The Age of Coumarins in Plant–Microbe Interactions

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Coumarins are a family of plant-derived secondary metabolites that are produced via the phenylpropanoid pathway. In the past decade, coumarins have emerged as iron-mobilizing compounds that are secreted by plant roots and aid in iron uptake from iron-deprived soils. Members of the coumarin family are found in many plant species. Besides their role in iron uptake, coumarins have been extensively studied for their potential to fight infections in both plants and animals. Coumarin activities range from antimicrobial and antiviral to anticoagulant and anticancer. In recent years, studies in the model plant species tobacco and Arabidopsis have significantly increased our understanding of coumarin biosynthesis, accumulation, secretion, chemical modification and their modes of action against plant pathogens. Here, we review current knowledge on coumarins in different plant species. We focus on simple coumarins and provide an overview on their biosynthesis and role in environmental stress responses, with special attention for the recently discovered semiochemical role of coumarins in aboveground and belowground plant–microbe interactions and the assembly of the root microbiome.

Keywords: Coumarins • Iron homeostasis • Microbiome • Plant–microbe interactions • Scopoletin • Secondary metabolism.

Introduction

In nature, plants are constantly exposed to a plethora of threats and unfavorable environmental conditions. Plants adapt and respond to these continuous challenges, therewith minimizing diseases, abiotic stresses and nutrient deficiencies. To cope with these environmental stresses, plants have evolved sophisticated adaptive strategies, such as inducible structural and physiological modifications, a highly effective immune system, and the capacity to produce an impressive arsenal of stress-protective secondary metabolites (Dixon 2001, Dodds and Rathjen 2010, Senthil-Kumar and Mysore 2013). Plant secondary metabolites display an enormous structural diversity. They can be produced in planta from various primary metabolites or their biosynthetic intermediates, either constitutively or in response to different biotic or abiotic stresses. Most of the secondary metabolites are derived from the isoprenoid, phenylpropanoid, alkaloid or fatty acid/polyketide biosynthesis pathways (Dixon 2001). Metabolites deriving from the phenylpropanoid pathway are often involved in structural or chemical defenses.

For example, the cell wall-fortifying compounds lignin, cutin and suberin form structural barriers that inhibit pathogen invasion (Doblas et al. 2017). Other phenylpropanoid derivatives such as flavonoids, anthocyanins and tannins participate in other aspects of environmental stress adaptation, or in plant growth and physiology (Vogt 2010). More specifically, flavonoids emerged as important mediators of the chemical communication between leguminous plants and beneficial nitrogen-fixing rhizobia. In this mutualistic interaction, root-secreted flavonoids act as chemoattractants for rhizobia and activate genes required for nodulation, which established the initial paradigm for the role phenylpropanoid-derived metabolites in beneficial plant–microbe interactions (Fisher and Long 1992, Phillips 1992). In the past decades, the phytoalexin family of antimicrobial coumarins emerged as important players in the plant's chemical defense strategy (Dixon 2001, Gnonlonfin et al. 2012), and more recently in adaptive plant responses to iron (Fe) deficiency (Tsai and Schmidt 2017) and the interaction between plant roots and beneficial microbes in the root microbiome (Stringlis et al. 2018b). Here, we review the current knowledge on coumarin accumulation, distribution and regulation during pathogen infection and zoom in on their emerging role in aboveground and belowground plant–microbe interactions and Fe uptake.

Plant Coumarins

Coumarins are named after the plant *Coumarouna odorata* (now *Dipteryx odorata*), from which the simplest member of this class of compounds, basic coumarin, was first isolated by Vogel in 1820 (Soine 1964, Borges et al. 2005). Coumarins are secondary metabolites that are present in a wide range of higher plants but have also been detected in some microorganisms and animal species (Soine 1964, Harborne 1999, de Lira et al. 2007). In the plant kingdom, coumarins occur in both monocotyledonous and dicotyledonous plant species and are produced in high levels in the plant families *Umbelliferae*, *Rutaceae*, *Compositae*, *Leguminosae*, *Oleaceae*, *Moraceae* and *Thymelaeaceae* (Harborne 1999, Bourgaud et al. 2006, Matos et al. 2015). The model plant *Arabidopsis thaliana* (hereafter: Arabidopsis), a member of the *Brassicaceae* family, is also capable of producing a suite of coumarins, which opened new avenues for their functional characterization in plant–microbe interactions (Bednarek et al. 2005, Kai et al. 2006, Strehmel et al. 2014). Coumarins

are present in different plant organs including leaves, fruits, flowers and roots, but also in the exudates of plants roots (Peters and Long 1988, Perez and Ormeno-Nunez 1991, Harborne 1999, Fourcroy et al. 2014, Schmidt et al. 2014, Ziegler et al. 2017, Tsai et al. 2018). Coumarins have been extensively studied in the past decades and were found to display pharmacological activities that range from antimicrobial, molluscicidal, antiviral (including anti-HIV), anticancer, antidepressant, antioxidant, anti-inflammatory and anti-coagulant to cardiovascular (Borges et al. 2005).

Coumarins are polar structures that are present in plants in their free state or in the form of glycosides. Their ability to absorb UV light results in their characteristic blue fluorescence (**Fig. 1a**). Some coumarins can be structurally altered by natural light due to their photosensitivity (Soine 1964, Gnonlonfin et al. 2012). Coumarins are 1,2-benzopyrones that consist of a benzene ring linked to a pyrone ring and are produced via the general phenylpropanoid pathway (Harborne 1999, Bourgaud et al. 2006). The structural core of coumarins is 2H-1-benzopyran-2-one or "basic coumarin" (**Fig. 1b**). Based on modifications of this core, coumarins can be classified into complex and simple coumarins. Complex coumarins are produced by the addition of heterocyclic compounds on the basic coumarin core and are further classified into furanocoumarins, pyranocoumarins, phenylcoumarins, dihydrofurocoumarins and biscoumarins (Medina et al. 2015). The focus of this review will be on simple coumarins, including scopolin, scopoletin, esculin, esculetin, umbelliferone, fraxetin and sideretin (**Fig. 1b**), which play diverse roles in the interaction of plants with biotic and abiotic environmental stress factors.

