phytophthora* and *Pythium*. Elicitins have features of oomycete MAMPs, as they have been shown to induce localized cell death, salicylic acid-, jasmonic acid- and ethylene-dependent accumulation, as well as systemic resistance in several responding plant genotypes, across various families (Derevnina et al., 2016; Vleeshouwers et al., 2006). The Solanaceous plants *Nicotiana benthamiana*, *N. tabacum* and *S. microdontum* mount a specific cell death response upon treatment with the INF1 elicitin (Du et al., 2015; Huitema et al., 2005; Kamoun et al., 1998; Vleeshouwers et al., 2006). All elicitins share a highly conserved 98-amino-acid elicitin domain, which contains six cysteine residues at conserved positions for the formation of disulfide bridges (Derevnina et al., 2016; Jiang et al., 2006). Elicitins of the canonical class ELI-1 consist of a signal peptide for extracellular targeting, which is cleaved off upon secretion, and the elicitin domain only. Elicitins of all other classes possess an additional C-terminal extension, which is of variable length and is putatively involved in plasma membrane or cell wall anchoring.

ELR, an RLP from the wild potato species *Solanum microdontum*, mediates recognition of various elicitins of diverse *Phytophthora* species (Du et al., 2015). The recognition spectrum ranges from elicitins belonging to class ELI-1 (e.g. INF1, ParA1), ELI-2 (e.g. INF2A) and class ELI-4 (e.g. INF5, INF6) (Du et al., 2015; Jiang et al., 2006) (Figure S1). In contrast to bacterial flagellin, EF-Tu and NLPs (Bohm et al., 2014; Felix et al., 1999; Kunze et al., 2004), elicitins do not seem to share a conserved stretch of amino acids (Fig S1a), but rather the conserved overall structure of all elicitins is likely recognized by the extracellular LRRs of ELR (Derevnina et al., 2016). In line with this, the elicitins that are recognized by ELR share rather low amino acid similarities (Du et al., 2015) (Figure S1b).

ELR lacks a cytoplasmic kinase domain and is thus incapable of downstream signaling by itself. However, recently, it was shown that ELR physically associates with the RLK SUPPRESSOR

OF BIR1-1 (SOBIR1) in a ligand-independent manner, and associates with the RLK SOMATIC EMBRYOGENESIS RECEPTOR KINASE 3 (SERK3), also referred to BRI1-ASSOCIATED KINASE 1 (BAK1), upon ELR activation by elicitors (Domazakis et al., 2018; Du et al., 2015). Both SOBIR1 and SERK3 are required for ELR function and for triggering resistance to pathogens, including *Phytophthora infestans* (Chaparro-Garcia et al., 2011; Domazakis et al., 2018; Liebrand et al., 2014; Peng et al., 2015).

In this study, we provide evidence that ELR physically associates with INF1 elicitor, using *in planta* and *in vitro* approaches. Furthermore, we tested various additional elicitors of different *Phytophthora* species and found a correlation between physical interaction of the elicitor with ELR and the occurrence of an ELR-mediated cell death response. Our data render ELR a genuine PRR, which associates with various members of the elicitor family of *Phytophthora* species.

Materials and methods

Plant materials

Nicotiana benthamiana plants were grown from seeds and *Solanum hjertingii* genotype 349-3 (HJT349-3) plants were clonally propagated *in vitro* on Murashige and Skoog (MS) medium, supplemented with 20% w/v sucrose as described before (Du et al., 2015; Murashige and Skoog, 1962). Seedlings of *N. benthamiana*, as well as well-rooted HJT349-3 plantlets, were transferred to pots with disinfected soil in a climate-regulated greenhouse compartment at Unifarm, Wageningen University & Research (22/18°C and 16/8 h light day/night regime at 70% relative humidity) where they were grown for 3 weeks prior to agroinfiltration

experiments. The transient protein expression and cell death assays were performed under the same conditions.

