Physiological and Molecular Plant Pathology 94 (2016) 156–166

Contents lists available at ScienceDirect

Physiological and Molecular Plant Pathology

journal homepage: www.elsevier.com/locate/pmpp

Signals that stop the rot: Regulation of secondary metabolite defences in cereals

Jacqueline Meyer [a, b], Shane L. Murray [a, c], Dave K. Berger [b, *]

[a] Centre for Proteomic and Genomic Research, Upper Level, St Peter's Mall, Cnr Anzio and Main Road, Observatory, 7925, Cape Town, South Africa
[b] Department of Plant Science, Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, P/Bag X20, Hatfield, 0028, Gauteng, South Africa
[c] Department of Molecular and Cell Biology, University of Cape Town, Private Bag, Rondebosch, 7701, Cape Town, South Africa

ARTICLE INFO

Article history:
Received 28 February 2015
Received in revised form
11 May 2015
Accepted 18 May 2015
Available online 20 June 2015

Keywords:
Cereals
Secondary metabolism
Phytoalexins
Phytoanticipins
PTI
Priming
Plant hormones

ABSTRACT

Plants accumulate a vast arsenal of chemically diverse secondary metabolites for defence against pathogens. This review will focus on the signal transduction and regulation of defence secondary metabolite production in five food security cereal crops: maize, rice, wheat, sorghum and oats. Recent research advances in this field have revealed novel processes and chemistry in these monocots that make this a rich field for future research.

1. The role of secondary metabolites in defence in cereal crops

Cereal crops such as maize (*Zea mays*), wheat (*Triticum aestivum*), oat (*Avena sativa*), rice (*Oryza sativa*) and sorghum (*Sorghum bicolor*) are consumed throughout the world and are crucial components of the world's caloric intake. In 2013, cereal production exceeded approximately 55 million tonnes in Africa alone (www.fao.org/worldfoodsituation/csdb/en/) – with maize the main produce followed by wheat and rice (statistics.amis-outlook.org/data/index.html). Viruses, bacteria, fungi, and herbivores are biotic stressors that threaten crop yields and economic stability. Therefore, it is crucial that plant defence mechanisms are understood in order to develop sustainable crop enhancement programs. Plants are sessile organisms that lack circulating cells, such as macrophages in mammals, whose purpose is to recognise non-self molecules and elicit an immune response. Instead, each plant cell acts autonomously and is programmed to recognise and respond to pathogens [1]. The mechanisms by which plants defend themselves include the production of secondary metabolites with antimicrobial properties and these responses are controlled by signal transduction pathways [2].

Early researchers in the field coined the terms “phytoanticipins” and “phytoalexins” for antimicrobial compounds involved in constitutive and *de novo* defence in crop plants, respectively [3]. Phytoanticipins are compounds produced constitutively in cereals and are involved in above and below ground protection [4–6]. They are preformed and stored as inactive, conjugated molecules in the vacuole [6,7]. When a plant is challenged by a pathogen, these molecules are activated and rapidly released in order to fight off the invader [7–9]. They are especially relevant during infection by necrotrophic pathogens, which rely on tissue injury and host cell

Abbreviations: PRRs, pattern-recognition receptors; PAMPs, pathogen-associated molecular patterns; PTI, PAMP-triggered immunity; ROS, reactive oxygen species; VIGS, virus induced gene silencing; SAR, systemic acquired resistance; JA, jasmonic acid; Et, ethylene; SA, salicylic acid; BX, benzoxazinoids; CPS, copalyl diphosphate synthase; ABA, abscisic acid; BTH, benzothiadiazole derivative; PAL, phenylalanine ammonia lyase; AMF, arbuscular mycorrhizal fungus; VOC, volatile organic compounds; y1, yellow seed1.

