Plant defence against aphids: the PAD4 signalling nexus

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Abstract

In Arabidopsis thaliana, PHYTOALEXIN DEFICIENT 4 (PAD4) functions as a key player in modulating defence against the phloem sap-feeding aphid Myzus persicae (Sülzer), more commonly known as the green peach aphid (GPA), an important pest of a wide variety of plants. PAD4 controls antibiosis and antixenosis against the GPA. In addition, PAD4 deters aphid feeding from sieve elements on Arabidopsis. In the past few years, substantial progress has been made in dissecting the role of PAD4 and its interaction with other signalling components in limiting aphid infestation. Several key genes/mechanisms involved in providing aphid resistance/susceptibility in Arabidopsis regulate the aphid infestation-stimulated expression of PAD4. Together, PAD4 and its interacting signalling partners provide a critical barrier to curtail GPA colonization of Arabidopsis.

Key words: Aphids, PAD4, Arabidopsis, plant defence.

Introduction

Plants utilize a plethora of defence responses, including molecular and biochemical mechanisms, to protect themselves from various biotic stresses. In Arabidopsis thaliana, which has long been used as a model plant to study plant stress response, the PHYTOALEXIN DEFICIENT 4 (PAD4) gene functions as a critical signalling component in defence against various pathogens (Glazebrook, 2005; Wiermer et al., 2005) as well as the green peach aphid (GPA; Myzus persicae Sülzer), a phloem sap consuming insect pest that causes considerable damage to a wide variety of plants (Louis et al., 2012c; Louis and Shah, 2013). PAD4 interacts with its signalling partner ENHANCED DISEASE SUSCEPTIBILITY 1 (EDS1) to provide resistance against pathogens (Feys et al., 2005). Interaction of PAD4 with EDS1 yields a nucleo-cytoplasmic PAD4–EDS1 complex that promotes accumulation of the plant defence signalling molecule salicylic acid (SA) and regulation of several defence-related genes that contribute to disease resistance. In contrast, the PAD4-mediated resistance to the GPA does not require EDS1 or SA (Moran and Thompson, 2001; Mewis et al., 2005; Pegadaraju et al., 2005, 2007; Louis et al., 2012a; Lei et al., 2014), thus unveiling a distinct, previously undefined mechanism involving PAD4 in defence against aphid infestation.

PAD4: A key regulator in providing defence against aphid infestation

PAD4 orchestrates antibiotic and antixenotic defences against aphids. Antibiosis involves mechanisms that influence the physiology of the aphids to adversely affect their growth, development and/or reproduction (Smith, 2005). On the other hand, antixenosis contributes to deterrence of aphid feeding and/or settling on the host plant (Painter, 1951; Kogan and Orman, 1978). The Electrical Penetration Graph...
(EPG) technique has provided a useful approach to study the influence of plant genotypic differences on GPA feeding behaviour. EPG analysis confirmed that a PAD4-exerted defence mechanism limits aphid feeding from the sieve elements (Pegadaraju et al., 2007; Louis et al., 2012a). The GPA spent significantly longer time in the sieve elements of the pad4 mutant compared with the wild type (WT) plant. PAD4 was also required for the accumulation of antibiosis activity. Petiole exudates, which are enriched in vascular sap, collected from the pad4 mutant were deficient in antibiosis activity compared with the petiole exudates obtained from the WT plants (Louis et al., 2010a, 2012a).

GPA infestation promotes PAD4 expression in the vascular tissues

GPA infestation resulted in the rapid induction of PAD4 expression in WT Arabidopsis leaves (Pegadaraju et al., 2005 2007; Couldridge et al., 2007; Louis et al., 2010a; Lei et al., 2014). Moreover, PAD4 expression was induced in and around the vascular tissues of GPA-infested leaves (Louis et al., 2012b). These results, in conjunction with the EPG studies, suggest that PAD4 expression in the vasculature is required for limiting GPA colonization. However, PAD4 expression was also observed in cells other than the vascular tissues (Louis et al., 2012b). Thus, a function for PAD4 operating in non-vascular tissues in Arabidopsis defence against the GPA cannot be ruled out. Generating transgenic Arabidopsis plants that specifically express PAD4 in the phloem will be useful to further characterize the role of PAD4 in phloem-based resistance to aphids.

