Published in "Plant & Signaling Behavior 5(9): 1099 - 1101, 2010"
which should be cited to refer to this work.

Indolic secondary metabolites protect Arabidopsis from the oomycete pathogen *Phytophthora brassicae*

Klaus Schlaeppi* and Felix Mauch
Department of Biology; University of Fribourg; Fribourg, Switzerland

Key words: Arabidopsis, disease resistance, *Phytophthora brassicae*, secondary metabolites, indolic glucosinolates, camalexin

*Correspondence to: Klaus Schlaeppi;
Email: klaus.schlaeppi@unifr.ch

Addendum to: Schlaeppi K, Abou-Mansour E, Buchala A, Mauch F. Disease resistance of Arabidopsis to ***Phytophthora brassicae*** is established by the sequential action of indole glucosinolates and camalexin. Plant J 2010; In press; PMID: 20230487; DOI: 10.1111/j.1365-313X.2010.04197.x.

The model plant *Arabidopsis thaliana* contains a large arsenal of secondary metabolites that are not essential in development but have important ecological functions in counteracting attacks of pathogens and herbivores.[1,2] Preformed secondary compounds are often referred to as phytoanticipins and metabolites, that are synthesized de novo in response to biotic stress are known as phytoalexins.[3] Camalexin is the typical phytoalexin of Arabidopsis. It has antimicrobial activity towards some pathogens and was shown to be an important component of disease resistance in several plant pathogen interactions.[4] Glucosinolates (GS) are characteristic phytoanticipins of the Brassicaceae family including Arabidopsis. GS are best known as repellents or attractants for herbivorous insects and their predators whereas their antimicrobial potential has received relatively little attention.[5] The GS are glucosides and the biologically active aglycone is released upon biotic stress by glucohydrolase enzymes commenly called myrosinases. Because an Arabidopsis mutant susceptible to the oomycete pathogen *Phytophthora brassicae* shows a partial deficiency in both camalexin and iGS accumulation we became intrigued by the role of these secondary compounds in disease resistance.[6,7] Our results show that disease resistance of Arabidopsis to *P. brassicae* is established by the combined action of iGS and camalexin.

Indole Glucosinolates and Camalexin are Important Components of Disease Resistance

Arabidopsis reacts to an infection by *P. brassicae* with a coordinated upregulation of genes of the biosynthetic pathways leading to the indolic compounds camalexin and indole GS (iGS). We investigated the possible defensive role of indolic secondary metabolites using sets of camalexin mutants, iGS-related mutants and double mutants with combined defects (see **Fig. 1A** for an overview). The biosynthetic mutants *cyp71A13* and *cyp71B15* (*pad3*) are deficient in camalexin production.[8,9] The *myb51* mutant accumulates about 50% of wildtype iGS, whereas the mutant *pen2* is compromised in the pathogen-induced hydrolysis of iGS.[10-12] The double mutant *cyp79B2 cyp79B3* fails to produce indol-3-aldoxime (IAOx) which serves as a common precursor of iGS and camalexin biosynthesis.[13] Consequentely, iGS and camalexin are not produced in *cyp79B2 cyp79B3*. The double mutants *myb51 pad3* and *pen2 pad3* have both defects in camalexin production and a deficiency in iGS biosynthesis or hydrolysis, respectively. The mutants were infected with zoospores of *P. brassicae* and the disease resistance phenotype was scored based on symptom development. **Figure 1B** illustrates that single defects in either the iGS- or the camalexin pathway have a minor effect on disease resistance. The pathogen causes slightly enhanced disease symptoms (corresponding to a reduced disease resistance phenotype: R-) but further progression into the leaf tissue is halted and no spores are produced.