Binary vectors for *Agrobacterium tumefaciens*-mediated transient transformation

Construction of pBin-KS-*p35s:ELR-eGFP*, pBin-KS-*p35s:Cf-4-eGFP*, pBin-KS-*p35s:eGFP* and pCB302-3-*p35s:INF1* has been described earlier (Du et al., 2015; Liebrand et al., 2013). Full length elicitor constructs of INF1, INF1^{C23S}, ParA1, CRY2, INF2A, INF2B, INL1 and PYU1 were synthesized, with their native signal peptide (Sp) replaced with the pathogenesis-related protein (PR1) Sp from tobacco as well as two N-terminal HA-tags, separated by spacers (Figs. S1a, S2a), as previously described (Domazakis et al., 2017). The PR1Sp2HA elicitor sequences were flanked by AttL1 and AttL2 recombination sites to facilitate direct Gateway recombination cloning (Invitrogen). The constructs were synthesized in pUC57 vector (GenScript). From those pUC57 constructs, the elicitor domains of INF2A (amino acid (aa) 21-118), INF2B (aa 21-118), CRY2 (aa 21-118) and INL1 (aa 19-104) (elicitor domains were defined according to (Jiang et al., 2006)) were PCR-amplified using Phusion DNA polymerase (Thermo Fisher Scientific) and primers listed in Table S1, and were cloned into pENTR/D-TOPO (Invitrogen) (Figs. S1a, S2a). Then, together with INF1, INF1^{C23S}, ParA1 and PYU1, they were recombined into the pK7WG2 binary vector by a Gateway LR reaction (Invitrogen). Final constructs were transformed into *A. tumefaciens* strain AGL1.

Agrobacterium transient cell death assays

For elicitor cell death induction assays, young, fully expanded leaves of 3 week-old *N. benthamiana* or *N. tabacum* SR1 plants, were agroinfiltrated with pK7WG2-empty vector (EV), pCB132:*p35s-INF1* and pK7WG2:*p35s-PR1Sp2HA-elicitors* constructs, at an OD₆₀₀ of

0.2. Similarly, *Solanum hjertingii* 349-3 plants were co-infiltrated with 1:1 mixtures of *A. tumefaciens* carrying pK7WG2:*p35s-ELR* (OD₆₀₀=0.15), in combination with pK7WG2-EV, pCB132:*p35s-INF1* or pK7WG2:*p35s-PR1Sp2HA-elicitins* constructs (OD₆₀₀=0.1). Leaves were visually examined for cell-death at 3-5 dpi. Cell death quantifications were performed as described previously (Du and Vleeshouwers, 2014).

Co-immunoprecipitations and immunoblotting

In planta protein-protein interaction studies were performed by transient expression of both proteins of interest in young fully expanded leaves of 3 week-old *N. benthamiana* plants using *Agrobacterium*-mediated transformation, followed by co-immunoprecipitation. At 2 days post infiltration, leaves were collected, snap-frozen in liquid nitrogen and ground to a fine powder. Protein extraction, co-immunoprecipitation and western blot analysis with α GFP-HRP and α HA-HRP was performed as described (Domazakis et al., 2018). Immunoprecipitations were performed using GFP-trap_MA (50% slurry) (Chromotek) and Pierce Anti-HA magnetic beads (Thermo Fisher Scientific).

Production and purification of recombinant proteins

Recombinant His-HA-INF1 and the unrelated His-HA-SCR74, a 74 amino acid cysteine-rich protein from *P. infestans* with similarity to the PcF protein from *Phytophthora cactorum fragaria* (variant B3b, NCBI accession number AY723717.1), were synthesized in a codon-optimized version for expression in *Pichia pastoris* (GenScript). Codon-optimized coding regions were designed to carry N-terminal His-HA tags, as described (Fig. S2b) (Domazakis et al., 2017). The coding regions were synthesized with flanking *StuI* and *KpnI* restriction sites to facilitate cloning and were obtained in the pUC57 vector. Coding regions were cloned in frame