PAD4 promoted senescence contributes to defence against the GPA

Aphids alter host source–sink relationship such that an uninterrupted supply of nutrients is available to the insect. By contrast, senescence acts as a defence mechanism against aphids (Pegadaraju et al., 2005). Leaf senescence results in the removal of nutrients from the aphid-infested leaves, thereby countering the source–sink alterations promoted by aphid colonization. PAD4 is required for promoting premature leaf senescence in GPA-infested plants, which is characterized by the up-regulation of a subset of SENESCE-ASSOCIATED GENE (SAG) expression, and increased chlorophyll loss and cell death (Pegadaraju et al., 2005; Louis et al., 2012a; Lei et al., 2014). The onset of cell death in response to GPA infestation was delayed in the pad4 mutant, compared with WT plants (Pegadaraju et al., 2005). In contrast, ectopic expression of PAD4 from the cauliflower mosaic virus (CaMV) 35S promoter rapidly induced cell death in response to GPA infestation in the 35S:PAD4 plant (Pegadaraju et al., 2007). Senescence also results in alterations of the redox status (Khanna-Chopra, 2012). A recent study showed that H2O2 content increased in GPA-infested Arabidopsis leaves, and this increase in H2O2 was associated with resistance (Lei et al., 2014). PAD4 was required for this increase in H2O2 in GPA-infested leaves (Lei et al., 2014). These studies suggest that PAD4-dependent leaf senescence- and cell death-associated mechanisms potentially contribute to the accumulation of factors that are detrimental for the attacking aphids.

An acyl hydrolase motif is required for PAD4 function in antibiosis and in deterring GPA feeding from the sieve elements

The PAD4 protein contains a triad of Ser (S), Asp (D), and His (H) residues that form the catalytic triad of many α/β fold acyl hydrolases that include lipases (Blow, 1990; Jirage et al., 1999; Feys et al., 2005). However, as yet, no lipase activity has been demonstrated for PAD4. The GPA population size was larger on Arabidopsis plants expressing mutant versions of PAD4 [PAD4(S118A) and PAD4(D178A)] in which Ser118 and Asp178 were substituted by Ala, than on WT plants. Furthermore, aphids spent more time in the sieve elements of the PAD4(S118A) compared with WT plants, and petiole exudates collected from the PAD4(S118A) plant lacked the PAD4-regulated antibiosis activity, thus suggesting that S118 is essential for the involvement of PAD4 in providing feeding deterrence and antibiosis activity against aphids (Louis et al., 2012a). However, PAD4(S118A) and PAD4(D178A) did not deter insect settling, SAG expression, and cell death in response to GPA infestation, thus suggesting the presence of at least two PAD4 containing molecular activities in defence against the GPA (Louis, 2011; Louis et al., 2012a).

Host lipids and their relationship with the PAD4-mediated defence pathway

Similar to PAD4, MYZUS PERSICAE-INDUCED LIPASE1 (MPL1) expression was induced in response to GPA infestation in Arabidopsis foliage (Louis et al., 2010b). However, unlike PAD4, MPL1 was not required for antixenosis. Like PAD4, the MPL1 protein contains the Ser-Asp-His triad of catalytic site residues that are conserved in α/β fold acyl hydrolases. The MPL1 protein, which exhibits lipase activity, was required only for antibiosis against the GPA (Louis et al., 2010b). Whether the lipase activity of MPL1 is indeed required for antibiosis will require additional experiments with plants expressing mutant forms of MPL1 in which the putative catalytic triad amino acid residues have been altered. Comparison of GPA feeding behaviour revealed that there was no significant difference in the total amount of time spent by the GPA in the sieve element phase on the mpl1 null mutant and WT plants, suggesting that the absence of MPL1 function in the mpl1 mutant does not affect aphid feeding behaviour. Petiole exudates of the mpl1 mutant lacked an antibiosis factor that is present in similar exudates of WT plants. PAD4 and MPL1 do not affect the GPA infestation-induced expression of each other (Louis et al., 2010b). Furthermore, ectopic expression of PAD4 and MPL1 from the CaMV 35S promoter in mpl1 and pad4 plants, respectively, rescued the antibiosis deficiency of the mpl1 and pad4 mutants, indicating that MPL1 and PAD4 contribute to two parallel antibiosis mechanisms and the elevated levels of one component/mechanism can overcome the deficiency of the
other (Fig. 1; Louis et al., 2010b; J Louis and J Shah, unpublished data). However, the existing evidence does not allow us to rule out the possibility that PAD4 or a PAD4-dependent factor alters the molecular activity of MPL1, and thereby contributes to MPL1-dependent antibiosis against aphids.