* Corresponding author.
E-mail addresses: jmnm2201@gmail.com (J. Meyer), shane.murray@uct.ac.za (S.L. Murray), dave.berger@fabi.up.ac.za (D.K. Berger).

http://dx.doi.org/10.1016/j.pmpp.2015.05.011
0885-5765/

death for effective infection [7]. Benzoxazinoids (BXs) are the predominant phytoanticipin in the major Poaceae crop plants, maize and wheat, but are absent from oat, rice and sorghum [10–13]. Interestingly, unlike Poaceae crop plants, where BXs are limited to seedlings or young plants, dicot plants accumulate BXs during all developmental stages in both above and below-ground parts [14]. BXs are associated with maize resistance to both fungal diseases (i.e. northern and southern corn leaf blight and corn smut), and insect pests (i.e. the European corn borer and the maize plant louse) [15–18].

An array of phytoalexins are produced in cereal crop plants in response to pathogens and belong to several chemically diverse classes of compounds, including, but not limited to, flavonoids, terpenoids and saponins [19–22]. Flavonoid phytoalexins, such as avenanthramides in oat [23], 3-deoxyanthocyanidins in sorghum [24] and sakuranetin in rice [25] are phenolic metabolites that originate from phenylalanine [26]. Plants rely on these phenols for growth and reproduction, pigmentation and as defensive molecules against pathogens [26]. Terpenoid phytoalexins in rice (oryzalexins, phytocassanes and momilactones) [25] as well as maize (kauralexins and zealexins) [27,28] are synthesised via the isoprenoid pathway. Saponins are glycosylated triterpenoids, protective molecules that are absent in all cereals except the genus *Avena* and likewise synthesised via the isoprenoid pathway using the precursor mevalonate [23,29,30]. Dicotyledonous plants, such as *Arabidopsis thaliana*, tobacco and cotton, also produce terpenoid phytoalexins [20,31]. More discussions about phytoalexin production in the model dicot, *A. thaliana* are extensively reviewed by Ahuja, Kissen and Bones (2012) [20]. The biosynthesis of monocot terpenoids is catalysed by a large class of enzymes termed terpene synthases (TPS), and these enzymes have been credited with the diversity of terpenoids [32–34]. Terpenoids function as plant hormones, vitamins, pigments and, critically, in plant–pathogen interactions [22].

Many secondary metabolites exhibit anti-microbial properties, as illustrated in sorghum, which produces 3-deoxyanthocyanidins, red-coloured flavonoid phytoalexins at the site of *Colletotrichum sublineolum* colonisation [24,35,36]. A combination of these red/orange-coloured compounds, known as apigeninidin and luteolinidin, are synthesised in the cytoplasm of epidermal sorghum cells in response to *C. sublineolum* [24,37]. The compounds accumulate in inclusion bodies which enable their translocation to the infected area, where they undergo structural modifications and release their content, killing both the pathogen as well as the plant cells [38].

The definitions of phytoalexins and phytoanticipins are based on the mode of regulation and synthesis of the compound and not necessarily by the chemical structure, which makes the terminology imprecise since the same compound can act as both a phytoalexin and a phytoanticipin depending on the host plant or even the organ of the host plant [7,39]. As an example, sakuranetin, a flavonoid compound, acts as a phytoalexin in rice, where it accumulates in response to rice blast and bacterial blight disease [40–44]. However, in blackcurrants, sakuranetin is constitutively present in the leaves, and is therefore also defined as a phytoanticipin [45]. Likewise, momilactone A, a diterpenoid compound in rice, is constitutively present in rice seed husks and roots, but induced in rice leaves upon infection with rice blast [41].

In this review, we firstly describe the diversity of defensive secondary metabolites (primarily phytoalexins and phytoanticipins) in the economically-important cereal crops rice, maize, wheat, sorghum and oat. We furthermore discuss how signalling pathways influence accumulation of antimicrobial secondary metabolites, and conclude with a "future perspectives" section in which on-going research questions are identified.

