



# At the frontier; RXLR effectors crossing the *Phytophthora*–host interface

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Plants are constantly beset by pathogenic organisms. To successfully infect their hosts, plant pathogens secrete effector proteins, many of which are translocated to the inside of the host cell where they manipulate normal physiological processes and undermine host defense. The way by which effectors cross the frontier to reach the inside of the host cell varies among different classes of pathogens. For oomycete plant pathogens – like the potato late blight pathogen *Phytophthora infestans* – it has been shown that effector translocation to the host cell cytoplasm is dependent on conserved amino acid motifs that are present in the N-terminal part of effector proteins. One of these motifs, known as the RXLR motif, has a strong resemblance with a host translocation motif found in effectors secreted by *Plasmodium* species. These malaria parasites, that reside inside specialized vacuoles in red blood cells, make use of a specific protein translocation complex to export effectors from the vacuole into the red blood cell. Whether or not also oomycete RXLR effectors require a translocation complex to cross the frontier is still under investigation. For one *P. infestans* RXLR effector named IPI-O we have found a potential host target that could play a role in establishing the first contact between this effector and the host cell. This membrane spanning lectin receptor kinase, LecRK-I.9, interacts with IPI-O via the tripeptide RGD that overlaps with the RXLR motif. In animals, RGD is a well-known cell adhesion motif; it binds to integrins, which are membrane receptors that regulate many cellular processes and which can be hijacked by pathogens for either effector translocation or pathogen entry into host cells.

**Keywords:** RXLR–dEER, *Phytophthora*, effector, oomycetes, RGD, lectin receptor kinase, LecRK-I.9

## INTRODUCTION

Although plants have an effective immune system, plant pathogens have evolved mechanisms to overcome plant resistance, and are thus able to invade and cause disease. Vital to the success of plant pathogens is their ability to secrete so-called effectors that manipulate host plant defense (reviewed by Stassen and van den Ackerveken, 2010; De Jonge et al., 2011). Ergo, the pathogen can colonize plant cells with less hindrance. In the “arms race” plants acquired specific resistance (R) proteins that directly or indirectly recognize effectors. This recognition leads to effector-triggered immunity (ETI) that blocks further growth of the pathogen and that is often associated with a hypersensitive response (HR; Jones and Dangl, 2006).

Oomycete plant pathogens are notorious for causing devastating diseases in a large variety of plants. The well-known and best studied species are the potato late blight pathogen *Phytophthora infestans*, the soybean stem and root rot pathogen *Phytophthora sojae*, and the downy mildew *Hyaloperonospora arabidopsidis* that infects *Arabidopsis*. Plant resistance to oomycetes is mostly governed by R proteins comprising a N-terminal nucleotide-binding site (NBS) domain and a C-terminal leucine-rich-repeat (LRR) domain. Since these NBS–LRR proteins are located intracellular, it was hypothesized that oomycete avirulence (Avr) proteins

should be translocated to the cytoplasm of the plant cell. Indeed, it has been shown that oomycetes secrete effectors that they deliver inside host cells. These intracellular effectors include Avr proteins that are recognized by their cognate R proteins (Rehmany et al., 2005; Whisson et al., 2007). They all share a signal peptide and a conserved N-terminal RXLR motif with often an dEER motif in its vicinity. These RXLR and dEER motifs were detected in 2004 during a *Phytophthora* genome annotation jamboree (Govers and Gijzen, 2006), a discovery that marked the beginning of a new episode in plant–pathogen interaction research. At that time, after many years of labor-intensive map based cloning procedures, only a few oomycete avirulence (Avr) genes were identified. As no conserved motifs were known for fungal Avr proteins the presence of a conserved motif in oomycete Avr proteins was unexpected. The number of RXLR–dEER effector genes in each of the three sequenced *Phytophthora* genomes is remarkably high, with 563 predicted for *P. infestans* (Haas et al., 2009) and 385 in *P. sojae* (Jiang et al., 2008). Significantly fewer RXLR–dEER effector genes were found in the genomes of two obligate oomycete pathogens sequenced so far, 115 in *H. arabidopsidis* and 25 in the white rust *Albugo laibachii* (Baxter et al., 2010; Kemen et al., 2011), whereas very few or none ( $\leq 2$ ) were found in two species with a necrotrophic life style, *Pythium ultimum*