Both PAD4 and MPL1 contribute to the suppressor of salicylic acid insensitivity (ssi2)-mediated heightened antibiosis against GPA (Louis et al., 2010a, 2010b). The SSI2 gene encodes a plastid-localized stearoyl-ACP desaturase, which catalyses the desaturation of stearic acid to oleic acid and alters the Arabidopsis membrane lipid composition (Shah et al., 2001; Kachroo et al., 2001; Nandi et al., 2003). In comparison to the WT plant, the aphid population was significantly reduced in the ssi2 mutant plant, which exhibits a spontaneous cell death phenotype and accumulates high levels of an antibiosis activity in petiole exudates (Pegadaraju et al., 2005; Louis et al., 2010a). MPL1 expression was constitutively higher in the ssi2 mutant compared with the WT plant. Furthermore, the heightened antibiosis activity in ssi2 was dependent on MPL1 function (Pegadaraju et al., 2005; Louis et al., 2010b). In contrast to the elevated expression of MPL1, basal expression of PAD4 was not higher in the ssi2 mutant compared with the WT plants, thus suggesting that ssi2 likely promotes PAD4-dependent antibiosis downstream of PAD4 transcript accumulation (Fig. 1; Louis et al., 2010a).

Recently, it was shown that foliar infestation of GPA results in the accumulation of 9-LOX5 transcript in roots (Nalam et al., 2013). LOX5, which encodes a 9-lipoxygenase, was found to promote aphid colonization. Indeed, the oxylipin 9-hydroxyoctadecenoic acid (9-HOD) was found to promote aphid colonization on Arabidopsis and promote insect fecundity on an artificial diet, thus suggesting that 9-LOX products probably have an effect on the insect (Nalam et al., 2012). Interestingly, LOX5 was also required for the GPA infestation-associated up-regulation of PAD4 expression (Nalam et al., 2013). Furthermore, 9-HOD application induced PAD4 expression in Arabidopsis leaves (Nalam et al., 2013), leading to the suggestion that while Arabidopsis utilizes LOX5-synthesized products to promote defences, the GPA has likely evolved to cue on LOX5-derived metabolites to facilitate feeding, growth, and reproduction (Fig. 1).

**TPS11-dependent trehalose metabolism and PAD4 interaction in mediating defence against aphids**

Trehalose, a non-reducing disaccharide, has a signalling function in plants to protect them from various stresses (Schluempmann et al., 2003; Paul et al., 2008; Fernandez et al., 2002). Trehalose is synthesized in the roots and/or related metabolites are probably translocated to the shoots through the vascular system where they enhance antibiosis against the aphids. Both PAD4 and MPL1 are required for heightened resistance to GPA in the ssi2 mutant. Cross-complementation experiments suggest that MPL1 likely functions independently of PAD4. However, available evidence does not rule out the possibility of a PAD4-dependent mechanism modulating MPL1 activity. Bik1, a receptor-like cytoplasmic kinase, suppresses PAD4 expression. Basal expression of PAD4 is elevated in bik1 mutants, which exhibit enhanced resistance against the GPA. PAD4 function is required for the bik1-conferring resistance against aphids. Aphid infestation results in ET accumulation, which has been implicated in antixenosis, in particular deterring aphid settling on Arabidopsis. The aphid infestation associated emission of ET was elevated in the bik1 mutant, but not in the pad4 and the bik1 pad4 double mutant, thus indicating that PAD4 is required for the full extent of ET emission and that PAD4’s involvement in repelling GPA is probably mediated through ET signalling. [Black lines ending in arrows represent positive effects, broken black lines ending in closed circle represent unknown mechanisms, broken black lines ending in arrow is indicative of constitutive expression, and red lines ending with perpendicular bar indicate repressive effects]. (This figure is available in colour at JXB online.)
Trehalose metabolism is also involved in promoting defence against the GPA (Singh et al., 2011; Hodge et al., 2013). In Arabidopsis, the Trehalose-6-phosphate Synthase 11 (TPS11) gene is involved in the transient up-regulation of trehalose accumulation in GPA-infested plants. Time-course analysis of TPS11 transcript accumulation in response to aphid infestation revealed that TPS11 expression is also transiently up-regulated in GPA-infested leaves and parallels the transient increase in trehalose levels in aphid-infested Arabidopsis WT leaves (Singh et al., 2011). Like PAD4, TPS11 also provided antibiotic and antixenotic defences against the GPA. In addition, EPG analysis revealed that aphids spent more time feeding from the sieve elements of tps11 null mutant compared with WT plants, thus suggesting that TPS11 obstructs the aphid’s ability to feed uninterrupted from the sieve elements (Singh et al., 2011).