with the α -mating factor in the pPink α -HC vector (Invitrogen), enabling protein secretion. The *P. pastoris* PichiaPink™ strain 1 (Invitrogen), was transformed by electroporation and high protein producing clones were selected using a high throughput screening method (Domazakis et al., 2017). For protein production, yeast cultures were first grown in 1 L of buffered glycerol complex medium (BMGY), at 28°C, and shaking at 250 rpm, till an OD₆₀₀ of 2-6 in a 3 L conical flask. Cells were then pelleted by centrifugation and were resuspended in 200 mL of buffered methanol complex medium (BMMY), containing 1% v/v MetOH at 28°C, and shaking at 300 rpm, for induction of protein production in a 2 L conical flask. Induction conditions were maintained for 48 hours with extra MetOH added to maintain 1% v/v, every 24 hrs. After 48 hours, the cultures were harvested, and the cells were removed by centrifugation. Supernatants were filter-sterilized through a 0.22 μ m syringe filter. A pressurized Amicon stirred cell concentrator (Millipore), combined with a 3 kDa exclusion filter, was used to concentrate the supernatants to a volume of ~10 mL. Purification of the expressed protein was performed using cation exchange. For this, a column was assembled containing SP Sepharose, for which the beads were equilibrated with 10 volumes of acetic acid buffer (50 mM acetic acid, pH 4.5). Concentrated yeast supernatants containing elicitors were diluted five times in the acetic acid buffer and were slowly loaded onto the column. The column was then washed with five volumes of the acetic acid buffer, and proteins were subsequently eluted by applying five volumes of elution buffer (10 mM Tris, 500 mM NaCl, pH 8) and collecting the eluate in several fractions. The protein-containing fractions were obtained by measuring A280 absorbance, SDS-PAGE and western blotting. Protein concentrations were determined using a BCA assay (Thermo Fisher Scientific).

In vitro binding assay

A. tumefaciens strains carrying ELR-eGFP or Cf-4-eGFP were infiltrated into leaflets of *N. benthamiana* plants at an OD₆₀₀ of 1 and 0.8, respectively. Two days after agroinfiltration, leaves were collected and snap-frozen in liquid nitrogen. Leaves were ground to a fine powder and RIPA extraction buffer was added to an amount of 2 mL/g of leaf material (Liebrand et al., 2012; Liebrand et al., 2013). Purified recombinant HisHA-INF1 or HisHA-Scr74 were incubated with 20 µl Pierce Anti-HA magnetic beads (Thermo Fisher Scientific) at an amount of 500 pmol each for 30 min. Subsequently, beads were washed four times with binding buffer (100 mM MES, 10 mM NaCl, 3 mM MgCl₂, pH=6 (Saur et al., 2016)). Then the extract containing ELR-eGFP or Cf4-eGFP was diluted at a ratio of 1:3 in the same binding buffer and 2 mL of that extract was applied to His-HA-INF1- or His-HA-SCR74-bound beads. The mixture was incubated for 1 hour at 4°C, after which co-immunoprecipitation was performed as described (Domazakis et al., 2017; Domazakis et al., 2018).

Results and discussion

ELR associates with INF1 *in planta*

To study whether ELR associates with INF1 in co-immunoprecipitation experiments, we generated an N-terminally tagged version of INF1. INF1 was designed with the tobacco pathogenesis-related protein 1 (PR1) signal peptide (Sp) for secretion, and a 2×HA tag (PR1Sp2HA-INF1) (Fig. S2a). To test whether ELR associates with INF1 *in planta*, we co-expressed PR1Sp2HA-INF1 with ELR-eGFP (Du et al., 2015), and used Cf-4-eGFP or eGFP only, as negative controls for ELR-eGFP. In addition, as another negative control for INF1, we included a cysteine mutant of INF1 (PR1Sp2HA-INF1^{C23S}), lacking in its overall structure one