2. Pathogen recognition, signal transduction and defence secondary metabolite synthesis

2.1. Pathogen recognition events that lead to secondary metabolite production

Plants boast a sophisticated, organ- and cell-specific surveillance system, which recognises pathogens and responds by triggering the innate immunity signal transduction pathway [2,46]. Essentially, the plant cell surface contains pattern-recognition receptors (PRRs) that identify pathogen-associated molecular patterns (PAMPs) [46]. PAMPs are conserved molecules, either secreted or surface-exposed, that are characteristic of pathogens. Examples of PAMPs recognised by plants include bacterial flagellin, fungal β-glucans and chitin [2,46]. Once PAMPs are perceived by a plant cell, a process termed PAMP-triggered immunity (PTI) is initiated [1]. This process involves a complex local and systemic intracellular signalling cascade promulgated through gene expression changes involving WRKY and TGA transcription factors [1]. In the interaction between cereals and fungi, β-glucan and chitin are representative fungal PAMPs and are polysaccharides that crosslink to form a scaffold within fungal cell walls [47]. There are several reports that β-glucan and chitin elicit a plant immune response in cereals leading to production of secondary metabolites [47–51]. Chitin fragments, such as N-acetylchitooligosaccharides, were shown to be a potent elicitor of momilactone accumulation in rice suspension cells, which leads to growth inhibition of fungi such as *Magnaporthe grisea* (responsible for rice blast fungus) [50]. Chitosan, a derivative of chitin, elicits both sakuranetin and momilactone production in rice seedlings [51]. An example of a PRR is OsCEBiP, a plasma membrane glycoprotein that acts as a receptor for chitin elicitors and has a demonstrated role in disease resistance of rice against *M. grisea* [49]. Upon chitin elicitor binding, OsCEBiP forms a complex with OsCERK1, a receptor kinase that is responsible for triggering downstream signalling (Fig. 1A) [52,53]. Phytoalexin production after chitin induction was lower in an *OsCEBiP* gene-specific knockdown line than the wild-type [53]. This observation was later replicated in an *OsCERK1* knock-down line [52]. Furthermore, expression of defence-related genes was suppressed, including a key gene required for the biosynthesis of diterpenoid phytoalexins [52]. Similarly, β-glucan was shown to elicit production of momilactones as well as small amounts of oryzalexins in rice suspension cells [47]. Upon exposure to β-glucan from *Colletotrichum graminicola* (responsible for anthracnose), the expression of putative phytoalexin biosynthetic genes in maize were up-regulated by more than 150-fold [54]. Interestingly, the same study showed that *C. graminicola* ostensibly attempts to evade maize PAMP-elicited defence responses by down-regulating its β-glucan production during the biotrophic growth phase to establish a compatible interaction [54]. Another study in maize demonstrated phytoalexin accumulation after wounded stems were treated with a pectinase elicitor derived from *Rhizopus microsporus* [27]. Pectin is a component of plant cell walls and a target for fungal pectinolytic enzymes during pathogen attack [55]. Likewise, momilactone accumulation was shown to be accelerated by the over expression of selenium-binding protein homologue gene (*OsSBP*), a receptor for the fungal elicitor, cerebroside from *M. grisea*, which triggered resistance to *M. grisea* and *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) (the causal organism of bacterial blight disease) [56]. Thus, PAMPs – and PRR recognition of PAMPs – set in motion PTI-specific signalling pathways that lead to accumulation of antimicrobial secondary metabolites.

Plant-derived molecules have also been shown to act as elicitors and provoke an immune response [57–60]. ZmPep1 is a peptide encoded by the maize gene *ZmPROPEP1*, whose expression is

