Lectin receptor kinase 79, a putative target of the *Phytophthora infestans* effector IPI-O

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Protection of plants against pathogen attack is governed by a variety of defense strategies. The outcome of a plant-pathogen interaction is determined by the virulence characteristics of the pathogen and the defense barriers present in the host. Pathogens have the capacity to suppress host defense responses, resulting in a situation in which a pathogen can thrive and reproduce on host tissue (basal compatibility). During evolution plants acquired specific resistance (R) proteins that can recognize pathogen-specific factors, so-called effectors. This recognition leads to effector triggered immunity (ETI) and is often associated with a hypersensitive response (HR) that blocks further growth of the pathogen. Although the majority of the R proteins described to date are intracellular nucleotide-binding leucine-rich-repeat (NBS-LRR) proteins (DeYoung and Innes, 2006), ETI can also be based on membrane associated receptors, such as Cf-like LRR proteins or receptor-like kinases (RLKs). Examples of the latter are rice Xa21 that confers resistance to the bacterial pathogen *Xanthomonas oryzae* pv. *oryzae* and the Arabidopsis ERECTA protein. ERECTA not only functions as signalling component in plant organ development but also in ETI to *Ralstonia solanacearum* (Godiard et al., 2003). Other well-known Arabidopsis RLKs that can activate plant defense pathways are FLS2 and EFR. These RLKs are receptors for the pathogen associated molecular patterns (PAMPs) flg22 and EF-Tu, respectively, and thus activate PAMP triggered immunity (PTI) (Gomez-Gomez and Boller, 2002; Zipfel et al., 2006).

Pathogens produce numerous PAMPs and effectors that can trigger ETI or PTI, or can reprogram host cells in order to promote infection. *Phytophthora* species contain large families of highly divergent effectors sharing a conserved motif named RxLR-dEER. This motif is thought to play a role in translocating these effectors into host cells. How plant cells perceive RxLR-dEER effectors is virtually unknown.

The cell wall – plasma membrane continuum

In plants as well as animals, the extracellular matrix (ECM) is the first line of exposure to invading pathogens. In animals contacts between the ECM and the intracellular actin cytoskeleton are established by integrins, a family of transmembrane receptors that play a role in many diffferent signalling pathways. Integrins are also chosen by pathogens as a target for binding and their role in animal innate immune responses has been emphazised in several reports (Isberg and Van Nhieu, 1994; Pribila et al., 2004). Extracellular domains of integrins can bind to a variety of ECM glycoproteins that possess a conserved RGD tripeptide motif. The fact that membrane to matrix interactions can be easily disrupted by adding synthetic RGD-peptides shows that RGD mediating interactions are important for the ECM-plasma membrane continuum (Canut et al., 1998; Gronowicz and Derome, 1994). Also in plants RGD-peptides are capable to disrupt cell wall-plasma membrane adhesions but remarkably, extensive studies did not result in the identification of integrin homologs in plants. Canut et al. (1998) showed however, that *Arabidopsis thaliana* contains high affinity RGD-binding sites in the plasma membrane pointing towards functional homologs of integrins in plants. Surprisingly one of these binding sites had a very high affinity for IPI-O, an RxLR-dEER effector from the potato late blight pathogen *Phytophthora infestans*. IPI-O has an RGD tripeptide motif that overlaps with the RxLR motif and since the affinity for IPI-O was lost when mutated into RGA or RGE, the RGD motif seems to be crucial for binding (Senchou et al., 2004). To identify proteins that potentially interact with the RGD motif in IPI-O a phage display screen was performed resulting in two heptamers that could inhibit plasma membrane RGD-binding activity in Arabidopsis and disrupt cell wall-plasma membrane adhesions. Blasting the Arabidopsis proteome with these peptide sequences led to the identification of Lectin Receptor Kinase 79 (LecRK79) as a putative effector target of IPI-O (Gouget et al., 2006).

The role of lectin receptor kinase 79 in plant defense

Lectin receptor kinases (LecRKs) are found to be wide-spread in higher plants. For example, *Arabidopsis thaliana* contains 43 LecRKs, divided over several classes. The extracellular domains of LecRKs have features of soluble legume lectins which are believed to be involved in the recognition of simple sugars. Sequence analysis showed however, that LecRKs lack an essential aspartic residue (Asp81) in their sugar-binding sites, whereas molecular modeling showed a good conservation of the overall structure of legume lectins. This suggests that LecRKs are unlikely to bind simple sugar molecules but are more likely to recognize complex glycans. To test the hypothesis that LecRK79 is the effector target of IPI-O we set out to address the role of LecRK79 in plant defense.

As a first step we analysed expression of *LecRK79* in Arabidopsis plants that were exposed to various biotic agents and abiotic stress factors using a GUS-reporter-aided approach. The promoter of the Arabidopsis *LecRK79* gene was fused to a GUS reporter gene and introduced into Arabidopsis Col-0. Homozygous $P_{LecRK79}$::*GUS* transgenic lines were selected and used for histochemical staining to monitor GUS activity indicative for active expression of *LecRK79*. When growing the transgenic $P_{LecRK79}$::*GUS* plants without applying stress, GUS activity was detected in the main veins of rosette leaves and cotyledons and in all hypocotyl cell layers. In roots GUS activity was observed in the apex and stele of apical roots, root primordia and in lateral and adventive roots. These observations point to a basal level of *LecRK79* expression in several cell types.