Biosynthesis of Simple Coumarins

The 2H-1-benzopyran-2-one structural core of coumarins is derived from cinnamic acid and is formed via the ortho-hydroxylation of cinnamates, trans/cis isomerization of the side chain and lactonization (Soine 1964, Gestetner and Conn 1974). The first step of the coumarin biosynthesis pathway is ortho-hydroxylation of cinnamates that branches off from lignin biosynthesis. The *CCoAOMT1* gene encodes caffeoyl-CoA O-methyltransferase 1, which is required for the production of feruloyl CoA and participates in the biosynthesis of both lignin and the simple coumarin scopoletin in Arabidopsis roots (Kai et al. 2008, Vogt 2010). The conversion of feruloyl CoA to the UV-fluorescent coumarin scopoletin is catalyzed by the Fe(II)- and 2-oxoglutarate-dependent dioxygenase (2OGD) feruloyl CoA ortho-hydroxylase 1 (F6′H1) (Kai et al. 2006, Kai et al. 2008). Arabidopsis mutant *f6′h1* is strongly impaired in the production of the coumarins scopolin, scopoletin, esculin, esculetin, fraxin, fraxetin and sideretin (Kai et al. 2008, Schmid et al. 2014, Rajniak et al. 2018, Tsai et al. 2018). Recent studies in Arabidopsis revealed that scopoletin is converted into fraxetin via the activity of scopoletin 8-hydroxylase (S8H) (Rajniak et al. 2018, Siwinska et al. 2018, Tsai et al. 2018). Fraxetin is further oxidized by a cytochrome P450 (CYP) enzyme (CYP82C4) leading to the production of sideretin (Rajniak et al. 2018). Upon their production, coumarins are found in their aglycone form or they can be modified by the activity of UDP-glucose-dependent glucosyltransferases (UGTs) to glycosylated forms like scopolin and esculin (Chong et al. 2002). Glycosylated coumarins are stored in the vacuole. In response to various stresses, disruption of the cells can bring the glycosylated forms in contact with β-glucosidases in the cytosol (**Fig. 2**).
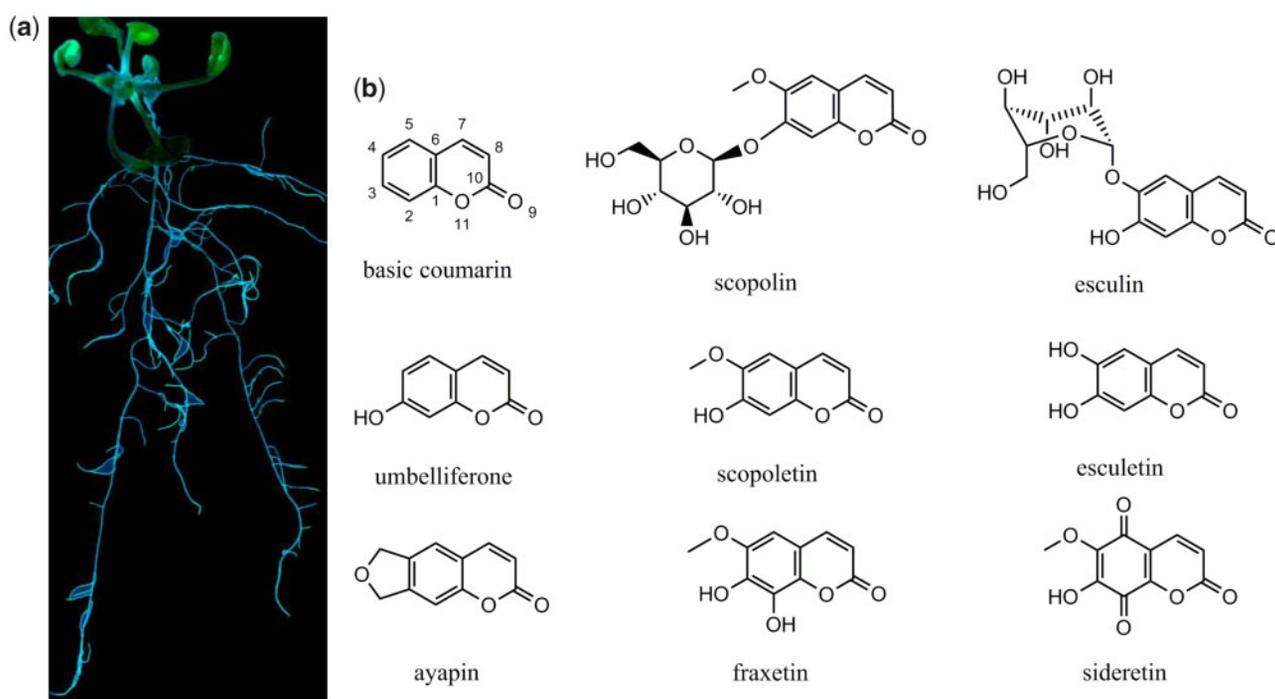

Fig. 1 (a) Visualization of fluorescent coumarins produced by roots of Fe-starved *Arabidopsis thaliana* Col-0 plants. (b) Chemical structures of representative plant-derived simple coumarins and of coumarin ayapin, whose role is discussed in this review.

β-Glucosidases belonging to the family 1 glycoside hydrolases catalyze the hydrolysis of the b-glucosidic bond between a carbohydrate moiety and the basic coumarin core resulting in bioactive coumarin aglycone forms, such as scopoletin and esculetin (Morant et al. 2008, Ahn et al. 2010).

Coumarins and Their Role in Fe Stress

Fe is an essential element for all life on Earth, including plants and their associated microbes (Aznar et al. 2015). Although Fe is abundant in most soils, it is mainly present in the form of ferric oxide (Fe^{3+}), which is poorly soluble at neutral and alkaline pH, thus drastically reducing its bioavailability (Hindt and Guerinot 2012). Hence, plants growing in nature oftentimes develop Fe deficiency. Dicotyledonous plants deal with Fe limitation via a number of adaptive processes collectively referred to as "Strategy I". In the first step of Strategy I, plant roots release protons into the rhizosphere via the activity of the H^+-ATPase AHA2 that lowers the pH of the surrounding soil and increases the solubility of Fe^{3+}. Then, solubilized Fe^{3+} is reduced to
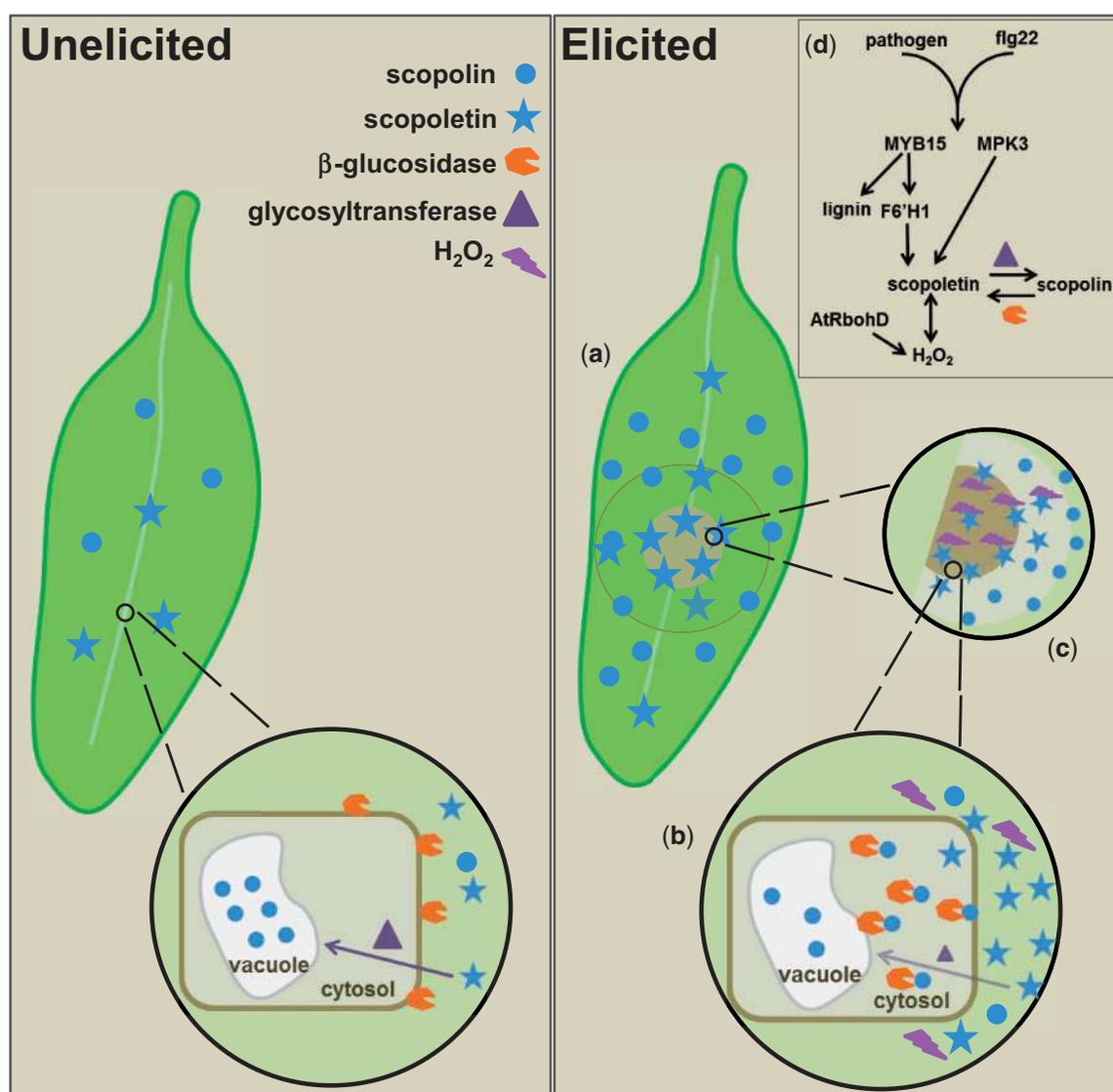