and *Aphanomyces euteiches* (Gaulin et al., 2008; Lévesque et al., 2010). Even more surprisingly than its conservation and its abundance in *Phytophthora* spp., is the clear resemblance of the RXLR motif with the RXLX<sup>E/Q</sup> host cell targeting motif in effector proteins of the malaria parasite *Plasmodium falciparum* known as the *Plasmodium* export element (PEXEL). Transfer into the erythrocyte cytoplasm enables the PEXEL effectors to remodel and reprogram the host cell to establish successful asexual replication of the malaria parasite (Templeton and Deitsch, 2005; Tilley et al., 2011). The RXLX<sup>E/Q</sup> motif was shown to be crucial for effector translocation across the parasitophorous vacuole membrane, which surrounds the malaria parasite in red blood cells (Hiller et al., 2004; Marti et al., 2004). This led to the hypothesis that oomycete RXLR effectors are also targeted into host cells, and stimulated many researchers to address the question how oomycete effectors are delivered into plant cells (Haldar et al., 2006).

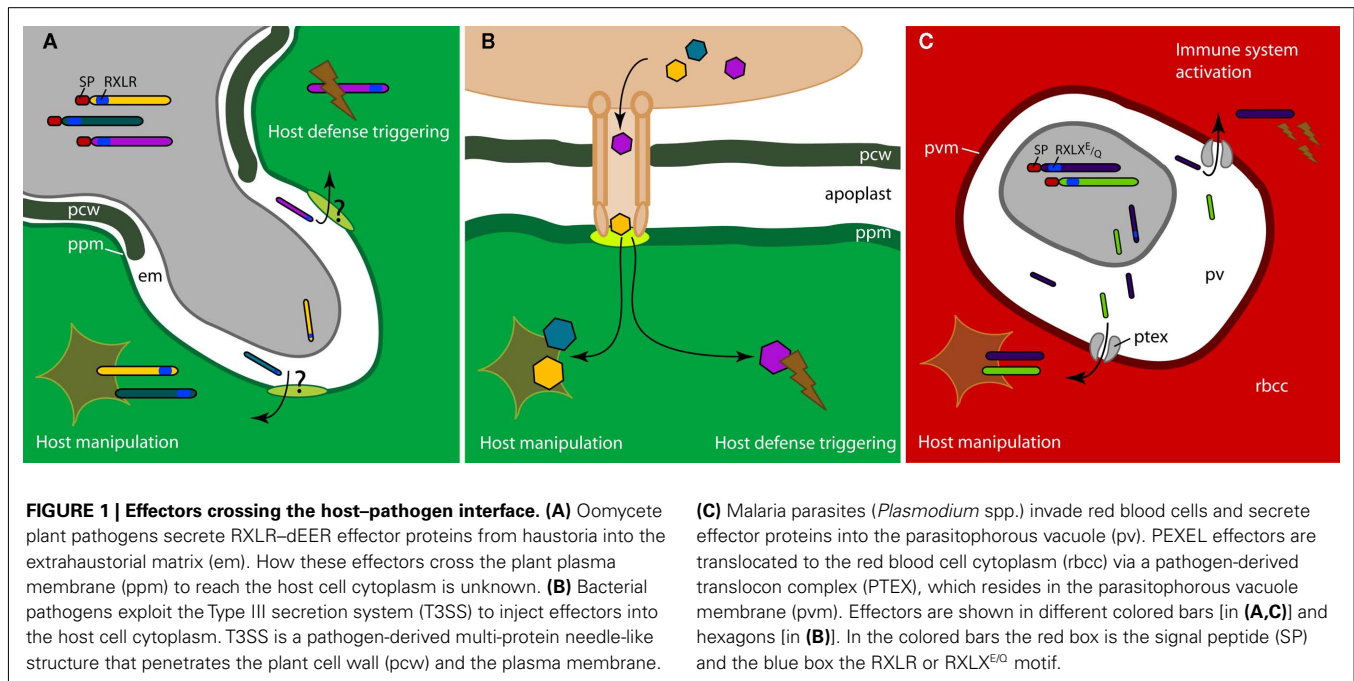
### CROSSING THE FRONTIER; THE ROLE OF THE RXLR MOTIF

Whisson et al. (2007) were the first to show that the RXLR-dEER domain is indeed required for effector translocation into host plant cells. *P. infestans* transformants expressing *Avr3a* in which either the RXLR or dEER motifs were mutated, were shown to be no longer detected by the resistance protein R3a, and as a result did not trigger an HR. Transient co-expression of the *R3a* gene with variants of *Avr3a* lacking the signal peptide sequence and with a mutated or deleted RXLR-dEER domain showed, however, that R3a-mediated cell death was not affected, and therefore it was concluded that the RXLR-dEER domain in *Avr3a* is needed for effector translocation to the host cytoplasm (Whisson et al., 2007). A similar approach was used for *P. sojae Avr1b-1*, the avirulence counterpart of the soybean *R* gene *Rps1b* and the first oomycete *Avr* gene that was cloned (Shan et al., 2004). The phenotype of a *P. sojae* strain changed from virulent to avirulent on *Rps1b* plants when complemented with the full length *Avr1b-1* gene, but remained virulent when transformed with *Avr1b-1* with mutations in RXLR and dEER. On the other hand, delivery of *Avr1b-1* by particle bombardment for transient expression of the *Avr* gene in *Rps1b*-leaves triggered cell death even when the RXLR or dEER motifs were mutated or absent, and hence, a role for these motifs in delivery of *Avr1b* was apparent (Dou et al., 2008).

Although the two studies described above provided convincing evidence that the RXLR-dEER domains of *Avr3* and *Avr1b* are crucial for uptake, the mechanisms underlying effector uptake by host cells remained unclear. To gain further insight in this process Dou et al. (2008) investigated what happens when *Avr1b*, produced *in planta* upon bombardment with an *Avr1b-1* construct, has a signal peptide. Whereas secreted *Avr1b* triggered *Rps1b*-mediated cell death with the same efficiency as *Avr1b* lacking its signal peptide, secreted *Avr1b* with mutated RXLR and dEER motifs triggered no response. This suggested that the motifs mediate re-entry of *Avr1b* into the cell and, more strikingly, that the entry does not require the presence of the pathogen (Dou et al., 2008). Using similar approaches and supported by immunolocalization, Rafiqi et al. (2010) showed that also internalization in host cells of two *Avr* proteins produced by the flax rust fungus