When the transgenic plants were subjected to heat- or cold treatments or exposed to high salinity no increase in GUS activity was found when compared to untreated control plants. Also treatment with the hormone salicylic acid resulted in a GUS staining profile comparable to that in water treated plants. However, treatment with methyl jasmonate showed a strong induction of GUS activity, suggesting that genes in the jasmonate signalling pathway are involved in activating *LecRK79* expression. Since wounding of plant tissue induces jasmonate signalling, we also tested the effect of wounding on *LecRK79* expression. Wounding did not reveal a visible change in *LecRK79* expression suggesting that wounding does not activate or repress all pathways that are regulated by jasmonate. In summary, these experiments show that the Arabidopsis *LecRK79* promoter is not highly responsive to abiotic stresses.

To reveal changes in *LecRK79* expression resulting from biotic stress the $P_{LecRK79}$::*GUS* plants were inoculated with a variety of pathogens. Inoculation with the necrotrophic fungus *Botrytis cinerea* resulted in typical necrotic

lesions and was associated with a fast and strong induction of *LecRK79* expression in inoculated leaves. Inoculations with biotrophic pathogens were performed with strains that are either compatible or incompatible with Arabidopsis Col-0. Interestingly, in all cases clear differences were observed between compatible and incompatible interactions (Table 1). For example, in leaves infiltrated with an avirulent strain of *Pseudomonas syringae,* DC3000-*avrRPM1*, there was a strong induction of *LecRK79* expression. In the compatible interaction, however, the GUS staining was similar to the water control. Analysis of *LecRK79* expression by Q-RT-PCR showed comparable results. A similar response was observed when the plants were infected with *Phytophthora brassicae.* In an incompatible interaction with strain HH the expression was higher than in the compatible interaction with strain CBS686.95 (Fig.1). Moreover, expression of *LecRK79* was induced in a non-host interaction with *P. infestans*. In summary, expression of *LecRK79* is induced during interaction with a necrotrophic pathogen *B. cinerea* and in incompatible interactions with biotrophic pathogens. This expression pattern suggests that LecRK79 has a role in pathways leading to programmed cell death or HR.

Table 1. Induction of *LecRK79* expression in Arabidopsis during compatible and incompatible plant-pathogen interactions.

pathogen	**type**[a]	**strain**	**compatibility**[b]	***LecRK79* expression**[c]
Botrytis cinerea	N	IMI169558	**C**	+
Pseudomonas syringae pv. *tomato*	B	DC3000	**C**	-
		AvrRPT2	**I**	+
		AvrRPM1	**I**	+
Phytophthora infestans	B	IPO-0	**I**	+
		NL88069	**I**	+
Phytophthora brassicae	B	CBS686.95	**C**	-
		HH	**I**	+

[a] N: necrotrophic; B: biotrophic
[b] C: compatible interaction; I: incompatible interaction
[c] +: *LecRK79* expression is increased; - *LecRK79* expression stays at the basal level.

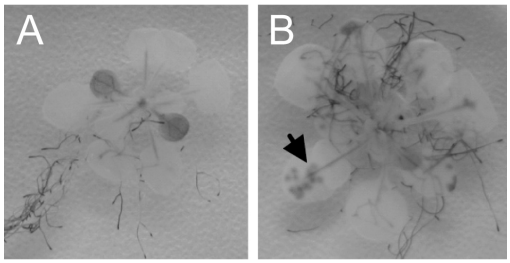

Fig. 1. Arabidopsis leaves were inoculated with *Phytophthora brassicae*. In an incompatible interaction with strain HH (A) a strong induction of expression was observed, while in a compatible interaction with strain CBS686.95 (B) no induction of expression was detected.

Concluding remarks

Cell-wall associated defense responses that are necessary to block invading pathogens are dependent on the functionality of the continuum between cell wall and plasma membrane. When adhesions between cell wall and plasma membrane are disrupted this continuum becomes unbalanced and effective defense is lost. Pathogens are able to reprogram host cells in order to promote infection. Destabilizing the cell wall-plasma membrane continuum could be an obvious step in this process. IPI-O is a pathogen effector that has the capacity to disrupt cell wall-plasma membrane adhesions, to interact with RGD-binding sites on the plasma membrane and to compete with RGD-containing proteins for those binding sites. Moreover, IPI-O has an RxLR-dEER motif that may function as a host cell targeting signal. The putative effector target of IPI-O, LecRK79 also plays an important role in mediating adhesions. The finding that expression of *LecRK79* is specifically induced in incompatible interactions suggests that LecRK79 has a role in defense. Possibly it counteracts the activity of a pathogen effector by either repairing cell wall-plasma membrane adhesions disrupted by the effector, or by preventing the effector to disrupt these adhesions. In a compatible interaction the effector may choose LecRK79 as a target for binding, possibly via RGD or the overlapping RxLR motif, and the basal level of LecRK79 may be sufficient to help the effector to enter the plant cell.

Acknowledgements

This reseach is supported by grants from the Netherlands Ministry of Agriculture, Nature and Food Quality (LNV427), EU-BioExploit (Food-CT-2005-513959) and the Netherlands-French bilateral exchange program Van Gogh (NWO-VGP 85-343).

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