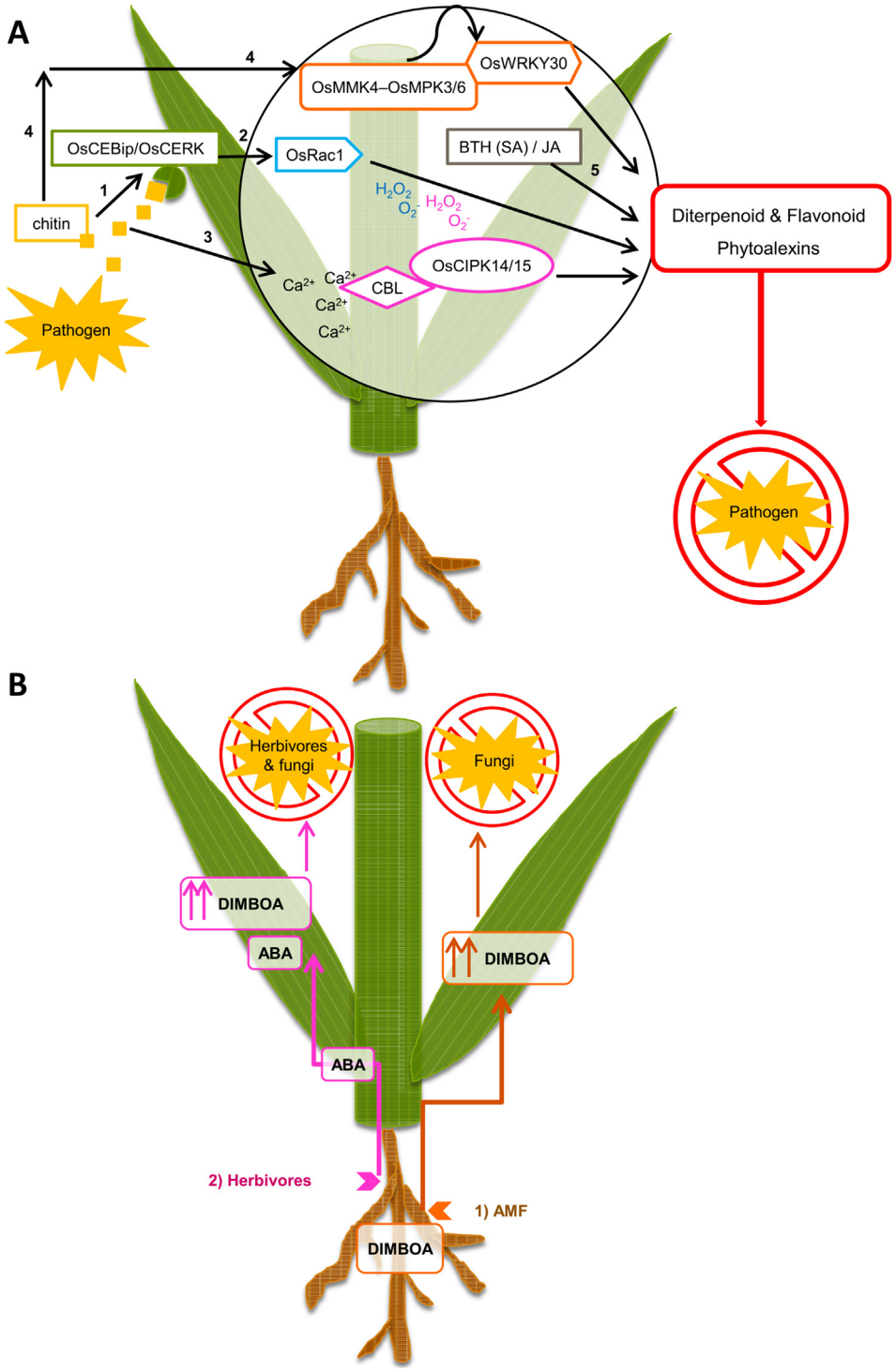

Fig. 1. A: A snapshot of signalling networks resulting in defensive secondary metabolite production in rice in response to chitin elicitation 1) The pattern-recognition receptors (PRRs), OsCEBiP and OsCERK1, identify the fungal pathogen-associated molecular pattern (PAMP), chitin, and 2) through phosphorylation of G-proteins such as OsRac1, regulate ROS production, leading to accumulation of momilactone. 3) Chitin recognition events trigger an influx of Ca^{2+} ions into the cytosol. Ca^{2+} is perceived by calcium sensors such as calcineurin B-like proteins (CBLs) which subsequently activate CBL interacting protein kinases (OsCIPK14/15) leading to an accumulation of momilactone and phytocassane. 4) Chitin binding leads to an induction of the MAPK cascade, OsMMK4 – OsMPK3/6, that regulate the transcription factor OsWRKY30, subsequently activating diterpenoid phytoalexin production. 5) Signalling via the phytohormones jasmonic acid (JA), and synthetic salicylic acid (SA) analogue, benzothiadiazole derivative (BTH), mediate production of diterpenoid and flavonoid phytoalexins. **B:** Dimboa participates in plant immune priming in maize 1) Symbiosis with mycorrhizal fungi (AMF) primes the defence responses against fungal pathogens. AMF prompts the production of DIMBOA in the roots, and the compound is translocated from the roots to the leaves where it provides protection against certain fungi. 2) Root herbivory leads to DIMBOA accumulation in the leaves, providing protection against fungal pathogens and herbivores. Abscicic acid (ABA) acts as a signalling molecule in this interaction.