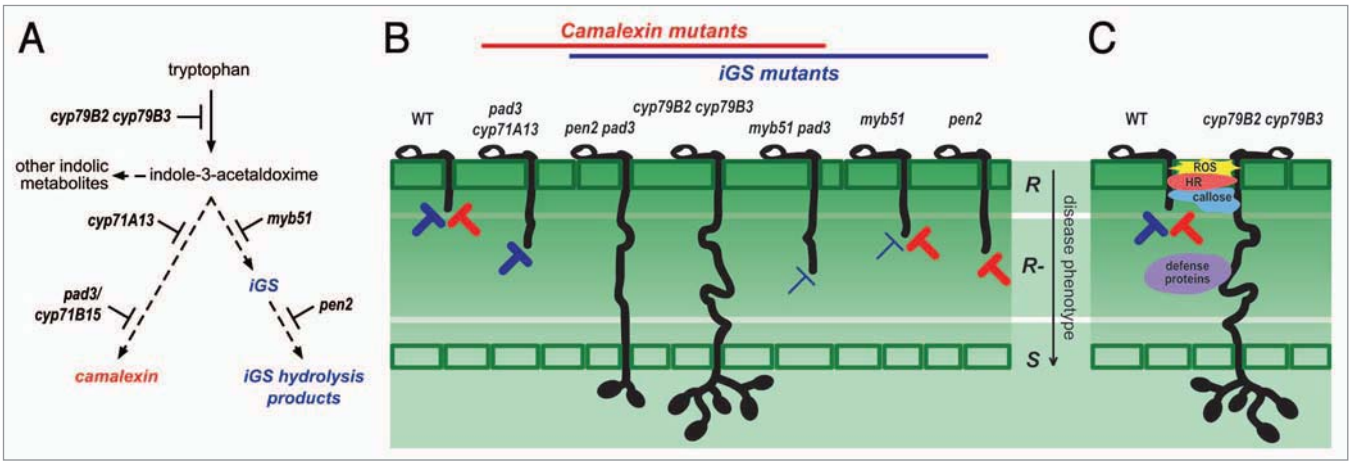

Figure 1. Arabidopsis mutants with combined deficiencies in camalexin and iGS metabolisms are susceptible to *P. brassicae*. Arabidopsis mutants with defects in camalexin biosynthesis (*cyp71A13* and *cyp71B15* (*pad3*)), iGS production or hydrolysis (*myb51* and *pen2*, respectively) and mutants with combined defects (*cyp79B2 cyp79B3*, *pen2 pad3* and *myb51 pad3*) were infected with *P. brassicae* and scored for disease resistance at 5 dpi. The defensive phytochemical pathways of iGS and camalexin are represented in blue and red, respectively. (A) Schematic representation of biosynthic pathways and corresponding mutants. (B) Disease resistance phenotypes are classified into resistant (R), reduced resistance (R-) and susceptible (S). The inhibitory action of the residual iGS accumulation of *myb51* is indicated by a lighter blue bar. (C) The *cyp79B2 cyp79B3* is susceptible despite activation of classical defence responses.

This finding indicates that the two chemical defenses of iGS and camalexin backup each other to ensure resistance. In contrast, the double mutants *pen2 pad3* and *cyp79B2 cyp79B3* with combined defects are susceptible (**Fig. 1B**). The susceptibility of both double mutants demonstrates that the combined lack of IAOx-derived secondary metabolites, predominantly iGS and camalexin, is sufficient to compromise disease resistance. Hence, iGS and camalexin are gentically identified as important components of Arabidopsis disease resistance to *P. brassicae*.

Interestingly, the double mutant *cyp79B2 cyp79B3* with the most severe phenotype has no defects in other classical defence-associated responses. It shows wildtype-like accumulation of reactive oxygen species (ROS), hypersensitve cell death response (HR), callose production and stress hormone signaling. Hence, *cyp79B2 cyp79B3* is susceptible despite the activation of many other defence responses (**Fig. 1C**). This conclusion emphasizes the importance of IAOx-derived secondary metabolites as disease resistance mechanisms of Arabidopsis to *P. brassicae*.

Indole Glucosinolates and Camalexin Act Sequentially

Analytical and microscopical results of the interaction of Arabidopsis with *P. brassicae* allow an explanation of the interplay of iGS- and camalexin-based chemical defences. Camalexin is synthesized in response to the pathogen and the timing of accumulation suggests that camalexin becomes important at later stages of defence. Microscopic analyses of early infection events (6 hpi) revealed that *P. brassicae* penetrates the leaf epidermis of iGS mutants much more efficiently compared to wildtype and camalexin mutants. The hydrolysis of the preformed iGS inhibits pathogen entry into the leaf indicating that iGS have an early defensive role in penetration resistance. This does not exclude a role of iGS at later stages of the interaction. The resistant *myb51* mutant shows an enhanced penetration phenotype. The double mutant *myb51 pad3* remains resistant despite reduced penetration resistance and camalexin deficiency (**Fig. 1B**). Hence, the residual iGS levels of *myb51* appear to be sufficient to protect from disease. Taken together, disease resistance of Arabidopsis to *P. brassicae* is mainly established by the combined and sequential activity of the two chemical defences of iGS and camalexin.