Trehalose application induced the expression of PAD4 in Arabidopsis WT leaves. Furthermore, the GPA infestation-associated induction of PAD4 was delayed in the tps11 null mutant, suggesting a significant contribution of TPS11 to the timely activation of PAD4 expression in response to aphid infestation (Singh et al., 2011). In agreement with a function for TPS11 in promoting PAD4 expression, higher basal expression of PAD4 was observed in the 35S:TPS11 and otsB transgenic plants, which contained elevated levels of trehalose, compared with WT plants. Taken together, the available evidence suggests that TPS11-dependent trehalose metabolism contributes to PAD4-mediated defence against aphids (Fig. 1). However, it was also shown that TPS11 and trehalose provided defence against aphids, independently of PAD4, by modulating carbon metabolism and activating starch accumulation in response to aphid infestation (Singh et al., 2011). It has been suggested that the plants might activate starch accumulation as a counter-defence mechanism to combat aphid attack (Singh et al., 2011).

BIK1, a receptor-like kinase, and its interaction with PAD4 upon aphid infestation

Very recently, a receptor-like cytoplasmic kinase (RLCK) BOTRYTIS-INDUCED KINASE 1 (BIK1) was shown to control defence against aphids by negatively regulating PAD4 expression. These receptor-like kinases are elicited when plants are attacked by various microbes and herbivores (Bent and Mackey, 2007; Boller and Felix, 2009; Prince et al., 2014). Unlike PAD4, aphid feeding did not significantly induce the expression of BIK1 in Arabidopsis WT leaves (Couldridge et al., 2007; Lei et al., 2014). Relative expression of BIK1 was comparable between uninfested and aphid-infested WT plants. Loss of BIK1 function in the bik1 mutant provided both antibiotic and antixenotic defences against aphids. In addition, aphids reared on the bik1 mutants, compared with WT plants, excreted less honeydew, a digestible waste, thus indicating reduced nutrient uptake. The body weight of aphids reared on the bik1 mutant was also significantly reduced compared with aphids reared on WT plants (Lei et al., 2014). Compared with the WT plants, the enhanced resistance against GPA in the bik1 mutant was accompanied by elevated levels of H$_2$O$_2$ accumulation, and enhanced cell death and callose deposition in response to GPA infestation (Lei et al., 2014).

The bik1-conferred resistance against the GPA was SA independent. Aphid numbers were comparable between bik1 and bik1 sid2 plants or bik1 and bik1 nahG plants, which express the bacterial NahG-encoded salicylate hydroxylase that degrades SA and thus does not accumulate elevated levels of SA. Furthermore, comparable aphid numbers were observed on the WT and the SA-deficient sid2 and nahG plants (Lei et al., 2014). It was also shown that GPA infestation induced accumulation of H$_2$O$_2$ and cell death in the bik1 mutant. However, SA was not required for these bik1-conferred phenotypes, thus supporting previous studies which inferred that SA was not critical in mediating defence against the GPA (Moran and Thompson, 2001; Mewis et al., 2005; Pegadaraju et al., 2005; Louis et al., 2010a).