Fig. 2 Coumarin accumulation and regulation in unelicited and elicited leaves with coumarins scopolin and scopoletin used as an example. In healthy leaves, scopolin and scopoletin accumulate to low basal levels. Due to the activity of glucosyltransferases, unstable and toxic scopoletin is converted to the glycosylated form scopolin. Scopolin is transferred within cells and stored in the vacuoles, spatially separated from β-glucosidases. Following defense activation in leaves by a pathogen or elicitor, scopoletin accumulates in the infected tissue and scopolin in the surrounding tissue (a). When scopolin is released from the vacuoles, it is subjected to the activity of the β-glucosidases that convert it to scopoletin (b). Then scopoletin exerts its antimicrobial activity and scavenges H_2O_2 in the infected tissues therewith restricting cell death (c). In infected Arabidopsis, MYB15 regulates F6′H1 activity and the subsequent accumulation of lignin and scopoletin (d). MPK3 is also found to be required for scopoletin accumulation in infected tissues. Produced scopoletin becomes oxidized by H_2O_2, which is generated by the activity of AtRbohD. Depending on the environmental cues, plants can control scopoletin levels by converting it to scopolin via the activity of glucosyltransferases and converting scopolin back to scopoletin by β-glucosidases (d).

ferrous Fe^{2+} by the plasma membrane protein FERRIC REDUCTION OXIDASE 2 (FRO2), after which it can be transported from the soil environment into the root epidermis by the high-affinity IRON-REGULATED TRANSPORTER1 (IRT1) (Hindt and Guerinot 2012, Kobayashi and Nishizawa 2012, Grillet and Schmidt 2017).

The release of phenolics in the rhizosphere by plants experiencing Fe limitation was suggested as another mechanism that facilitates Fe mobilization and uptake by plant roots (Dakora and Phillips 2002). Jin et al. (2007) observed that phenolic compounds released by roots of red clover facilitated the mobilization of sparingly available Fe from the rhizosphere soil or from the root apoplast. More recently, different studies in Arabidopsis have uncovered that the Fe-mobilizing phenolic compounds are root-secreted coumarins and that they have an important role in Fe acquisition, particularly under alkaline conditions where Fe availability is low (Rodriguez-Celma et al. 2013, Fourcroy et al. 2014, Schmid et al. 2014, Schmidt et al. 2014, Fourcroy et al. 2016, Rajniak et al. 2018, Siwinska et al. 2018). As mentioned above, F6′H1 is essential for the production of these coumarins (Schmid et al. 2014), while the ABC transporter PDR9 (ABCG37) is required for their secretion into the rhizosphere (Fourcroy et al. 2014, Fourcroy et al. 2016).

The biosynthesis of coumarins is transcriptionally regulated (Tsai and Schmidt 2017). Under conditions of Fe starvation, the bHLH transcription factor FER-LIKE IRON DEFICIENCY-INDUCED TRANSCRIPTION FACTOR (FIT) is activated, which subsequently regulates the expression of *FRO2*, *IRT1* and *F6′H1* (Colangelo and Guerinot 2004, Schmid et al. 2014). Arabidopsis *fit* mutants are impaired in coumarin production (Schmid et al. 2014). Upstream of F6′H1, a cascade of phenylpropanoid biosynthesis genes also becomes activated in response to Fe deficiency, including those encoding phenylalanine ammonia-lyase (PAL), coumarate:CoA ligase 4CL1 and 4CL2, CCoAOMT1 and hydroxycinnamoyl-CoA shikimate/quinate hydroxycinnamoyltransferase (HCT), which provide the phenylpropanoid precursors for the biosynthesis of coumarins (Fourcroy et al. 2014, Siso-Terraza et al. 2016, Tsai and Schmidt 2017). The major coumarins produced in Arabidopsis in response to Fe deficiency are scopoletin, esculetin, fraxetin and sideretin and the coumarin glycosides, scopolin, esculin and fraxin. In vitro studies showed that coumarins participate in Fe acquisition by chelation and/or reduction of Fe^{3+} (Schmid et al. 2014, Schmidt et al. 2014, Siso-Terraza et al. 2016, Rajniak et al. 2018), which is subsequently reduced to Fe^{2+} by FRO2 and transported into root cells via IRT1 (Fourcroy et al. 2016). Interestingly the coumarin profile of Fe-deficient Arabidopsis plants is dependent on the pH status of their growth substrate. At pH 7.5, roots accumulate more scopolin, scopoletin, fraxetin, isofraxidin and coumarinolignans than at pH 5.5 (Siso-Terraza et al. 2016). In Arabidopsis root exudates, scopoletin and sideretin are the most abundant coumarins at pH levels below 6.0 (Siso-Terraza et al. 2016, Rajniak et al. 2018, Stringlis et al. 2018b), but at more alkaline pH levels fraxetin becomes more abundant (Siso-Terraza et al. 2016).

Under Fe-limited growth conditions, Arabidopsis coumarin mutants *f6′h1* and *s8h* are smaller and more chlorotic compared to wild-type plants (Fourcroy et al. 2014, Schmid et al. 2014, Rajniak et al. 2018). Supplementing the growth substrate with esculin, esculetin, fraxetin and sideretin rescued the chlorotic phenotypes of both *f6′h1* and *s8h* (Schmid et al. 2014, Rajniak et al. 2018), indicating that these coumarins or their metabolized derivatives can alleviate Fe deficiency symptoms. Interestingly, scopoletin could only rescue the chlorotic symptoms of *f6′h1* but not that of *s8h* plants, suggesting that scopoletin needs to be metabolized via S8H to become capable of alleviating Fe deficiency (Rajniak et al. 2018). The capacity of specific coumarins to chelate and mobilize Fe is variable and depends on the presence of a catecholic moiety in their structure. A catecholic moiety is characterized by the presence of two adjacent hydroxy groups in the benzene ring of the coumarin structure, which resembles the structure of microbial catechol-type siderophores that function in the chelation and uptake of Fe by the microbes that secrete them (Neilands 1995, Verbon et al. 2017). Esculetin, fraxetin and sideretin are catecholic coumarins and possess a high Fe-mobilization capacity. The non-catecholic coumarins scopoletin, esculin and fraxin are incapable of chelating Fe themselves, possibly because the catechol moiety is not accessible to Fe^{3+} (Schmid et al. 2014, Siso-Terraza et al. 2016, Rajniak et al. 2018).

Coumarins also emerged as key components in the interplay between the Fe deficiency response and induced systemic resistance (ISR), a well-characterized systemic immune response that is triggered upon colonization of the roots by beneficial microbes in the rhizosphere (Pieterse et al. 2014, Verbon et al. 2017, Stringlis et al. 2018b). Fe deficiency response genes, including *FIT*, *FRO2* and *IRT1*, are induced in Arabidopsis roots upon colonization by ISR-inducing rhizobacteria and fungi, even when plants are growing under Fe-sufficient conditions (Zamioudis et al. 2015, Martinez-Medina et al. 2017). During the interaction of roots with ISR-inducing microbes, FIT regulates the expression of the Arabidopsis root-specific transcription factor MYB72 which in turn controls the expression of the β-glucosidase gene *BGLU42* (Van der Ent et al. 2008, Zamioudis et al. 2014, Zamioudis et al. 2015, Verbon et al. 2017, Stringlis et al. 2018b). Both MYB72 and BGLU42 were identified as key components in the onset of ISR in the roots (Van der Ent et al. 2008, Pieterse et al. 2014, Zamioudis et al. 2014). The role of MYB72 was linked to the production and secretion of coumarins, as mutant *myb72* plants appeared to be impaired in coumarin biosynthesis and secretion (Zamioudis et al. 2014, Stringlis et al. 2018b). Using mutant *bglu42* plants, it was shown that BGLU42 activity is required for the processing of scopolin into scopoletin and its subsequent secretion into the rhizosphere (Stringlis et al. 2018b). This is in line with Ahn et al. (2010) who showed that the root-expressed Arabidopsis β-glucosidases BGLU21, BGLU22 and BGLU23 can specifically hydrolyze scopolin to form scopoletin. Hence, removal of the sugar moiety of coumarin glycosides by BGLU42 and possibly other β-glucosidases is a crucial step to enable their secretion into the rhizosphere (Zamioudis et al. 2014, Tsai and Schmidt 2017). Interestingly, MYB72, together with its closest homolog MYB10, was also found to be required for the survival of Arabidopsis plants growing in alkaline soils (Palmer et al.