can occur in the absence of the pathogen. As with *Avr1b*, uptake is dependent on features in the N-terminal region of the *Avr* proteins; but unlike *Avr1b*, the rust *Avr* proteins have no obvious conserved motifs. For *P. sojae Avr1b* autonomous uptake in plant cells was also demonstrated with *Avr1b*-GFP fusion proteins synthesized in *Escherichia coli* (Dou et al., 2008). Soaking root tips of soybean seedlings for 12 h in a solution containing partly purified fusion protein resulted in uptake of the *Avr1b*-GFP protein into the root tip cells up to 10 cell layers. Also in this assay mutations in the RXLR or dEER motifs abolished uptake. These experiments suggested that the RXLR-dEER domain is all that is needed from the pathogen to enable translocation of oomycete effectors into host cells (Dou et al., 2008). This is quite different from the situation in certain Gram-negative bacteria that make use of the Type III secretion system (T3SS). These bacteria provide a suite of building blocks for a complex translocation machinery consisting of a multi-protein needle-like structure through which effectors are injected into the host cell cytoplasm (Figures 1A,B).

The next question to be answered is how RXLR-dEER effectors orchestrate their own translocation. If it is true that no other pathogen components are required, an issue that is still under debate, are the pathogens then actively recruiting host-derived components to support effector entry into host cells? Or do they possibly exploit an existing uptake machinery? According to Kale et al. (2010) the RXLR motif is capable to interact with phosphoinositides (PPIs) at the host plasma membrane (PM), in particular phosphatidylinositol-3-phosphate (PI3P). In filter-binding and liposome-binding assays they showed interaction of the *Avr* protein *Avr1b* and two other members of the *P. sojae* RXLR family, *Avh5* and *Avh331*, with PI3P. Since mutations in RXLR resulted in loss of binding it was concluded that the interaction with PPIs is specific. A puzzling issue, however, was the dogma that PPIs always reside on the cytoplasmic site of eukaryotic cell membranes whereas the RXLR effectors approach the PM from the outside. To address this Kale et al. (2010) exploited highly specific PPI biosensors, proteins composed of lipid-binding domains that target either PI3P or PI4P fused to a fluorescent tag. When exposed to soybean root suspension cultures they observed fluorescence with the PI3P biosensor, but not with the PI4P biosensor, on the extracellular leaflet of the PM, suggesting that PI3P is externally exposed. In a recent study Yaeno et al. (2011) also reported binding of an RXLR effector to PPI. However, in contrast to Kale et al. (2010), they found that mutations in the RXLR motif of *Avr1b* do not interfere with PI3P binding. Instead they observed that a positively charged lysine-rich patch in the C-terminus of *Avr1b*, that is conserved in the *P. infestans* homolog *Avr3a*, is responsible for the binding to PPI. The PPI binding to the C-terminus of *Avr3a*, which is thought to take place inside host cells, seems to be required for accumulation of *Avr3a* and for suppressing host immunity mediated by *Avr3a* via interaction with the E3 ubiquitin ligase CMPG1 (Yaeno et al., 2011). To what extent PPI binding plays a role in the activity of other RXLR effectors remains to be investigated. Taken together the data that are currently available on the role of PPI binding are conflicting and fragmented, and the frontier-crossing journey of RXLR effectors is still a black box.