Our findings fit the concept of 'pre- and postinvasion defences' formulated in the context of Arabidopsis interactions with non-adapted powdery mildew fungi.[11,12] Arabidopsis *pen* (penetration) mutants permit non-host powdery mildew species to invade epidermal cells but the pathogen is still inhibited by postinvasive defence mechanisms including camalexin. These studies identified iGS as an important preinvasion defence. Comparable to our results, increased epiphytic hyphal growth as a sign for breakdown of non-host resistance was only observed in the double mutants *pen2 pad3* and *cyp79B2 cyp79B3*. In addition, indolic compounds were recently reported to partially explain other disease resistance related phenomena.[14,15]

Conclusions

Arabidopsis disease resistance to *P. brassicae* relies on the sequential action of the two chemical defences of iGS, traditionally recognized as anti-herbivore compounds, and the phytoalexin camalexin. These double-layered chemical defences backup each other and ensure disease resistance. iGS have an early function in penetration resistance and are likely to contribute to the inducible camalexin defence for late pathogen arrest. Because the iGS- and camalexin mutant *cyp79B2 cyp79B3* is susceptible despite other functional defence responses, we can deduce that these indolic secondary metabolites are of major importance for disease resistance to *P. brassicae*.

References

1. d'Auria JC, Gershenzon J. The secondary metabolism of *Arabidopsis thaliana*: growing like a weed. Curr Opin Plant Biol 2005; 8:308-16.
2. Dixon RA. Natural products and plant disease resistance. Nature 2001; 411:843-7.
3. Van Etten HD, Mansfield JW, Bailey JA, Farmer EE. Two classes of plant antibiotics—phytoalexins versus phytoanticipins. Plant Cell 1994; 6:1191-2.
4. Glawischnig E. Camalexin. Phytochemistry 2007; 68:401-6.
5. Halkier BA, Gershenzon J. Biology and Biochemistry of Glucosinolates. Annu Rev Plant Biol 2006; 57:303-33.
6. Roetschi A, Si-Ammour A, Belbahri L, Mauch F, Mauch-Mani B. Characterization of an Arabidopsis-Phytophthora pathosystem: resistance requires a functional PAD2 gene and is independent of salicylic acid, ethylene and jasmonic acid signalling. Plant J 2001; 28:293-305.
7. Schlaeppi K, Bodenhausen N, Buchala A, Mauch F, Reymond P. The glutathione-deficient mutant *pad2-1* accumulates lower amounts of glucosinolates and is more susceptible to the insect herbivore *Spodoptera littoralis*. Plant J 2008; 55:774-86.
8. Nafisi M, Goregaoker S, Botanga CJ, Glawischnig E, Olsen CE, Halkier BA, et al. Arabidopsis cytochrome P450 monooxygenase 71A13 catalyzes the conversion of indole-3-acetaldoxime in camalexin synthesis. Plant Cell 2007; 19:2039-52.
9. Schuhegger R, Nafisi M, Mansourova M, Petersen BL, Olsen CE, Svatos A, et al. CYP71B15 (PAD3) catalyzes the final step in camalexin biosynthesis. Plant Physiol 2006; 141:1248-54.
10. Gigolashvili T, Berger B, Mock HP, Muller C, Weisshaar B, Flugge UI. The transcription factor HIG1/MYB51 regulates indolic glucosinolate biosynthesis in *Arabidopsis thaliana*. Plant J 2007; 50:886-901.
11. Lipka V, Dittgen J, Bednarek P, Bhat R, Wiermer M, Stein M, et al. Pre- and postinvasion defenses both contribute to nonhost resistance in Arabidopsis. Science 2005; 310:1180-3.
12. Bednarek P, Pislewska-Bednarek M, Svatos A, Schneider B, Doubsky J, Mansurova M, et al. A glucosinolate metabolism pathway in living plant cells mediates broad-spectrum antifungal defense. Science 2009; 323:101-6.
13. Zhao Y, Hull AK, Gupta NR, Goss KA, Alonso J, Ecker JR, et al. Trp-dependent auxin biosynthesis in Arabidopsis: involvement of cytochrome P450s CYP79B2 and CYP79B3. Genes Dev 2002; 16:3100-12.
14. Truman WM, Bennett MH, Turnbull CG, Grant MR. Arabidopsis auxin mutants are compromised in systemic acquired resistance and exhibit aberrant accumulation of various indolic compounds. Plant Physiol 2010; 152:1562-73.
15. Consonni C, Bednarek P, Humphry M, Francocci F, Ferrari S, Harzen A, et al. Tryptophan-derived metabolites are required for antifungal defense in the Arabidopsis *mlo2* mutant. Plant Physiol 2010; 152:1544-61.