induced by fungal infection [57]. ZmPEP1 stimulates biosynthesis and accumulation of BXs in leaves [57]. In maize, pre-treatment with ZmPep1 prior to infection resulted in enhanced resistance to *Bipolaris maydis* (causal organism of southern leaf blight) and *C. graminicola* [57]. Restricted lesion spread and disease severity, which lead to a reduction in subsequent cell death, was observed [57]. Likewise, pre-treatment of wheat with plant cell-wall-derived oligosaccharides, such as oligogalacturonides, resulted in reduced growth of *Blumeria graminis*, which is a parasite responsible for powdery mildew in wheat [58]. Simultaneously, observed accumulation and activation of the first enzyme in the phenylpropanoid pathway, phenylalanine ammonia lyase (PAL), lead the authors to presume that these molecules contribute to phytoalexin production and have resistance-inducing potential [58]. There is also evidence that the fungal sugar, trehalose, can elicit wheat defence responses to powdery mildew through activation of PAL; three consecutive applications of trehalose reduced *B. graminis* infection in wheat by up to 95% [59,60].

The wheat *Tsn1* gene confers sensitivity to the *Stagonospora nodorum* effector, ToxA (SnToxA) and mutants lacking a functional receptor show resistance to *S. nodorum* [61]. Recently, Du Fall and Solomon (2013) reported that accumulation of the phytoanticipin, DIMBOA, several DIMBOA precursors as well as a novel phytoalexin, monoamine serotonin, was increased in wheat in response to SnToxA [62]. Subsequent *in vitro* application of the compounds inhibited the growth and spore germination of the fungus [62]. Another example of a dominant "susceptibility locus" is found in oats, where the locus *Vb* confers susceptibility in the presence of victorin, a toxin secreted by *Helminthosporin victoriae* [63]. The same locus, incidentally, is linked to a dominant inherited trait, *Pc-2*, which mediates resistance to crown rust in oats through accumulation of high levels of avenacins [63].

Thus, in summary, perception of a fungal PAMP, effector, or toxin will activate plant receptors that subsequently trigger downstream signal transduction pathways. Current data suggests that there are several defence signalling pathways leading to phytoalexin and phytoanticipin production in cereals (Fig. 1A). We discuss these below.

2.2. Chemical triggers after pathogen recognition

Once pathogen recognition has taken place, for example as a result of PAMP or effector detection, some proteins in the plasma membrane are phosphorylated and there is an influx of Ca^{2+} ions into the cytosol [64]. The plasma membrane becomes depolarised, Cl^- and K^+ ions are transported out and an influx of H^+ ions take place, which causes the cytoplasm to acidify [64]. This movement of ions occurs instantaneously once an effector is perceived. Ca^{2+} is one of the critical messenger ions and activates transcription factors, which regulate downstream gene expression. In sorghum, transcriptome analysis revealed that calcium signalling genes were upregulated as well as key biosynthetic genes for flavonoid phytoalexins in response to *Bipolaris sorghicola* [65]. Yang et al. (2004) demonstrated that application of calcium channel blockers diminished the accumulation of avenanthramides, which led them to believe that calcium ions were critical to the activation of oat phytoalexin production [66]. Signals from cellular Ca^{2+} are interpreted by calcium sensors, such as calcineurin B-like proteins (CBLs), which subsequently activate CBL interacting protein kinases (CIPKs) [67]. The expression of two rice CIPKs, *OsCIPK14* and *OSCIPK15*, was induced by N-acetylchitooligosaccharides in rice cells and resulted in rapid accumulation of momilactones and phytocassanes (Fig. 1A) [67].