## FACING THE FRONTIER; PHYTOPHTHORA RXLR–dEER VERSUS PLASMODIUM PEXEL

For PEXEL effectors the frontier-crossing journey is much better pictured. The fact that the RXLR motif is highly similar to the RXLX<sup>E/Q</sup> translocation motif present in *Plasmodium* effectors and the observations that the RXLR and RXLX<sup>E/Q</sup> motifs are functionally interchangeable (Bhattacharjee et al., 2006; Grouffaud et al., 2008), raise questions about potential similarities in uptake machineries. Although PEXEL motif-containing proteins are shown to be taken up in the absence of the pathogen (Kale et al., 2010), a *Plasmodium* translocon complex (PTEX) is described to work as an active export machine to deliver PEXEL effectors into the cytosol of the erythrocyte host cell (Figure 1C; De Koning-Ward et al., 2009). Components comprising the PTEX complex are an AAA<sup>+</sup>-ATPase, the exported protein EXP2, thioredoxin 2, and two novel *Plasmodium* proteins, PTEX88 and PTEX150. In the model it is assumed that also host-derived proteins assist this translocon complex. So far, BLAST searches did not reveal obvious homologs of PTEX components in *Phytophthora* genomes (K. Bouwmeester and H. J. G. Meijer, unpublished results). This makes it less likely that *Plasmodium* and *Phytophthora* share highly conserved translocon machineries, although the existence of an analogous translocon complex for shuttling *Phytophthora* effectors is still conceivable.

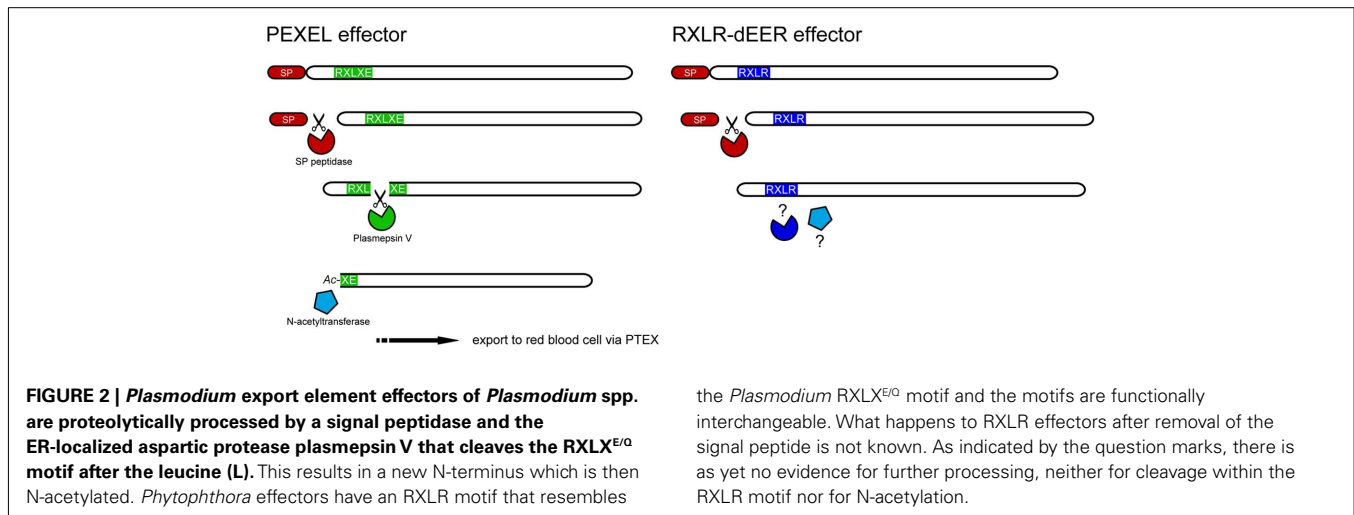
Intriguingly, it was reported that the PEXEL motif is not involved in a direct interaction with the PTEX, but is functioning as a recognition site for cleavage (Figure 2; Chang et al., 2008; Boddey et al., 2009). Cleavage of the RXLX<sup>E/Q</sup> motif was shown to occur after the third residue (RXL↓), and when the conserved L residue was mutated, export was prevented (Boddey et al., 2009). Plasmepsin V, an endoplasmic reticulum (ER) aspartic protease (AP), was found responsible for PEXEL cleavage (Boddey et al., 2010; Russo et al., 2010). APs are well represented in oomycetes.