of the three disulfide bridges and a suppressed cell death response (Kamoun et al., 1997). After performing a co-immunoprecipitation experiment, we found that ELR specifically immunopurifies with INF1 (Fig. 1a). As expected, Cf-4 and eGFP did not associate with INF1, whereas the INF1^{C23S} mutant did show a severely reduced interaction with ELR, which is in line with the reduced cell death phenotype of this INF1 mutant (Kamoun et al., 1997). Interestingly, we noticed that INF1 migrates as a double band in the total protein extract, while after immunoprecipitation of ELR, only a single band is found (Fig. 1a, inputs). We propose that the upper band of INF1 corresponds to the unprocessed version of the protein, while the lower band, representing INF1 that associates with ELR, corresponds to the mature INF1 protein from which the Sp has been cleaved off. Alternatively, INF1 could be modified when expressed *in planta*, as has been suggested for *P. cryptogea* β -CRY (Uhlikova et al., 2016). In conclusion, these data show that ELR specifically associates with INF1 *in planta* and that this interaction occurs with the mature form of the elicitor, likely in the plant apoplast.

ELR associates with INF1 *in vitro*

To further validate the association between ELR and INF1, we designed an *in vitro* binding assay in which purified INF1 protein was tested for interaction with ELR. For this, His-HA-tagged INF1, as well as a His-HA tagged version of the unrelated protein SCR74, were produced in *Pichia pastoris* (Domazakis et al., 2017; Liu et al., 2005) and purified using cation exchange chromatography. To test for association, ELR-eGFP and Cf-4-eGFP were transiently expressed in *N. benthamiana* as described above, and were tested for interaction with the effector proteins. First, the His-HA-INF1 and His-HA-SCR74 proteins were immuno-absorbed to anti-HA beads. *N. benthamiana* extract, containing either ELR-eGFP or Cf-4-eGFP, was applied to the beads after three times dilution in binding buffer. After incubation at room temperature for 1 hour, and thorough washing with binding buffer we found that ELR-eGFP is

specifically being captured from the plant extract by INF1, but not by the unrelated protein SCR74 (Fig. 1b). In contrast, as expected, Cf-4-eGFP was not interacting with either of these effectors. We confirmed that the recombinant His-HA-INF1 is recognized in *N. benthamiana* similar to untagged INF1, while His-HA-SCR74 does not trigger an HR (Fig. 1c). These data show that ELR specifically interacts with INF1, and this finding is in accordance with our *in planta* observations.

ELR associates *in planta* with ELI-1 elicitors

In contrast to other PRRs that recognize small epitopes of their matching MAMP and often do not trigger cell death, ELR seems to recognize some kind of structural domain of elicitors, and does induce cell death in plants (Derevnina et al., 2016; Du et al., 2015). To determine whether the cell death-inducing activity of the various elicitors is associated with their ability to bind to ELR, we generated affinity-tagged versions of diverse elicitor domains from various *Phytophthora* and one *Pythium* species. These included the N-terminally -HA-tagged elicitor domains of INF1, INF2A, INF2B and INL1 from *P. infestans*, ParA1 from *P. parasitica*, CRY2 from *P. cryptogea* and PYU1 from *Pythium ultimum*. The tested elicitors belong to different classes, INF1, INF2A, INF2B, ParA1 (classes ELI-1 and ELI-2), INL1 (class ELL) and PYU1, representing an outgroup (Fig. S1c), and share 23-94% amino acid identity to INF1 (Fig. S1b). To test the cell death-inducing activities of the various elicitors, agro-infiltration was performed in combination with ELR, in *S. hjertingii*, a wild *Solanum* genotype that does not respond to elicitors (Fig. 2a, b). We found that the tagged elicitors INF1, ParA1, CRY2 (belonging to the ELI-1 family) and INF2A (belonging to the ELI-2 family), induced cell death when co-expressed with ELR, at 3 days post-infiltration, similar to what was reported before (Du et al., 2015) (Fig. 2a, b). The INF1^{C23S} mutant, INF2B, INL1 and PYU1 did not trigger cell death (Fig. 2a, b).