After cellular Ca^{2+} ions are received and interpreted, extant NADPH oxidase activity is initiated, which results in ROS production [64]. ROS are mediators of the plant immune response. ROS produced at the pathogen penetration site are in two main forms, superoxide and hydrogen peroxide (H_2O_2). ROS originates from NADPH-oxidases on the cell membrane and peroxidases associated with the cell wall [68]. Once ROS are produced, it usually implies successful recognition of a pathogen by the host, resulting in an oxidative burst and restriction of infection. Various studies have shown an induction of ROS-related genes in conjunction with phytoalexin biosynthesis and resistance to pathogens [43,69–71]. In maize, phytoalexin biosynthetic genes as well as ROS-related genes were upregulated in response to *Phytophthora cinnamomi* [69], and similarly in rice, where infection with bacterial blight caused accumulation of diterpenoid phytoalexins as well as regulated ROS production [72]. Maize lines transformed with a wheat *oxalate oxidase (OxO)* gene exhibited resistance to herbivory [73]. *OxO* catalyses the conversion of oxalate into H_2O_2 and CO_2 [73]. Interestingly, DIMBOA synthesis was reduced in the transgenic line and the authors speculated that this was due to a metabolic diversion of building blocks from DIMBOA to the shikimate pathway that resulted in high levels of phenolic compounds [73]. The examples given above indicate that phytoalexin accumulation is dependent on ROS production and subsequent signal transduction.

Curiously, it has been shown that necrotrophic fungal pathogens promote *in planta* ROS production in order to advance their growth and pathogenicity on a plant [74]. Vargas et al. (2012) also speculated that *C. graminicola* uses local oxidative bursts to further its progress through the maize cell wall [70]. Interestingly, in transgenic lines overexpressing a bacterial effector gene, phytoalexin accumulation was shown to increase in parallel with decreased H_2O_2 and increased anti-oxidant enzymes which are known ROS scavengers [75]. This phenomenon was also demonstrated in transgenic rice lines overexpressing a fungal PRR gene [56]. This may reflect a novel role for phytoalexins at later stages of infection when they have accumulated to sufficient levels so that pathogen ingress has been limited, and therefore they participate in a negative feedback "ROS-quenching" mechanism to prevent an overreaction by the plant cell to the pathogen.

Defence signalling is also mediated through small G-proteins. OsRac1, a Rac-like small G-protein has been shown to regulate ROS production in rice cell cultures through NADPH oxidase [71,76]. OsRac1 is a crucial component of N-acetylchitooligosaccharide elicited signalling, which is tightly regulated by the OsCEBiP/OsCERK1 receptor complex [77]. OsCEBiP/OsCERK1 transmits signals to OsRac1 through phosphorylation of OsRacGEF1 (OsRac1 guanine nucleotide exchange factor) (Fig. 1A) [77]. It was shown that rice which overexpressed *OsRac1* was resistant to *M. grisea* and *Xoo* [71]. Concomitantly, the authors reported a 180-fold increase of the rice phytoalexin, momilactone A [71], which thus indicates that phytoalexin accumulation is positively regulated by G-protein signal transduction in rice.

2.3. Signal transduction through MAPK cascades

MAPK cascades are stepwise mediators of PAMP signals, which ultimately result in activation of defence responses. A cascade commonly consists of at least three kinases: a MAPK kinase kinase (MAPKKK), a MAPK kinase (MAPKK) and a MAPK. Chitin oligosaccharides elicit a rice MAPK cascade commencing with OsMKK4 [48]. OsMKK4 subsequently phosphorylates two rice MAPKs (OsMPK3 and OsMPK6), thereby initiating a signalling cascade leading to activation of secondary metabolite biosynthesis (Fig. 1A) [48]. The authors demonstrated that activation of OsMPK6 is a crucial target for OsMKK4 driven cell death [48]. Additionally OsMKK4 up-regulates the expression of genes belonging to the