A recent study showed that each of the three sequenced *Phytophthora* species has 11–12 AP genes distributed over five clades (Kay et al., 2011). One clade with *Phytophthora* AP10, AP11, and AP12 clusters with Plasmepsin V, but whether or not these are functional RXLR or PEXEL proteases remains to be tested.

An additional step in PEXEL processing is N-acetylation of the new N-terminus, which could well be a crucial step to facilitate translocation to the red blood cell (Chang et al., 2008; Boddey et al., 2009; Figure 2). Both cleavage and N-acetylation are executed early in the trafficking pathway within the ER of *Plasmodium*, but not in the parasitophorous vacuole or during export to the erythrocyte, as was concluded based on inhibition of ER-to-Golgi protein transport with brefeldin A. As yet, the N-acetyltransferase in *Plasmodium* remains to be identified. So far, there are no indications for cleavage of oomycete RXLR effectors or N-acetylation and it is therefore questionable whether RXLR–dEER effectors from oomycetes are processed in a similar way.

## OPENING THE FRONTIER; THE ROLE OF THE RGD MOTIF

One of the 563 predicted *P. infestans* effectors that carries an RXLR motif is IPI-O. The encoding gene *ipiO* (with *ipi* referring to *in planta* induced) was one of the first oomycete genes that was isolated and studied in detail (Pieterse et al., 1993). At that time, the assignment of a putative function of *ipiO* in pathogenesis was solely based on its expression pattern, i.e., high expression *in planta* versus low or no expression in *in vitro* culture. Attempts to determine a function of IPI-O by gene silencing or overexpression failed (Van West, 2000). The finding that IPI-O is a potential Avr factor came many years later when *ipiO* was tested in a high-throughput effector genomics screening aimed at identifying novel pairs of *P. infestans* Avr genes and host plant R genes (Vleeshouwers et al., 2008). Subsequently, we confirmed that *ipiO* is *Avr-blb1*, the counterpart of the late blight R gene *Rpi-blb1* which originates



from the nightshade *Solanum bulbocastanum* (Champouret et al., 2009).

In the pre-genomic era comparative analysis that could have led to the discovery of the RXLR-dEER domain in IPI-O was not yet feasible; at that time the only potentially interesting feature that could be distinguished in IPI-O was the presence of a cell attachment motif (Pieterse et al., 1994). This motif consisting of the tripeptide sequence Arg-Gly-Asp (RGD) partly overlaps with the RXLR motif in IPI-O, i.e., RXLRGD. The RGD motif is found in a large number of extracellular proteins, such as the mammalian glycoproteins fibronectin and vitronectin that reside in the extracellular matrix (ECM). A wide variety of these proteins binds to integrins, a family of transmembrane receptors that function in “inside-out” and “outside-in” signaling and regulate many cellular processes. The fact that membrane-to-matrix interactions can be easily disrupted by adding synthetic RGD peptides shows that these interactions are important for the ECM-PM continuum (Gronowicz and Derome, 1994; Canut et al., 1998). Integrins are also chosen by pathogens as a target for binding and their role in animal innate immune responses has been emphasized in several reports (Isberg and Van Nhieu, 1994; Pribila et al., 2004; Stewart and Nemerow, 2007). Integrins, for instance, interact with the RGD-containing penton base proteins of adenoviruses (reviewed in Mercer et al., 2010). Following this interaction integrins initiate clathrin-mediated endocytosis and promote virus internalization. It was demonstrated, however, that the RGD motif is not necessary for initial virus attachment *per se*; adenoviruses with deleted RGD motifs could still attach but were impaired in endosomal internalization and in escape to the host cytoplasm (Shayakhmetov et al., 2005). Another example can be found in *Helicobacter pylori*, a bacterium causal to stomach inflammation, that exploits integrins for effector translocation into the host cell (Kwok et al., 2007). This process is dependent on CagL, a RGD-containing protein that is situated on the surface of the pilus and interacts in an RGD-dependent manner with the integrin  $\alpha 5\beta 1$ . Upon interaction, various downstream components are activated that were shown to be essential for effector translocation. Other potent effectors of integrin function are the disintegrins from snake venoms