2013), pointing to a dual role of MYB72 in both Fe mobilization and ISR. Although MYB10 acts redundantly with MYB72 in Fe acquisition (Palmer et al. 2013), MYB72 alone seems to be sufficient for the onset of ISR (Van der Ent et al. 2008, Segarra et al. 2009).

Coumarins are not only released into the rhizosphere in response to Fe limitation, also conditions of phosphate (Pi) starvation triggers the exudation of coumarins by plant roots (Pant et al. 2015, Ziegler et al. 2016). Recently, Chutia et al. (2019) studied the coumarin profiles in Arabidopsis plants experiencing Fe deficiency, Pi deficiency or both deficiencies. They found that Pi deficiency and Fe deficiency stimulate different coumarin profiles, and that a combination of both nutrient deficiencies affects the coumarin profiles produced by the single nutrient deficiencies. This suggests that fine-tuning of the coumarin profiles depends on both Fe and Pi nutrition (Chutia et al. 2019).

Role of Coumarins in Aboveground Plant–Microbe Interactions

Plant-derived compounds with a role in chemical defense are generally categorized either as phytoanticipins, which are constitutively produced and thus pre-existing in plant tissues, or phytoalexins, which are produced de novo upon infection and are typically not detected in healthy tissues (Dixon 2001). In the past 50 years, coumarins have been extensively studied in various plant species and their role as phytoanticipins or phytoalexins are well documented. In this section, we provide an overview of the role that simple coumarins play in the interaction between plants and phytopathogens or pathogenic elicitors.

Coumarin accumulation in response to pathogen attack and their role in disease resistance

Studies in many different plant species have shown that coumarins can accumulate in response to infection by a diversity of pathogens, including viruses, bacteria, fungi and oomycetes (summarized in Table 1). Already in 1972, researchers observed that inoculation of tobacco mosaic virus (TMV) on leaves of the TMV-resistant cultivar Nicotiana tabacum cv. Xanthi resulted in the accumulation of coumarins in developing local necrotic lesions (Tanguy and Martin 1972). Production of scopoletin was also reported in leaves of the rubber tree Hevea brasiliensis during infection by the fungus Microcyclus ulei (Giesemann et al. 1986). In another setup, cell cultures of H. brasiliensis treated with the oomycete defense elicitor elicitin produced high levels of scopoletin. When rubber tree leaves were sprayed with these oomycete elicitors, increased resistance towards the oomycete pathogen Phytophthora palmivora was observed (Dutsadee and Nunta 2008). Similarly, in young leaves of the vegetable fiber crop jute mallow (Corchorus olitorius), coumarins accumulated in response to inoculation with spores of the fungal pathogen Helminthosporium turcicum (Abou Zeid 2002). One of the identified coumarins was scopoletin, which inhibited growth of H. turcicum in vitro. Similar observations were done in other plant species, including Arabidopsis (Kai et al. 2006), tobacco (Vereecke et al. 1997) and broccoli (Brassica oleracea cv. italics) (Vereecke et al. 1997, Tortosa et al. 2018). The related studies are listed in Table 1.

The extent and timing of coumarin accumulation has often been associated with the level of disease resistance. For instance, clones of the Hevea rubber tree that are resistant to the fungus M. ulei and the oomycete P. palmivora accumulated scopoletin faster and in a longer-lasting manner in response to pathogen infection than the susceptible clones (Garcia et al. 1995, Churngchow and Rattarasarn 2001). Also in the plane tree Platanus occidentalis, resistance against the fungal leaf pathogen Ceratocystis fimbriata f. sp. platani was associated with an increased accumulation of the coumarins scopoletin and umbelliferone at the site of infection, while in the susceptible tree Platanus acerifolia a delayed accumulation of these coumarins was observed (El Modafar et al. 1995). Similar associations between coumarin accumulation and disease resistance have been found in a range of plant species, including elm (Valle et al. 1997), sunflower (Prats et al. 2006, Prats et al. 2007), cultivated and wild tobacco (Gasser et al. 1988, Goy et al. 1993, El Oirdi et al. 2010, Sun et al. 2014) and tomato (Sade et al. 2015). The related studies are listed in Table 1.

Antimicrobial activity of coumarins against phytopathogens

In most of the studies presented in Table 1, the identified coumarins were tested in vitro for their activity against different plant pathogens. For instance, scopoletin displayed antifungal activity in vitro against M. ulei and two other fungal leaf pathogens of rubber tree: Colletotrichum gloeosporioides and Corynespora cassiicola, resulting in reduced spore germination and germ tube elongation (Garcia et al. 1995). In in vitro growth experiments, the oomycete pathogen P. palmivora showed a higher sensitivity to scopoletin than the tested fungal pathogens (Churngchow and Rattarasarn 2001). Because basic coumarin was previously reported to inhibit cellulose biosynthesis in higher plants (Hara et al. 1973), the structural differences in the cellulose-based cell walls of oomycetes and the chitin-based cell walls of fungi was coined to be related to the higher coumarin sensitivity of oomycetes. In vitro bioassays further demonstrated that scopoletin is highly toxic to the fungi O. ulmi, Cercospora nicotianae, Botrytis cinerea, Alternaria alternata, the oomycete Phytophthora parasitica var. nicotianae, the bacteria Pseudomonas syringae pv. tabaci and P. syringae pv. syringae, and the virus TMV (Goy et al. 1993, Valle et al. 1997, El Oirdi et al. 2010, Sun et al. 2014). In the cases of O. ulmi and B. cinerea, this coumarin had an inhibitory effect on spore germination but not on mycelium growth (Valle et al. 1997, El Oirdi et al. 2010). The antimicrobial activity of coumarins was found to depend on the number and the polarity of the oxygen substituents in the benzene ring (Kayser and Kolodziej 1999). In the case of scopoletin, the presence of a methoxy (-O-CH$_3$) and a hydroxy group (-OH) in the benzene ring may explain its toxicity. Another explanation for the antimicrobial effect of aglycone coumarins like scopoletin compared to their

Table 1 Coumarin accumulation in different plant species in response to chemical or biological elicitation

Plant species (tissue/organ)	Elicitor	Coumarins	References
Hevea brasiliensis (leaves, cell cultures)	*Microcyclus ulei; Phytophthora palmivora;* elicitin	Scopoletin	Churngchow and Rattarasarn (2001), Dutsadee and Nunta (2008), Garcia et al. (1995), Giesemann et al. (1986)
Platanus occidentalis (leaves)	*Ceratocystis fimbriata* f. sp. *platani*	Scopoletin, umbelliferone	El Modafar et al. (1995)
Ulmus pumila (cell cultures)	*Ophiostoma ulmi*	Scopoletin	Valle et al. (1997)
Helianthus annuus (leaves, petals)	*Puccinia helianthi; Sclerotinia sclerotiorum;* ASM	Scopolin, scopoletin, ayapin	Prats et al. (2006), Prats et al. (2007), Prats et al. (2002)
Matricaria chamomilla (leaves)	Salicylic acid	Umbelliferone, herniarin	Pastirova et al. (2004)
Pisum sativum (leaves)	BTH	Scopoletin	Barilli et al. (2015)
Ipomoea tricolor (cuttings)	*Fusarium oxysporum* f. sp. *batatas*	Scopolin, scopoletin	Shimizu et al. (2005)
Corchorus olitorius (leaves)	*Helminthosporium turcicum*	Scopoletin	Abou Zeid (2002)
Brassica oleracea (leaves)	*Xanthomonas campestris* pv. *campestris*	Basic coumarin	Tortosa et al. (2018)
Solanum lycopersicum (leaves)	TYLCV	Scopoletin	Sade et al. (2015)
Nicotiana tabacum (leaves, cell cultures, roots)	*Alternaria alternata; Botrytis cinerea; Thielaviopsis basicola;* TMV; 2,4-D; β-megaspermin; Cytokinins; MeJA; Oligo-sulphated galactan Poly-Ga	Scopolin, scopoletin, esculin, fraxetin	Chong et al. (1999), El Oirdi et al. (2010), Gasser et al. (1988), Grosskinsky et al. (2011), Santhanam et al. (2019), Sharan et al. (1998), Sun et al. (2014), Taguchi et al. (2000a), Tanguy and Martin (1972), Vera et al. (2011)
Arabidopsis thaliana (leaves, roots, cell cultures)	*Fusarium oxysporum* f. sp. *batatas; Paenibacillus polymyxa* BFKC01; *Pseudomonas fluorescens* S101; *Pseudomonas simiae* WCS417; *Pseudomonas syringae* pv. *tomato, Pythium sylvaticum,* flg22, 2,4-D	Scopolin, scopoletin, esculin, esculetin	Bednarek et al. (2005), Chaouch et al. (2012), Chezem et al. (2017), Kai et al. (2006), Schenke et al. (2011), Simon et al. (2010), Simon et al. (2014), Stringlis et al. (2018b), Van de Mortel et al. (2012), Zhou et al. (2016)

glycosylated forms may be the lack of an elongated side chain, which makes it easier for aglycone coumarins to cross microbial cell walls and exert their toxic effect (Rauckman et al. 1989).