To investigate whether the cell death-inducing activity of the various elicitors, correlates with their ability to associate with ELR, we performed an *in planta* co-immunoprecipitation (Co-IP) experiment of ELR-eGFP with the different affinity-tagged elicitors. N-terminally-HA-tagged INF1 and INF1^{C23S} were included as positive and negative controls, respectively (Fig. 2c). We found that, besides with INF1, ELR also interacts with ParA1, which belongs to the same ELI-1 class (Fig. S1c) (Fig. 2c).

For CRY2 and INF2A (Fig. S1a, b) that share lower amino acid identities to INF1 (74 % and 52%, respectively), we did not detect association with ELR (Fig. 2a, b). Since these elicitors do trigger cell death when co-expressed with ELR (Du et al., 2015), we anticipate that there should be interaction, however, we were unable to detect it in our Co-IP assay. Potentially this may be explained by reduced binding affinity of ELR to CRY2 and INF2A, in line with reduced cell death (Fig. 2b). INF2b, INL1 and PYU1 that do not cause cell death in agro-co-infiltrations with ELR, also do not show association with ELR. Overall, this study demonstrates that ELR is functioning as a genuine PRR and associates with elicitors beyond those produced by *P. infestans*, both *in vivo* and *in vitro*.

Fig. 1. ELR associates with INF1 elicitor *in planta* and under *in vitro* conditions. (a) N-terminally HA-tagged INF1 wild type (PR1Sp2HA-INF1^{WT}; WT) and the INF1^{C23S} cysteine mutant (PR1Sp2HA-INF1^{C23S}; C23S) were co-expressed with ELR-eGFP, Cf-4-eGFP or eGFP only, in leaves of *Nicotiana benthamiana*. Total protein was extracted at 2 days post agro-infiltration and was subjected to immunoprecipitation using anti-GFP beads to capture ELR-eGFP, Cf-4-eGFP and eGFP. The immunopurified proteins were detected with anti-GFP, while their interaction with INF1 was assessed with anti-HA antibodies. Ponceau S staining of RuBisCO indicates equal protein loading. The results shown here are representative from three biological repeats. (b) Purified recombinant His-HA-INF1 or His-HA-SCR74 protein (500 pmol each) was immuno-absorbed to anti-HA beads for 30 min. Subsequently, beads were washed with binding buffer and diluted extract from leaves of transiently transformed *N.*

benthamiana expressing ELR-eGFP or Cf-4-eGFP, was added to the beads (panel: input). After a 1h incubation, proteins were separated on SDS-PAGE gels and western blotting was performed. The immunopurified effector proteins His-HA-INF1 and His-HA-SCR74 were detected with anti-HA antibodies, while the interaction with the eGFP-tagged receptors was assessed with anti-GFP antibodies (panel: IP-immobilized effector). Results that are shown are representative for two biological repeats. (c) Functionality test of a 1 μ M concentration of recombinant His-HA-INF1 and His-HA-SCR74 proteins in *N. benthamiana*, as compared to INF1 produced by *P. infestans*, at a similar concentration (all proteins were diluted in Milli-Q water).

Fig. 2. ELR associates with elicitors *in planta*. Diverse elicitors (ParA1, CRY2, INF2A, INF2B, and PYU1) from *P. parasitica*, *P. cryptogea*, *P. infestans* and *Pythium ultimum* as well as *P. infestans* elicitin-like INL1 were tested for recognition by ELR, together with INF1 and INF^{C23S} as a positive and a negative control, respectively. (a) N-terminally HA-tagged elicitin recognition in *Solanum hjertini* 349-5, upon their transient co-expression with ELR. Elicitin recognition by ELR results in cell death. (b) Quantification of the cell death response from the experiment shown in (a). (c) Co-immunoprecipitation of ELR with N-terminally HA-tagged elicitors. Total protein was extracted at 2 days post agro-infiltration of ELR in combination with

the various elicitors, and was subjected to immunoprecipitation using anti-GFP beads, to capture ELR. Immunopurified ELR was detected with anti-GFP, while interaction with the elicitors was assessed with anti-HA antibodies. Ponceau S staining of RuBisCO indicates equal protein loading. Results that are shown are representative of three biological repeats.