of various viper species (Calvete et al., 2007; Matsui et al., 2010). Disintegrins are usually short cysteine-rich peptides that contain a RGD motif on an exposed hairpin loop. Disintegrins selectively bind and activate integrin receptors, and hence compete with their natural RGD-containing ligands, such as fibrinogen and Von Willebrand factor, subsequently disturbing platelet aggregation and cell-ECM adhesion. Several RGD-disintegrins – such as contortrostatin and jarastatin – have been shown to activate the focal adhesion kinase FAK that regulates actin dynamics, and to trigger phosphoinositide 3-kinase and MAPK pathways upon integrin-binding (Coelho et al., 2001; Oliva et al., 2007).

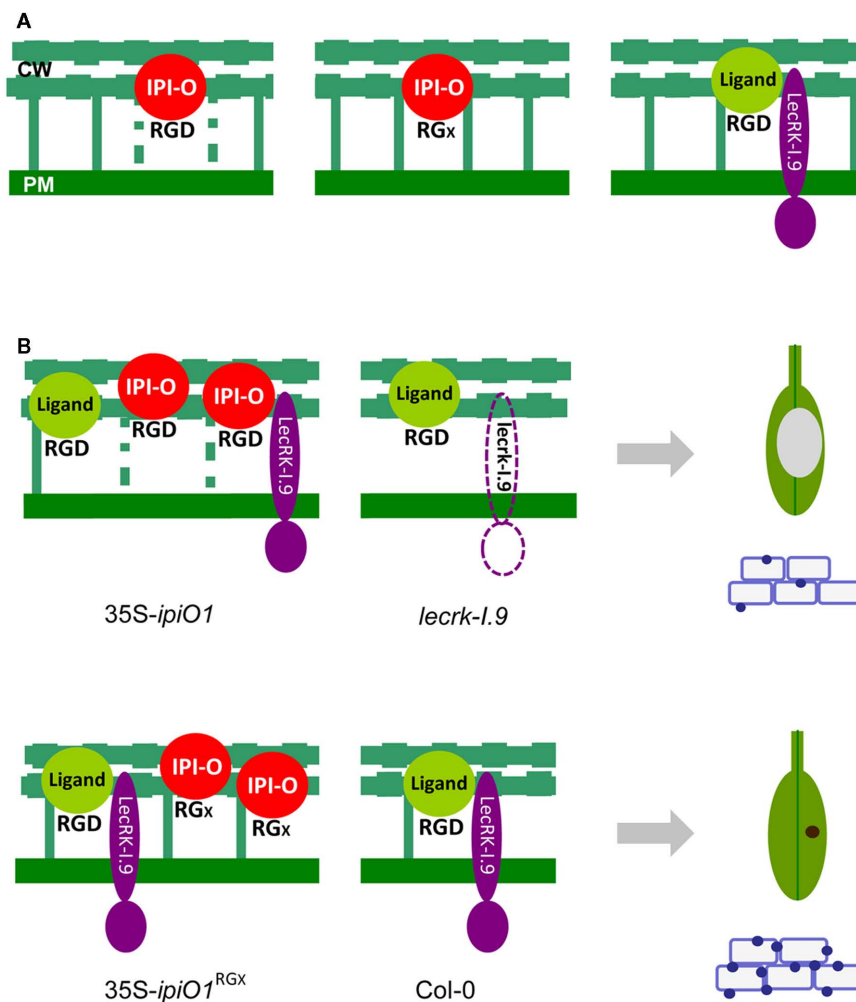
It is conceivable that also plant pathogens take advantage of the RGD motif for manipulating their hosts. One example comes from the RGD-containing ToxA protein produced by *Pyrenophora tritici-repentis* and *Stagonospora nodorum*, two fungi that cause leaf spot disease on wheat. These pathogens require the host-selective toxin ToxA to induce necrosis in host cells (Sarma et al., 2005; Friesen et al., 2008). The ToxA gene encodes a pre-protein of which the signal peptide (pre-region) and the N-terminal pro-region are both cleaved by the fungus prior to secretion of the mature 13.2 kDa protein. The RGD motif in ToxA was found to be required for internalization into wheat mesophyll cells (Meinhardt et al., 2002; Manning et al., 2008) and it was proposed that internalization occurs by means of receptor-mediated endocytosis. This is in line with the finding that a number of genes encoding endocytosis-related proteins and lectin receptor kinases were significantly upregulated in wheat treated with ToxA (Pandolova et al., 2009). Other studies reported that exogenously applied RGD peptides can change the structure and behavior of plant cells. When added, adhesions between the cell wall (CW) and plasma membrane (PM) were easily disrupted, and resulted in reduced levels of phytoalexins and phenolic compounds (Schindler et al., 1989; Canut et al., 1998; Kiba et al., 1998; Gao et al., 2007). Moreover, Mellersh and Heath (2001) showed that RGD-treated cowpea plants were attenuated in their defense response, which correlated with enhanced fungal penetration efficiency.