Coumarin accumulation in response to elicitors or hormones

Application of elicitors or priming agents is an alternative approach used in agriculture to enhance the defense potential of plants against various pathogens. Among the efforts to reduce rust disease incidence in sunflower caused by the fungus *Puccinia helianthi* is exogenous application of the priming agent acibenzolar-S-methyl (ASM) (Prats et al. 2002). Metabolome analysis of ASM-treated leaves demonstrated an increased accumulation of the coumarins scopolin, scopoletin and ayapin inside the leaves and a significant secretion of scopoletin to the leaf surface. This was associated with the inhibition of *P. helianthi* spore germination and appressorium formation, suggesting the involvement of these coumarins in the ASM-induced priming against sunflower rust (Prats et al. 2002). Elevated accumulation of the coumarins scopoletin, esculetin and other phenylpropanoid compounds was also observed in tobacco plants sprayed with the priming agent oligo-sulphated galactan Poly-Ga, contributing as such to enhanced resistance against TMV (Vera et al. 2011).

Microbe-associated molecular patterns (MAMPs), such as bacterial flagellin and fungal chitin, are well-known elicitors of the plant's innate immune system (Pel and Pieterse 2013). In Arabidopsis cell cultures, treatment with the defense elicitor flg22, a 22-amino-acid peptide derived from bacterial flagellin (Felix et al. 1999), induced the production of scopoletin (Schenke et al. 2011). The MYB-type transcription factor MYB15 was shown to be required for flg22-mediated production of scopoletin and formation of lignin (Chezem et al. 2017). Moreover, mutant *myb15* plants showed reduced expression of *F6′H1*, accumulated less scopoletin, and displayed reduced lignification in response to flg22 treatment. As a result, mutant *myb15* and *f6′h1* plants were more susceptible to the bacterial pathogen *P. syringae* pv. *tomato*, highlighting the potential role of scopoletin in MAMP-triggered immunity. The mitogen-activated protein kinase (MAPK) MPK3 is an important player in the immune response that is activated downstream of flg22 (Meng and Zhang 2013). Overexpression of MPK3 resulted in increased expression of *F6′H1* and enhanced production of scopoletin (Genot et al. 2017) confirming the notion that coumarins play a role in the plant's innate immune response (**Fig. 2**).

Salicylic acid (SA) and jasmonic acid (JA) are two major plant defense hormones with important roles in induced resistance to pathogens and pests (Pieterse et al. 2012). In some studies, SA was shown to be an elicitor of coumarin

biosynthesis. For instance, application of SA to roots of chamomile (*Matricaria chamomilla*) resulted in the accumulation of coumarins umbelliferone and herniarin in the leaves (Pastirova et al. 2004). In pea, application of the SA mimic benzo(1,2,3,)thiadiazole-7-carbothioic acid S-methyl ester (BTH) and DL-β-aminobutyric acid (BABA) reduced the frequency of infection by the rust pathogen *Uromyces pisi* (Barilli et al. 2010). These applications were accompanied by an increased production of total phenolic compounds (Barilli et al. 2010). BTH increased the levels of scopoletin and the antimicrobials pisatin and medicarpin in leaves. This was more pronounced in a *U. pisi* resistant pea genotype compared to a susceptible one (Barilli et al. 2015). Application of scopoletin, medicarpin or pisatin on leaves of pea reduced spore germination and appressoria formation of the fungus, confirming that these compounds are involved in BTH- and BABA-induced pea resistance against *U. pisi* (Barilli et al. 2015). Treatment of tobacco cell suspensions with methyl jasmonate (MeJA) resulted in the accumulation of scopolin and scopoletin, with scopolin being mostly inside the cells and scopoletin in the culture filtrate. These data suggested a role for MeJA in eliciting coumarin biosynthesis and that formation of scopolin is required in the cells before its conversion and release in the filtrate as scopoletin (Sharan et al. 1998). In line with this, the higher resistance of young leaves of wild tobacco *Nicotiana attenuata* to *A. alternata* infection compared to the mature leaves is associated with the accumulation of higher levels of JA, scopolin and scopoletin (Sun et al. 2014). Interestingly, no scopoletin was detected in infected JA-deficient plants, while its induced accumulation was restored upon exogenous application of MeJA (Sun et al. 2014). Findings from the same group showed that intact JA signaling is also required for the production of scopolin in *A. alternata* infected wild tobacco leaves (Li and Wu 2016).

Apart from SA and JA, other hormones have also been implicated in coumarin accumulation and plant resistance to pathogens. Taguchi et al. (2000a) explored the effect of synthetic auxin 2,4-dichlorophenoxyacetic acid (2,4-D) on the accumulation of coumarins in tobacco cells. They observed that scopoletin was taken up by 2,4-D-treated cells and converted to scopolin in the cytoplasm before being stored in the vacuoles. Conversely, scopoletin uptake was abolished when cells were treated with the auxin inhibitor *p*-chlorophenoxyisobutyric acid (PCIB) (Taguchi et al. 2000a, Taguchi et al. 2001). Similar results were observed in Arabidopsis, where treatment of shoots with the synthetic auxin 2,4-D increased the levels of scopoletin and scopolin (Kai et al. 2006). Cytokinins are involved in tobacco resistance against the hemibiotrophic pathogen *P. syringae* pv. *tabaci* by, among other activities, inducing the production of scopoletin and the phytoalexin capsidiol, a terpenoid derived from the isoprenoid biosynthetic pathway (Grosskinsky et al. 2011). More recent findings support a role for ethylene (ET) in the biosynthesis of coumarin scopoletin (Sun et al. 2017).

Besides chemical defense elicitors, biological elicitors of defense have also been described to be associated with coumarins. Amongst the best-studied, biological defense elicitors are plant growth- and health-promoting fungi and rhizobacteria that upon colonization of plant roots induce a systemic, broad-spectrum immune response known as ISR (Pieterse et al. 2014, Martinez-Medina et al. 2016). In morning-glory plants (*Ipomoea tricolor* cv. heavenly blue), the non-pathogenic *Fusarium oxysporum* strain 101–2 reduced the wilting symptoms caused by the pathogen *F. oxysporum* f. sp. *batatas* strain O-17. This enhanced resistance was associated with an accelerated accumulation of the coumarins scopolin and scopoletin in the cuttings pre-inoculated with the beneficial fungus (Shimizu et al. 2005). A similar phenomenon was observed in Arabidopsis roots, which accumulated high levels of coumarins in response to elicitation of the roots by selected ISR-inducing rhizosphere bacteria (Van de Mortel et al. 2012, Zhou et al. 2016, Stringlis et al. 2018b).