Supplemental data

Fig. S1. Characteristics of the elicitors that are subject of this study. (a) The elicitor domains of seven elicitors from *Phytophthora* spp. and *Pythium ultimum* were aligned using COBALT, and BOXSHADE was used to visualize conservation. Amino acid residues identical in all sequences are highlighted in black, while those identical in >80% of the sequences are highlighted in grey. (b) Percentages of amino acid sequence identity of the elicitor domain between the different elicitors. (c) A neighbor-joining phylogenetic tree was created based on the elicitor domains of the seven elicitors using MEGA 5.1. Bootstrap support values (1,000 replicates) above 50% are given next to the branches.

Fig. S2. Overview of affinity-tagged elicitors used in this study. Effector constructs used for (a) transient expression *in planta* using *A. tumefaciens* and for (b) recombinant protein expression in *Pichia pastoris*. Pathogenesis-related protein 1a (PR1a) or native INF1 signal peptides (Sp) for extracellular targeting were used, while elicitors were carrying hemagglutinin (HA) or histidine (His) tags, as indicated. The 98-amino acid elicitor domains were used for all tested elicitors.

Table S1. Primers used in this study.

Acknowledgements

This work was supported by NWO-VIDI grant 12378 and COST Action SUSTAIN FA1208. We thank Sophien Kamoun for the always inspiring discussions, Prof. Dr. Evert Jacobsen for reviewing the manuscript, Isolde Bertram-Pereira for culturing *Solanum* plants, Henk Smid and Harm Wiegiersma for help in the greenhouse.

References

- Albert, I., Bohm, H., Albert, M., Feiler, C. E., Imkampe, J., Wallmeroth, N., Brancato, C., Raaymakers, T. M., Oome, S., Zhang, H., Krol, E., Grefen, C., Gust, A. A., Chai, J., Hedrich, R., Van den Ackerveken, G., and Nurnberger, T. (2015). An RLP23-SOBIR1-BAK1 complex mediates NLP-triggered immunity. *Nat Plants* **1**, 15140.
- Bohm, H., Albert, I., Oome, S., Raaymakers, T. M., van den Ackerveken, G., and Nurnberger, T. (2014). A conserved peptide pattern from a widespread microbial virulence factor triggers pattern-induced immunity in *Arabidopsis*. *PLoS Pathog* **10**, e1004491.
- Boutrot, F., and Zipfel, C. (2017). Function, discovery, and exploitation of plant pattern recognition receptors for broad-spectrum disease resistance. *Annu Rev Phytopathol* **55**, 257-286.
- Chaparro-Garcia, A., Wilkinson, R. C., Gimenez-Ibanez, S., Findlay, K., Coffey, M. D., Zipfel, C., Rathjen, J. P., Kamoun, S., and Schornack, S. (2011). The receptor-like kinase SERK3/BAK1 is required for basal resistance against the late blight pathogen *Phytophthora infestans* in *Nicotiana benthamiana*. *PLoS One* **6**, e16608.
- Couto, D., and Zipfel, C. (2016). Regulation of pattern recognition receptor signalling in plants. *Nat Rev Immunol* **16**, 537-52.
- Derevnina, L., Dagdas, Y. F., De la Concepcion, J. C., Bialas, A., Kellner, R., Petre, B., Domazakis, E., Du, J., Wu, C. H., Lin, X., Aguilera-Galvez, C., Cruz-Mireles, N.,