In our studies on the *P. infestans* effector IPI-O we also obtained strong evidence that the RGD motif is a functional determinant.



Similar to RGD peptides, exogenously added IPI-O has the capacity to disrupt CW–PM adhesions through its RGD motif. Plasmolysed *Arabidopsis* cells from a suspension culture showed convex pockets when exposed to recombinant IPI-O protein but concave pockets when exposed to recombinant IPI-O protein with RGD mutated into RGE or RGA (Senchou et al., 2004; **Figure 3A**). Transgenic *Arabidopsis* plants with constitutive expression of the *ipiO* gene develop normally but at the microscopic level we again observed concave forms of plasmolysis in hypocotyl cells reminiscent of a disrupted adhesion between the CW and PM, this in contrast to wild-type plants and transgenic plants expressing

a mutant form of *ipiO* (RGD > RGE; Bouwmeester et al., 2011; **Figure 3B**). Intriguingly, the *ipiO* expressing plants responded in a different manner to infection and elicitor treatment. They displayed gain of susceptibility to *Phytophthora brassicae*, and showed less callose deposition, thus suggesting that IPI-O functions as a suppressor of defense (Bouwmeester et al., 2011). Since also these phenotypes are RGD dependent, we conclude that RGD is essential for IPI-O to function at the host–pathogen interface. We imagine that plants perceive IPI-O either directly via the RGD motif or via a structural feature imposed on IPI-O by the RGD motif.



**FIGURE 3 | The *Phytophthora infestans* RXLR effector IPI-O and its putative host cell receptor *Arabidopsis* LecRK-1.9. (A)** Observed changes in *Arabidopsis* suspension cells upon exposure to IPI-O. The CW–PM continuum (shown as green vertical bars) is disturbed (dotted vertical bars) when IPI-O has an intact RGD motif (left panel) but when RGD is mutated into RGA or RGE the continuum is not disturbed (middle panel; Senchou et al., 2004). Model depicting the membrane-associated LecRK-1.9 in interaction with a putative RGD-containing extracellular ligand (right panel). Under normal circumstances (wild-type) the CW–PM continuum is in a balanced state. **(B)** Observed changes in the *Arabidopsis* phenocopy lines 35S-*ipiO1* and *lecrk-1.9* (top left and right panel, respectively). Both show gain of susceptibility to *Phytophthora brassicae* (the gray area on the leaf

depicts lesion growth) and reduced callose deposition (shown as blue dots at the cell boundaries). *Arabidopsis* lines expressing *ipiO* with a mutated RGD motif (35S-*ipiO1*<sup>RGGA</sup> or 35S-*ipiO1*<sup>RGGE</sup>) behave as the wild-type Col-0 accession (lower left and right panel, respectively); no lesion formation upon infection with *P. brassicae* (the brown dot represents a hypersensitive response) and efficient callose deposition (Bouwmeester et al., 2011). The model predicts that IPI-O competes with the endogenous ligand of LecRK-1.9 leading to less adhesions between CW and PM, and gain of susceptibility. A mutation in the RGD motif abolishes the competition resulting in a wild-type phenotype. In the absence of LecRK-1.9 the proper balance between endogenous ligand and receptor is disturbed and results in gain of susceptibility.