Basal expression of PAD4 and SAG13, a PAD4-regulated senescence-associated gene in Arabidopsis, were elevated in the bik1 mutants compared with the WT plant. Loss of PAD4 gene function in the bik1 mutant background compromised the bik1-mediated enhanced resistance to GPA. Aphid numbers were significantly higher on bik1 pad4 double mutant plants than on bik1 single mutant plants (Lei et al., 2014). Furthermore, aphid feeding induced accumulation of H$_2$O$_2$ production and cell death were compromised in bik1 pad4 plants compared with bik1 plants (Lei et al., 2014). Taken together, these data suggest that PAD4 is required for the bik1-conferred heightened resistance to aphids.

Studies have shown that ethylene (ET) signalling is required for providing enhanced resistance to aphids (Dong et al., 2004; Anstead et al., 2010; Zhang et al., 2011). Increased aphid repellence on bik1 mutant plants compared with WT plants at an early time period (6h post release) was mediated through the ET pathway (Lei et al., 2014). Mutations in the ETHYLENE INSENSITIVE 2 (EIN2) gene, a key component in the ET signalling pathway, resulted in attenuation of the bik1-conferred deterrence of GPA settling on the bik1 ein2 double mutant at an early time point compared with the bik1 single mutant plants (Lei et al., 2014). Similarly, as mentioned before, bik1 pad4 mutant plants were more attractive to aphids compared with bik1 plants. Furthermore, aphid infestation resulted in an elevated ET burst in the bik1 mutant compared with uninfested bik1 plants. ET release was significantly reduced in bik1 pad4 plants compared with bik1 plants before and after aphid infestation, suggesting that PAD4 is involved in promoting ET accumulation that potentially deters aphid settling on Arabidopsis. These results indicate that BIK1 negatively regulates PAD4 expression and ET production, whereas the aphid infestation-induced expression of PAD4 positively modulates the ET emission (Fig. 1).

PAD4 beyond Arabidopsis

Similar to Arabidopsis, in tomato (Solanum lycopersicum) plants GPA-infestation up-regulated the expression of SIPAD4, the tomato homologue of Arabidopsis PAD4 (Singh...
and Shah, 2012). Likewise, aphid infestation induced the expression of SITPSI1 and trehalose application up-regulated SIPAD4 expression in tomato leaves, thus suggesting that similar defence signalling pathways might be operating in both Arabidopsis and tomato. As in Arabidopsis, trehalose metabolism likely also contributes to defence against aphids in tomato independent of the PAD4 pathway by promoting the accumulation of starch that acts a defence mechanism to curtail GPA proliferation (Singh et al., 2011; Singh and Shah, 2012). Additional studies with different host plants are required to confirm how extensively the PAD4 and trehalose signalling pathways are conserved in providing defence against aphids.

Final remarks

The current evidence indicates that PAD4 is a critical node at which different signals converge to control Arabidopsis response to GPA infestation. Negative regulation of PAD4 expression, presumably by a BIK1-dependent mechanism, is likely released and a combination of inductive factors, including trehalose and 9-LOX-derived oxylipins, promote PAD4 expression in response to GPA infestation, thereby contributing to defences that limit GPA infestation on Arabidopsis. Although the mechanism by which these PAD4-activating processes are elicited in response to aphid infestation is unclear, it is likely that the elicitors present in the aphid saliva trigger these mechanisms. Indeed, several recent studies have demonstrated the ability of aphid salivary components to influence plant defence responses (Bos et al., 2010; Atamian et al., 2013; Elzinga et al., 2014; Rodriguez et al., 2014). Although PAD4 function in defence against the GPA has been studied in Arabidopsis, a PAD4 homologue in tomato was similarly found to respond to GPA infestation as well as trehalose application, thus suggesting that PAD4 function in limiting aphid infestation is likely engaged by plants beyond Arabidopsis. However, how PAD4, a nucleo-cytoplasmic protein, modulates host defences against aphids, and how PAD4 expression is regulated by TPS11-, LOX-5-, and BIK1-dependent mechanisms remains to be unravelled.

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