- Vleeshouwers, V. G. A. A., and Kamoun, S. (2016). Nine things to know about elicitors. *New Phytol* **212**, 888-895.
- Dodds, P. N., and Rathjen, J. P. (2010). Plant immunity: towards an integrated view of plant-pathogen interactions. *Nat Rev Genet* **11**, 539-48.
- Domazakis, E., Lin, X., Aguilera-Galvez, C., Wouters, D., Bijsterbosch, G., Wolters, P. J., and Vleeshouwers, V. G. A. A. (2017). Effectoromics-Based Identification of Cell Surface Receptors in Potato. In "Plant Pattern Recognition Receptors: Methods and Protocols" (L. Shan and P. He, eds.), pp. 337-353. Springer New York, New York, NY.
- Domazakis, E., Wouters, D., Visser, R. G. F., Kamoun, S., Joosten, M., and Vleeshouwers, V. (2018). The ELR-SOBIR1 Complex Functions as a Two-Component Receptor-Like Kinase to Mount Defense Against *Phytophthora infestans*. *Mol Plant Microbe Interact* **31**, 795-802.
- Du, J., Verzaux, E., Chaparro-Garcia, A., Bijsterbosch, G., Keizer, L. C., Zhou, J., Liebrand, T. W., Xie, C., Govers, F., Robatzek, S., van der Vossen, E. A., Jacobsen, E., Visser, R. G., Kamoun, S., and Vleeshouwers, V. G. A. A. (2015). Elicitor recognition confers enhanced resistance to *Phytophthora infestans* in potato. *Nat Plants* **1**, 15034.
- Du, J., and Vleeshouwers, V. G. A. A. (2014). The Do's and Don'ts of Effectoromics. In "Plant-Pathogen Interactions", Vol. 1127, pp. 257-268.
- Felix, G., Duran, J. D., Volko, S., and Boller, T. (1999). Plants have a sensitive perception system for the most conserved domain of bacterial flagellin. *Plant J* **18**, 265-276.
- Gust, A. A., and Felix, G. (2014). Receptor like proteins associate with SOBIR1-type of adaptors to form bimolecular receptor kinases. *Curr Opin Plant Biol* **21**, 104-111.
- Huitema, E., Vleeshouwers, V. G. A. A., Cakir, C., Kamoun, S., and Govers, F. (2005). Differences in intensity and specificity of hypersensitive response induction in *Nicotiana* spp. by INF1, INF2A, and INF2B of *Phytophthora infestans*. *Mol Plant Microbe Interact* **18**, 183-93.
- Jiang, R. H., Tyler, B. M., Whisson, S. C., Hardham, A. R., and Govers, F. (2006). Ancient origin of elicitor gene clusters in *Phytophthora* genomes. *Mol Biol Evol* **23**, 338-51.
- Jones, J. D., and Dangl, J. L. (2006). The plant immune system. *Nature* **444**, 323-9.
- Kamoun, S., van West, P., de Jong, A. J., de Groot, K. E., Vleeshouwers, V. G. A. A., and Govers, F. (1997). A gene encoding a protein elicitor of *Phytophthora infestans* is down-regulated during infection of potato. *Mol Plant Microbe Interact* **10**, 13-20.