Glucosyltransferase activity and accumulation of coumarins

Early studies in tobacco were highly instrumental in providing a mechanistic understanding of how plants control coumarin accumulation and distribution during pathogen infection. In response to avirulent pathogens, resistant tobacco plants develop a hypersensitive response (HR) that restricts pathogen growth in the infected plant tissue. This response is associated with SA accumulation and the subsequent induction of defense-related genes. Among the SA-responsive tobacco genes are the glucosyltransferase genes *TOGT1* and *TOGT2* (Horvath and Chua 1996). These genes are also induced in tobacco cell suspension cultures treated with the β-megaspermin elicitor from *Phytophthora megasperma* and in tobacco leaves inoculated with TMV, confirming their role in plant defense (Fraissinet-Tachet et al. 1998). *TOGT1* and *TOGT2* were heterologously expressed in *Escherichia coli* and tested for their substrate specificity towards a range of phenolic compounds. TOGT1 and TOGT2 displayed glucosyltransferase activity when scopoletin or esculetin were used as substrates, suggesting a role for these pathogen-induced TOGTs in coumarin metabolism (Fraissinet-Tachet et al. 1998). Indeed, elicitation of tobacco cells with β-megaspermin induced the accumulation of a TOGT, which was followed by rapid secretion of scopolin out of the cells (Chong et al. 1999), confirming that glucosyltransferase activity of TOGT plays a role in the conversion of scopoletin to scopolin (Fraissinet-Tachet et al. 1998). Interestingly, the released scopolin was converted back to scopoletin by the activity of a β-glucosidase. Moreover, scopoletin was shown to act as a H_2O_2 scavenger, possibly to control diffusion of H_2O_2 during the HR (Chong et al. 1999). Also in Arabidopsis the conversion of coumarins from the free form to the glycosylated form is catalyzed by the activity of UDP-glycosyltransferases (UGTs). The genome of Arabidopsis contains 120 UGT genes classified into 14 groups (A–N) according to the level of similarity of the conserved amino acid sequences (Ross et al. 2001). Following infection of Arabidopsis leaves by an avirulent strain of *P. syringae* pv. *tomato* (strain: *Pst-AvrRpm1*), the UGT genes *UGT73B3* and *UGT73B5* were significantly upregulated during the development of the HR (Langlois-Meurinne et al. 2005), highlighting that they are pathogen responsive.

The tobacco glucosyltransferase UDP-glucose: hydroxycoumarin 7-*O*-glucosyltransferase was found to be expressed in response to auxin and involved in the conversion of scopoletin to scopolin before its storage in the vacuoles (Taguchi et al. 2000b, Taguchi et al. 2001). Other studies confirmed that glucosyltransferase activity is important for disease resistance. Chong et al. (2002) generated TOGT-depleted tobacco plants by antisense expression of the *TOTG1* gene. They observed that these plants accumulated less scopolin and scopoletin and were less resistant to TMV. The reduced TMV resistance was linked to a longer-lasting ROS accumulation in the tissues surrounding the TMV infection site, supporting the ROS-scavenging role of scopoletin (Chong et al. 2002) (**Fig. 2**). These observations were confirmed in transgenic TOGT-overexpressing tobacco plants, in which increased glucosyltransferase activity in leaves and roots was accompanied by enhanced resistance against potato virus Y (PVY) (Matros and Mock 2004). Together, these findings point to a finely tuned regulation of the free and glycosylated forms of coumarins by the reciprocal activity of glucosyltransferases and β-glucosidases in the hours following defense elicitation in tobacco (**Fig. 2**).

Arabidopsis UGT mutants *ugt7b3* and *ugt7b5*, show a reduced level of resistance to the avirulent pathogen *Pst-AvrRpm1*, confirming a role for UGT-mediated coumarin modification in this plant–pathogen interaction (Langlois-Meurinne et al. 2005). Simon et al. (2014) investigated the role of free and glycosylated coumarins in the Arabidopsis-*Pst-AvrRpm1* interaction in the context of their ROS-scavenging capacity. They monitored the production of ROS and scopoletin in wild-type plants and the single and double mutants *ugt73b3*, *ugt73b5* and *ugt73b3/ugt73b5* following infection by *Pst-AvrRpm1* in the absence or presence of the ROS production inhibitor diphenyleneiodonium (DPI). From their study it was concluded that ROS produced in developing HR lesions is scavenged via a fast oxidation of scopoletin, therewith dampening toxic effects of the ROS in the HR-forming tissues (Simon et al. 2014). Hence, the coumarin glycosylating UGTs UGT73B3 and UGT73B5 may play a role in a ROS buffering mechanism in developing HR lesions that are initiated in response to infection by avirulent pathogens.

A metabolome analysis of Arabidopsis leaves infected with *Pst-AvrRpm1* was conducted to dissect the spatial metabolomic response of plants in infected and adjacent uninfected leaf tissues (Simon et al. 2010). SA, scopoletin and the phytoalexin camalexin (Zhou et al. 1999) strongly accumulated in HR-forming tissues, while scopolin was more abundant in the adjacent uninfected tissues (**Fig. 2**). Using the Arabidopsis mutant *cat2*, which is impaired in the ROS scavenger CATALASE-2 and displays upregulated ROS signaling, the authors studied the interaction between ROS and coumarin accumulation in infected and uninfected adjacent tissues. In mutant *cat2* plants, HR lesion formation and ROS accumulation was enhanced in comparison to wild-type plants. The scopolin content in adjacent uninfected tissues was also enhanced in *cat2*, but in infected tissues scopolin levels were similar to wild-type (Simon et al. 2010). The authors did not measure scopoletin levels in *cat2* mutants, however they suggested that higher scopolin

accumulation in uninfected adjacent tissues and the activity of β-glucosidases could facilitate the increased scopoletin production in infected tissues. Increased production of scopoletin could therefore aid plants in dealing with the oxidative stress caused by scavenging the ROS that accumulate in infected tissues.

To further understand the ROS-scavenging role of coumarins in defense responses to pathogens, Chaouch et al. (2012) characterized metabolic changes in mutants of *AtRbohD* and *AtRbohF* genes that have a role in ROS production and cell death. During the Arabidopsis-*Pst-AvrRpm1* interaction, the *atrbohF* mutant accumulated less SA and camalexin compared with wild-type and *atrbohD* mutant plants, but scopoletin levels accumulated to wild-type levels (Chaouch et al. 2012). By contrast, mutant *atrbohD* accumulated higher levels of scopoletin. Introduction of the *atrbohD* mutation in the *cat2* background did not affect SA levels but enhanced scopoletin and camalexin accumulation compared with the single mutant *cat2*. Introduction of the *atrbohF* mutation in the *cat2* background decreased SA and camalexin levels, but did not affect scopoletin accumulation (Chaouch et al. 2012). These data point to a role for AtRbohD in scopoletin accumulation during HR development following infection by *Pst-AvrRpm1* (**Fig. 2**). These immune components were also tested for their involvement in Arabidopsis defense priming by the defense elicitor phosphite, which provides protection against the oomycete pathogen *Hyaloperonospora arabidopsidis* (Massoud et al. 2012). Priming by phosphite was still effective in the SA, camalexin and scopoletin biosynthesis mutants tested and independent of AtRbohD-dependent ROS production, suggesting that ROS, camalexin and scopoletin are not components of phosphite-induced priming against *Hpa* infection in Arabidopsis (Massoud et al. 2012).