methylerythritol phosphate pathway as well as the diterpenoid phytoalexin biosynthetic pathway [48]. This rice MAPK cascade functions through WRKY transcription factors in response to biotic and abiotic stresses [78,79]. It was shown that OsMPK3/OsMPK6 directly phosphorylates OsWRKY30 and OsWRKY53 in rice (Fig. 1A) [78,79]. In another example, OsACDR1 (accelerated cell death and resistance 1) was identified as a MAPKKK connected to signalling in diterpenoid phytoalexin production [19].

2.4. Transcriptional activation of secondary metabolite biosynthesis genes

WRKY transcription factors are common regulators of transcription associated with plant defence responses [80], and the rice and maize genomes contain over a 100 *WRKY* genes each [81,82]. Transcription factors activate or repress downstream defence genes such as pathogenesis related (PR) genes and secondary metabolite biosynthetic genes [64]. In a recent study, the link between WRKY transcription factors and phytoalexins in rice was elucidated by data showing that OsWKRY45 is an integral part of benzothiadiazole (BTH)-induced priming of rice defences against *M. grisea* [83], which lead to up-regulation of momilactone, phytocassane and oryzalexin biosynthetic genes. In *OsWRKY45* overexpressing rice plants, pre-treatment with BTH resulted in an augmented response to *M. grisea*, both in speed and intensity [83]. On the other hand, OsWRKY76 is a transcriptional repressor of rice phytoalexin biosynthesis [84]. In transgenic rice plants overexpressing OsWRKY76, both flavonoid and diterpenoid phytoalexin accumulation was suppressed resulting in susceptibility to *M. grisea* [84].

W-boxes are recognised binding sites of WRKY proteins [84]. The presence of functional W-boxes in the promotors of rice diterpenoid biosynthetic genes further supports the involvement of WRKY genes in phytoalexin production [85]. Interestingly, Tao et al. (2009) demonstrated that allelic variation in *OsWRKY45* resulted in differential host–pathogen interactions [86]. Two alleles in different rice cultivars, designated as *OsWRKY45*-1 and *OsWRKY45*-2, encodes two proteins differing in 10 amino acids and have contrasting roles in rice resistance against *Xoo* and *X. oryzae* pv *oryzicola (Xoc)* [86]. Plants that overexpress *OsWRKY45*-1 are susceptible to bacterial infections, and when the gene is silenced, resistance is recovered. In contrast, high expression of *OsWRKY45*-2 results in resistance to both *Xoo* and *Xoc*, which is subsequently abolished if the gene expression is suppressed [86]. An increased accumulation of SA and JA was observed in conjunction with *OsWRKY45-1*-regulated resistance whereas only JA accrued with *OsWRKY45-2* regulation. Additionally, different defence-responsive genes were induced by each *WRKY* allele [86]. Notably, plants which overexpress either *OsWRKY45-1* or *OsWRKY45-2* have enhanced resistance to *M. grisea*. Taken together, their results indicate that *OsWRKY45-1* is a negative regulator of resistance to bacterial infection and *OsWRKY45-2* a positive regulator of resistance to bacterial infections but that both are positive regulators of resistance against *M. grisea* in conjunction with diterpenoid phytoalexin accumulation [86].

Other transcription factors similarly play a role in the induction of phytoalexins. Ibraheem et al. (2010) demonstrated through loss-of-function mutants that the accumulation of sorghum 3-deoxyanthocyanidin phytoalexins and resistance to *C. sublineolum* require a functional *yellow seed1* (*y1*) gene encoding a MYB transcription factor [87]. Further to that, they also demonstrated that in transgenic maize plants expressing *y1*, the flavonoid pathway yielding 3-deoxyanthocyanidin was activated and the maize plants were resistant to *C. sublineolum* [88].