## OPENING THE FRONTIER; THE ROLE OF HOST CELL RECEPTOR PROTEINS

The fact that several animal and human pathogens exploit integrin-mediated-uptake for host cell entry or effector delivery raises the question whether plant pathogens use similar strategies. Plants however, lack integrins. In the 1980s and 1990s several researchers attempted to identify plant integrins using immunological and biochemical approaches but failed. Also the first plant genome sequence did not reveal the occurrence of integrin homologs (The Arabidopsis Genome Initiative, 2000). It is conceivable though that plant pathogens use other types of host receptors for cell entry. In a search for functional homologs of integrins in plants Canut et al. (1998) showed that *Arabidopsis* contains high affinity RGD-binding sites in the PM. Consistently, one of these binding sites had a very high affinity for IPI-O. Since this affinity was lost when RGD in IPI-O was mutated into RGA or RGE, the RGD motif seemed to be crucial for binding (Senchou et al., 2004). Subsequently, Gouget et al. (2006) performed a phage display screen to identify proteins that potentially interact with IPI-O via the RGD motif. This resulted in two heptamers that could inhibit the RGD-binding activity to the *Arabidopsis* PM and disrupt CW-PM membrane adhesions, possibly by competing with naturally occurring RGD-based ligand-receptor contacts. Screening the *Arabidopsis* proteome for the occurrence of these two peptide sequences led to the identification of the *Arabidopsis* legume-like lectin receptor kinase LecRK-I.9, which has two amino acid motifs in the N-terminal region (ASY and PHPR) that are implicated in binding IPI-O via its RGD motif (Gouget et al., 2006). LecRK-I.9 belongs to a family of 45 members divided over nine clades and a few singletons (Bouwmeester and Govers, 2009). They are typical transmembrane receptors with an extracellular lectin domain and an intracellular kinase domain. As yet, little is known about the functions of LecRK-I.9 and other legume-like lectin receptor kinases, neither about their natural ligands. They are likely involved in monitoring CW integrity and it is conceivable that LecRK-I.9 interacts with endogenous RGD-containing proteins to balance CW-PM adhesions (Figure 3A). To elucidate the role of LecRK-I.9 at the *Phytophthora*-host interface we analyzed expression during compatible and incompatible interactions with *P. brassicae* isolates and determined the phenotypes of *Arabidopsis* LecRK-I.9 knock-out lines (*lecrk-I.9*) in disease assays. These studies convincingly demonstrated that LecRK-I.9 plays a

role in *Phytophthora* disease resistance. *LecRK-I.9* expression was induced in incompatible interactions (Bouwmeester et al., 2008) and two independent knock-out lines showed gain of susceptibility to *P. brassicae* and the bacterium *Pseudomonas syringae*. Strikingly, the *lecrk-I.9* lines behaved as perfect phenocopies of the *Arabidopsis* lines that constitutively express the RXLR effector gene *ipiO* suggesting that an overload of IPI-O disables LecRK-I.9 to properly guard the frontier (Figure 3B). *P. brassicae* does not have an *ipiO* homolog and hence cannot effectively disable LecRK-I.9. In contrast, *ipiO* is highly expressed in *P. infestans* hyphae invading potato leaves (Van West et al., 1998). Competition of IPI-O with potential endogenous RGD-containing ligands of LecRK-I.9 may destabilize the CW-PM continuum helping *P. infestans* to colonize host tissue. Loosening CW-PM adhesions might facilitate the formation of haustoria, feeding structures that are not only important for nutrient uptake but also for effector translocation to host cells. We hypothesize that *Phytophthora* uses LecRK-I.9 as a gateway to establish infection and that one of the functions of IPI-O is to mediate this early step in the infection process. Other anticipated functions of IPI-O are more downstream in the process when IPI-O has reached the cytoplasm.

## STRENGTHENING THE FRONTIER

In recent years comparative genomics and high-throughput functional analyses of *Phytophthora* effectors have boosted the discovery of translocation motifs but the molecular machinery that is used by oomycetes to deliver their armory into the host cell is still a black box. The recent identification of host components at the cell surface that bind translocation and adhesion motifs opened new avenues and the search for additional components is on-going. Insight into similarities and dissimilarities in transport mechanisms exploited by different groups of pathogens will help to shed light in the black box. The ultimate challenge is to find ways to block the uptake machinery thereby strengthening the frontier at the *Phytophthora*-host interface. Hopefully, this will provide novel strategies to control these notorious pathogens.

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