The Role of Coumarins in Belowground Plant–Microbe Interactions

Roots growing in the soil are in contact with a tremendous diversity of microbes, both pathogenic and beneficial, collectively known as the root microbiome (Berendsen et al. 2012). Pathogenic and beneficial microbes are able to colonize roots (Zamioudis and Pieterse 2012, Stringlis et al. 2018c), and thus plants need to discriminate the pathogenic ones from those that can promote plant growth and health. Selected members of the root microbiome can boost plant resistance by inducing ISR, a systemic immune response that is effective against a broad spectrum of aboveground attackers (Pieterse et al. 2014). Before successful colonization, both pathogenic and beneficial microbes need to compete for the same niches and efficiently use the exudates released by the roots. These exudates however consist of a cocktail of compounds, with some being a food source and others being deleterious for the microbes (Bais et al. 2006). Coumarins emerged as important players in the interaction of plants with members in its belowground root microbiome, either pathogenic or beneficial.
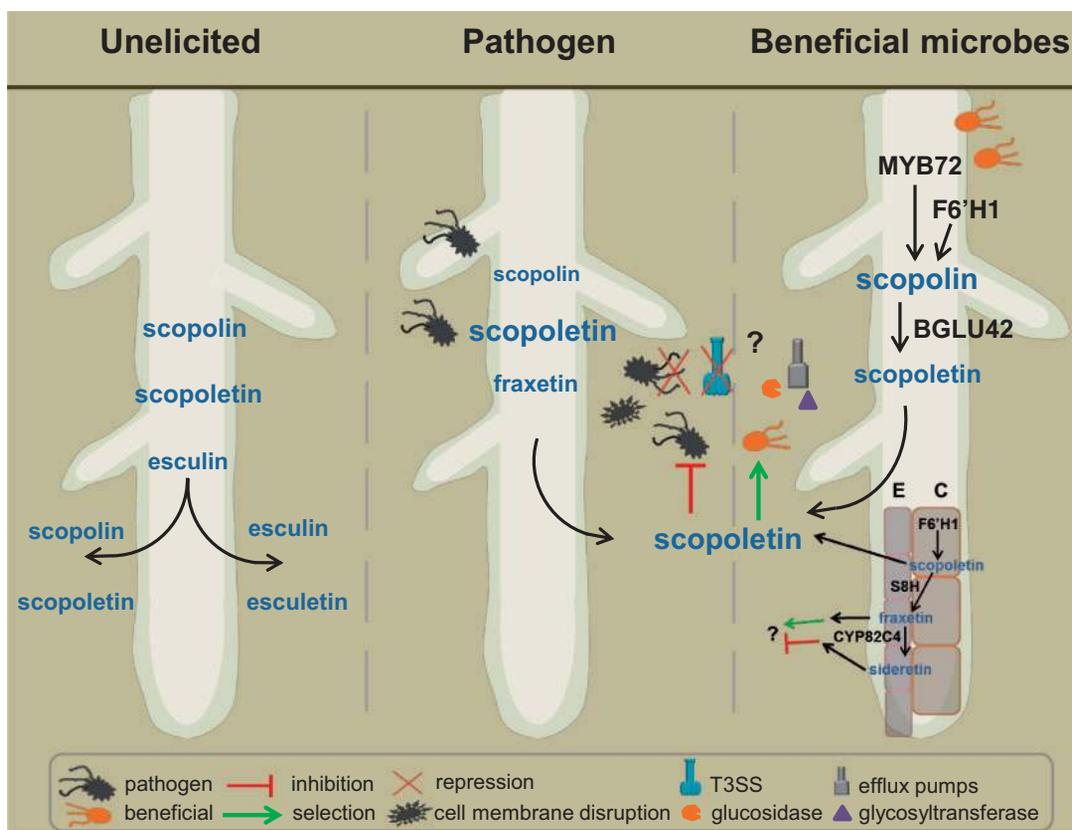
Fig. 3 Production of coumarins during belowground plant–microbe interactions, as suggested by studies in Arabidopsis and tobacco. Coumarins scopolin, scopoletin, esculin and esculetin are present at low basal levels in roots and exudates of healthy, unelicited Arabidopsis plants. In the case of root infection by a pathogen, scopolin levels decrease while scopoletin (and fraxetin) accumulates inside the roots and in root exudates. In roots colonized by beneficial *MYB72*-inducing microbes, scopolin production increases. Due to the activity of BGLU42, scopolin is converted to scopoletin, which is then released into the rhizosphere, where it may be further processed to fraxetin and sideretin. Coumarin biosynthesis relies on a functional F6'H1 and expression of *F6'H1* in the cortex (C) suggests that biosynthesis of this coumarin predominantly takes place in this cell layer (Schmid et al. 2014). Subsequently, scopoletin can be transferred to the epidermal cell layer (E) where due to the activity of S8H and CYP82C4 it can be converted to fraxetin or sideretin, respectively (Rajniak et al. 2018). In the rhizosphere, scopoletin either favors or inhibits the proliferation of different microbiome members (Stringlis et al. 2018b) but the role of fraxetin and sideretin in this context is unknown. Coumarins can negatively affect microbial growth by repressing motility, suppressing the activation of the type III secretion system (T3SS) or by disrupting microbial cell membranes.

Coumarins in the interaction with soil-borne pathogens

In unelicited Arabidopsis roots, scopolin and the lignin precursors coniferin and syringin are highly abundant (Bednarek et al. 2005). Upon infection of the roots by the oomycete pathogen *Pythium sylvaticum*, scopolin, coniferin and syringin were rapidly processed, probably to produce cell wall-fortifying lignin (from coniferin and syringin) and the antimicrobial coumarin scopoletin (from scopolin) (Bednarek et al. 2005). In wild tobacco *N. attenuata*, the antimicrobial coumarins scopoletin and fraxetin accumulated in roots in response to infection by the necrotrophic fungus *A. alternata* (Santhanam et al. 2019). These examples indicate that in analogy to their function in leaves, accumulation of specific coumarins in roots plays a role in defense against soil-borne pathogens (**Fig. 3**).

Phytopathogens rely on the activity of membrane-bound efflux pumps to detoxify plant-derived toxic compounds and effectively colonize their plant hosts (Martinez et al. 2009). In

tomato, plant-derived compounds including the coumarin esculetin were shown to induce the expression of two efflux pump-encoding genes in the soil-borne wilt bacterium *Ralstonia solanacearum*. Mutation of these efflux pump genes conferred enhanced sensitivity to the plant metabolites and a reduction of *R. solanacearum* virulence on the tomato host (Brown et al. 2007). Also, the coumarins daphnetin, esculetin, xanthotol and umbelliferone significantly inhibited *R. solanacearum* growth (Yang et al. 2016). Microscopical examination of *R. solanacearum* cells showed that daphnetin and esculetin caused disruption of the cell membrane, and daphnetin, esculetin and umbelliferone significantly inhibited biofilm formation. Moreover, the bacterial motility genes *fliA* and *flhC* were repressed by umbelliferone, esculetin and daphnetin, which may also contribute to the reduced virulence of *R. solanacearum* (Yang et al. 2016). In another study, coumarin and the phytoalexin resveratrol displayed antimicrobial activity against *R. solanacearum* both in vitro and in vivo and

contributed to tobacco resistance against this pathogen (Chen et al. 2016). Both compounds inhibited bacterial growth on agar plates, affected cell morphology and permeability of bacterial cell membranes, and suppressed swarming motility and biofilm formation. In line with this, tobacco roots pre-treated with coumarin and resveratrol showed reduced ahdesion and colonization by *R. solanacearum* and consequently developed less disease symptoms (Chen et al. 2016). Hence, coumarins can have profound effects on different fundamental life processes of microbes, explaining their versatility in plant immunity.

Another demonstration of the versatile role of coumarins in plant defense, is their observed effect on specific infection mechanisms of phytopathogens. Pathogenic bacteria employ a secretion system to inject effectors into the host cells in order to suppress immune responses and achieve colonization. This secretion system, known as the type III secretion system (T3SS), is well-characterized for its role in pathogenicity of bacteria belonging to the genera *Pseudomonas*, *Erwinia*, *Ralstonia* and *Xanthomonas* (Tampakaki et al. 2010, Galan et al. 2014). Interestingly, in the tobacco-*R. solanacearum* pathosystem, the coumarin umbelliferone was found to suppress the expression of T3SS regulatory and effector genes and inhibited *R. solanacearum* biofilm formation (Yang et al. 2017). Treatment of tobacco roots with umbelliferone prior to infection with *R. solanacearum*, significantly reduced *R. solanacearum* populations in tobacco roots and lowered disease levels (Yang et al. 2017).

Coumarins and their interaction with soil-inhabiting beneficial microbes

Coumarins also emerged as players in the interaction of plants with plant growth- and health-promoting microbes in the rhizosphere. A class of microbes that facilitate Pi uptake and enhance plant growth under Pi starvation conditions in about 80% of all terrestrial plant species are symbiotic arbuscular mycorrhizal fungal (AMF) (Oldroyd 2013, Cosme et al. 2018). *Nicotiana attenuata* plants with silenced calcium- and cal-modulin-dependent protein kinase (CCaMK), have a compromised interaction with AMF and show reduced growth compared to wild-type plants after inoculation with AMF (Wang et al. 2018). It appeared that upon AMF colonization, roots of this silenced line accumulated more fraxetin and scopoletin compared to wild-type plants (Wang et al. 2018). In similarity to Fe-starved plants, plants growing under Pi limitation also accumulate and excrete coumarins (Ziegler et al. 2016, Chutia et al. 2019). Hence, the enhanced coumarin production in AMF-colonized, CCaMK-silenced plants is probably the result of the activation of the Pi starvation response, while in wild-type plants this response is alleviated because of the functional AMF interaction.

