Indolic secondary metabolites protect Arabidopsis from the oomycete pathogen Phytophthora brassicae

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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 commonly 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 Consequently, 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.
Analytical and microscopical results of the interaction of Arabidopsis with P. brassicaceae 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. brassicaceae 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. brassicaceae 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. 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.

Conclusions

Arabidopsis disease resistance to P. brassicaceae 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. brassicaceae.
References