- Kamoun, S., van West, P., Vleeshouwers, V. G. A. A., de Groot, K. E., and Govers, F. (1998). Resistance of *Nicotiana benthamiana* to *Phytophthora infestans* is mediated by the recognition of the elicitor protein INF1. *Plant Cell* **10**, 1413-1426.
- Kunze, G., Zipfel, C., Robatzek, S., Niehaus, K., Boller, T., and Felix, G. (2004). The N terminus of bacterial elongation factor Tu elicits innate immunity in *Arabidopsis* plants. *Plant Cell* **16**, 3496-507.
- Liebrand, T. W., Smit, P., Abd-El-Halim, A., de Jonge, R., Cordewener, J. H., America, A. H., Sklenar, J., Jones, A. M., Robatzek, S., Thomma, B. P., Tameling, W. I., and Joosten, M. H. (2012). Endoplasmic reticulum-quality control chaperones facilitate the biogenesis of Cf receptor-like proteins involved in pathogen resistance of tomato. *Plant Physiol* **159**, 1819-33.
- Liebrand, T. W., van den Berg, G. C., Zhang, Z., Smit, P., Cordewener, J. H., America, A. H., Sklenar, J., Jones, A. M., Tameling, W. I., Robatzek, S., Thomma, B. P., and Joosten, M. H. (2013). Receptor-like kinase SOBIR1/EVR interacts with receptor-like proteins in plant immunity against fungal infection. *Proc Natl Acad Sci USA* **110**, 10010-5.
- Liebrand, T. W., van den Burg, H. A., and Joosten, M. H. (2014). Two for all: receptor-associated kinases SOBIR1 and BAK1. *Trends Plant Sci* **19**, 123-32.
- Liu, Z., Bos, J. I., Armstrong, M., Whisson, S. C., da Cunha, L., Torto-Alalibo, T., Win, J., Avrova, A. O., Wright, F., Birch, P. R., and Kamoun, S. (2005). Patterns of diversifying selection in the phytotoxin-like scr74 gene family of *Phytophthora infestans*. *Mol Biol Evol* **22**, 659-72.
- Murashige, T., and Skoog, F. (1962). A revised medium for rapid growth and bio assays with tobacco tissue cultures. *Physiol Plant* **15**, 473-497.
- Peng, K. C., Wang, C. W., Wu, C. H., Huang, C. T., and Liou, R. F. (2015). Tomato SOBIR1/EVR homologs are involved in elicitor perception and plant defense against the oomycete pathogen *Phytophthora parasitica*. *Mol Plant Microbe Interact* **28**, 913-26.
- Ranf, S. (2017). Sensing of molecular patterns through cell surface immune receptors. *Current Opinion in Plant Biology* **38**, 68-77.
- Ron, M., and Avni, A. (2004). The receptor for the fungal elicitor ethylene-inducing xylanase is a member of a resistance-like gene family in tomato. *Plant Cell* **16**, 1604-15.
- Saur, I. M., Kadota, Y., Sklenar, J., Holton, N. J., Smakowska, E., Belkhadir, Y., Zipfel, C., and Rathjen, J. P. (2016). NbCSPR underlies age-dependent immune responses to

- bacterial cold shock protein in *Nicotiana benthamiana*. *Proc Natl Acad Sci USA* **113**, 3389-94.
- Tang, D., Wang, G., and Zhou, J. M. (2017). Receptor kinases in plant-pathogen interactions: more than pattern recognition. *Plant Cell* **29**, 618-637.
- Uhlikova, H., Oboril, M., Klempova, J., Sedo, O., Zdrahal, Z., Kasparovsky, T., Skladal, P., and Lochman, J. (2016). Elicitin-Induced distal systemic resistance in plants is mediated through the protein-protein interactions influenced by selected lysine residues. *Front Plant Sci* **7**, 59.
- van der Burgh, A. M., and Joosten, M. H. A. J. (2019). Plant Immunity: Thinking Outside and Inside the Box. *Trends in Plant Science* **24**, 587-601.
- Vleeshouwers, V. G., Driesprong, J. D., Kamphuis, L. G., Torto-Alalibo, T., Van't Slot, K. A., Govers, F., Visser, R. G., Jacobsen, E., and Kamoun, S. (2006). Agroinfection-based high-throughput screening reveals specific recognition of INF elicitors in *Solanum*. *Mol Plant Pathol* **7**, 499-510.
- Wang, Y., Xu, Y., Sun, Y., Wang, H., Qi, J., Wan, B., Ye, W., Lin, Y., Shao, Y., Dong, S., Tyler, B. M., and Wang, Y. (2018). Leucine-rich repeat receptor-like gene screen reveals that *Nicotiana* RXEG1 regulates glycoside hydrolase 12 MAMP detection. *Nat Commun* **9**, 594.
- Zhang, L., Kars, I., Essenstam, B., Liebrand, T. W., Wagemakers, L., Elberse, J., Tagkalaki, P., Tjoitang, D., van den Ackerveken, G., and van Kan, J. A. (2014). Fungal endopolygalacturonases are recognized as microbe-associated molecular patterns by the *Arabidopsis* receptor-like protein RESPONSIVENESS TO BOTRYTIS POLYGALACTURONASES1. *Plant Physiol* **164**, 352-64.
- Zipfel, C. (2014). Plant pattern-recognition receptors. *Trends Immunol* **35**, 345-51.