Beneficial plant–microbe associations also include nonsymbiotic plant growth-promoting rhizobacteria (PGPR) and fungi (PGPF) of diverse genera. PGPR and PGPF can stimulate plant growth through degradation of soil pollutants, the production of phytostimulators, or by suppressing plant diseases or pests, either directly via antibiosis or indirectly via the elicitation of

ISR (Pieterse et al. 2014). Recently, coumarins emerged as important semiochemicals in the interaction between Arabidopsis and the well-characterized PGPR *Pseudomonas simiae* WCS417 (Berendsen et al. 2015). Colonization of Arabidopsis roots by WCS417 caused massive transcriptional changes, many of which overlapped with root transcriptional changes to Fe deficiency (Verhagen et al. 2004, Zamioudis et al. 2014, Zamioudis et al. 2015, Stringlis et al. 2018a), a phenomenon that was also observed in tomato (Martinez-Medina et al. 2017). This transcriptional overlap contained many genes with roles in the biosynthesis and excretion of Fe-mobilizing coumarins, including *F6′H1* (Rodriguez-Celma et al. 2013, Schmid et al. 2014), *MYB72* and *MYB10* (Palmer et al. 2013, Stringlis et al. 2018b), *S8H* (Rajniak et al. 2018, Siwinska et al. 2018), *CYP82C4* (Rajniak et al. 2018), *BGLU42* (Zamioudis et al. 2014) and *PDR9* (Fourcroy et al. 2014, Zamioudis et al. 2014).

The root-specific transcription factor gene *MYB72* was established to be important in both the onset of ISR and plant survival under Fe limiting conditions (Van der Ent et al. 2008, Palmer et al. 2013, Zamioudis et al. 2014, Zamioudis et al. 2015). Interestingly, *MYB72* overexpression was shown to upregulate many biosynthetic genes of the shikimate, phenylpropanoid and nicotianamine biosynthesis pathways, with coumarins being among the end-products of these pathways (Zamioudis et al. 2014). Upregulation of *MYB72* in response to WCS417 root colonization or conditions of Fe deficiency indeed both resulted in the accumulation of coumarins inside the roots and in root exudates (**Fig. 3**) (Zamioudis et al. 2014, Stringlis et al. 2018b). Among the target genes of the transcription factor, MYB72 was the β-glucosidase gene *BGLU42* (Zamioudis et al. 2014). Overexpression of *BGLU42* in Arabidopsis conferred enhanced resistance against *B. cinerea*, *H. arabidopsis* and *P. syringae*. pv. *tomato*, suggesting that BGLU42 activity is crucial for the development of ISR. BGLU42 activity was also found to be important for the excretion of fluorescent coumarins into the rhizosphere under Fe starvation conditions, where they are thought to play a role in the mobilization and uptake of Fe (Tsai and Schmidt 2017). Hence, BGLU42 seems to play a dual role in the plant growth- and health-promoting response of Arabidopsis to beneficial rhizobacteria and the Fe deficiency response. Accumulation of coumarins in roots was also observed during the interaction of Arabidopsis with the beneficial rhizobacteria *Paenibacillus polymyxa* BFKC01 (Zhou et al. 2016) and *Pseudomonas fluorescens* SS101 (Van de Mortel et al. 2012), suggesting that the role of coumarins in plant–beneficial microbe interactions is more general (**Fig. 3**).

Coumarins and root microbiome assembly

A recent study added a new dimension to the multifaceted role of coumarins in plant–microbe interactions. By studying the effect of coumarins on the structure of the root microbiome, it was shown that coumarins have an impact on microbial community composition in the rhizosphere (Stringlis et al. 2018b). In this study, the metabolome and metagenome of Arabidopsis wild-type and coumarin-deficient mutants was investigated. Following induction of *MYB72*, the most dominant compound

in Arabidopsis root exudates was scopoletin, while scopolin accumulated to high levels inside the roots. Subsequent metagenome analysis of the roots of wild-type and *f6′h1* mutant plants grown in natural soil revealed that these plants assembled distinct microbial communities, indicating that coumarins in root exudates play a role in shaping the root microbiome. In vitro testing of the antimicrobial effect of scopoletin on the beneficial *MYB72*-inducing microbes *P. simiae* WCS417 and *Pseudomonas capeferrum* WCS358 and the soil-borne fungal pathogens *Verticillium dahliae* and *F. oxysporum* f. sp. *raphani*, revealed that the beneficial rhizobacteria were tolerant to the antimicrobial activity of the exuded scopoletin, while scopoletin displayed a number of antimicrobial activities towards the pathogenic soil-borne fungi (Stringlis et al. 2018b). These data suggest that scopoletin is part of a selective mechanism in the rhizosphere employed by the plants that can deter pathogenic microbes and facilitate the proliferation of beneficial microbes in the same niches (**Fig. 3**).

Concluding Remarks

All the exciting findings on the role of coumarins in nutrient stress and plant–microbe interactions generated many new questions that await answers in the future. Nutrient stress, such as Fe deprivation, has been proposed to be a major driving force in the establishment of mutually beneficial host–microbe interactions (Bakker et al. 2018). Scopoletin, which is excreted into the rhizosphere during conditions of Fe starvation, shapes the root microbiome in Arabidopsis (Stringlis et al. 2018b), but the ecological relevance and underlying biological mechanisms remain to be uncovered. Recent data elucidated the biosynthesis pathway downstream of scopoletin, leading to fraxetin and sideretin, and provided insight into the bioactivity and role of these compounds in Fe nutrition of plants (Rajniak et al. 2018, Siwinska et al. 2018). It is tempting to speculate that these compounds can also affect microbiome composition. The availability of their biosynthetic mutants will be highly instrumental in future studies on their role in plant–microbe and plant–microbiome interactions. Initial findings via in vitro experiments (El Oirdi et al. 2010, Sun et al. 2014, Yang et al. 2017, Stringlis et al. 2018b) and metagenome analyses (Stringlis et al. 2018b) showed that diverse coumarins can have selective antimicrobial activities. In this context, it is critical to understand which molecular and chemical mechanisms are involved and to which functional groups of microbiota (e.g. pathogens vs. mutualists) are targeted. This knowledge will facilitate the targeted design of synthetic communities that could assist plant growth and disease resistance (Paredes et al. 2018) following coumarin selection.

Interestingly, genes encoding β-glucosidases and UGTs have also been found in soil- and plant-associated microbes (Thelen and Delmer 1986, Jorasch et al. 1998, Pathan et al. 2017). Hence, activity of their corresponding proteins together with multidrug efflux pumps (Brown et al. 2007) could be potential mechanisms of host-associated microbes to deal with the antimicrobial effects of coumarins. The soil is a source of many uncultured, unexplored and unidentified microbes. In order to unlock their functions in complex host–microbe interactions, culture-independent techniques, such as metagenome sequencing and comparative genomics will prove to be essential (Levy et al. 2018, Stringlis et al. 2018c). Another challenge for future coumarin research will be the detailed analysis of the spatial distribution and accumulation of coumarins during infection and/or colonization via advanced metabolomics methods. Such analyses will help to locate metabolic niches of specific coumarins and their specific effect on microbial proliferation (Zhalnina et al. 2018, Jacoby and Kopriva 2019).

The notion that plant coumarins can have a selective effect on host microbiota is not only relevant for plant–microbiome interactions. Recently, human gut bacteria were found to grow in the presence of the coumarins esculin and fraxin, meanwhile metabolizing and releasing the bioactive antimicrobial aglycones from these coumarins in their culture supernatants (Theilmann et al. 2017). Hence, gut microbes that convert glucosides of plant secondary metabolites to their deglycosylated bioactive aglycones have the potential to have an impact on human and animal health. Recent advances in research on the structure and functions of microbiota of plants, fish, animals and humans demonstrated numerous similarities on how microbiota improve host growth, nutrition and immunity (Brugman et al. 2018, Ikeda-Ohtsubo et al. 2018). The coumarin story nicely showcases how such small but widely abundant metabolites function in interkingdom host–microbiome interactions and affect growth and health of plants, animals and humans.

Disclosures

The authors have no conflicts of interest to declare.

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