In summary, once the pathogen is recognised, the defence signal is relayed through the action of Ca^{2+}-signalling, ROS, G-proteins and MAPK cascades, resulting in transcriptional activation of secondary metabolite biosynthesis genes, often mediated by WRKY transcription factors (Fig. 1A). In addition, phytohormones also play an important role in signalling that leads to defensive secondary metabolite production, and this will be discussed in the next section. Key phytohormones include jasmonic acid (JA), ethylene (Et) and salicylic acid (SA) [64,89].

3. Plant hormones act as messengers to induce secondary metabolite production

The phytohormones jasmonic acid (JA), ethylene (E) and salicylic acid (SA) are important messenger molecules in PTI-mediated signalling and defence responses, and the biosynthesis of phytoalexins and phytoanticipins in cereals (Table 1). In the following section, we review the role of these hormones in the biosynthesis of specific phytoalexins and phytoanticipins. Many of these studies have been based on the application of exogenous plant hormones, which is used as an experimental proxy for endogenous hormone levels [96]. However, this approach suffers several limitations, namely that (i) the absorbed hormone concentrations may not be biologically relevant; (ii) co-application of two hormones may not result in the same ratios inside the cells; and (iii) the chemical form of the hormone applied (e.g. jasmonate vs methyl jasmonate) may not be biologically relevant. Nevertheless much of current knowledge is based on this experimental strategy, although phytohormone metabolite profiling is being increasingly used in cereal–pathogen interactions [27,28].

3.1. Induction of phytoalexin accumulation by salicylic acid and jasmonates in sorghum

SA and JA have both been implicated in phytoalexin production in sorghum and wheat (Table 1) [35,93]. Generally it is accepted that SA mediates resistance against biotrophic pathogens, whereas JA/Et act against nectrotrophs and herbivores. However, this is not a definitive classification [97]. Global microarray analysis revealed that sorghum responds to exogenous SA and JA by up-regulating genes that form part of the phenylpropanoid pathway and generating 3-deoxyanthocyanidins, flavonoid phytoalexins [65]. Historically, based on studies in Arabidopsis, it has been thought that SA and JA act antagonistically; however, studies have both corroborated and contested this interaction in sorghum [24,35]. The accumulation of 3-deoxyanthocyanidins were induced in sorghum roots by JA but repressed by concurrent application of SA treatment [35]. However, Liu et al. (2010) reported varied degrees of

Table 1
Signalling hormones involved in secondary metabolite production in cereals.

Cereal	Secondary metabolite	Signal molecule[a]	Reference
Oat	Avenanthramides	SA^+	[90]
(Avena sativa)	Avenacins	BTH^+	
Maize	Kauralexin	$(JA+Et)^+$	[27]
(Zea mays)	Zealexin	$(JA+Et)^+$	[28]
	DIMBOA	ABA^+; $(JA+Et)^+$	[91,92]
Wheat	DIMBOA	JA^+	[93]
(Triticum aestivum)			
Rice	Sakuranetin	JA^+	[42,94,95]
(Oryza sativa)	Phytocassanes	JA^+	
	Oryzalexins	JA^+	
	Momilactones	JA^+	
Sorghum	3-deoxyanthocyanidins	JA^+	[65]
(Sorghum bicolor)		SA^+	[65]
		$(SA+JA)^{+-}$	[24,35,36]

[a] Jasmonic acid (JA); ethylene (E); salicylic acid (SA); abscisic acid (ABA); benzothiadiazole derivative (BTH); positively regulates (+); negatively regulates (−).

antagonism when JA and SA were applied simultaneously to sorghum seedlings [24]. Interestingly, application of SA to sorghum and avirulent strains of *Fusarium graminearum* to wheat heads resulted in the up-regulation of JA biosynthetic genes [65,98], thereby arguing that upon elicitation, plant hormones are synthesised *de novo*.

3.2. Jasmonic acid and ethylene have a synergistic effect in maize

JA/Et synergy has been demonstrated in maize [27,69]. Application of a combination of these phytohormones resulted in a build-up of kauralexins and zealexins, novel diterpenoid and sesquiterpenoid phytoalexins, respectively, which far exceeded the accumulation induced by each hormone individually [27]. Parallel studies in which maize stems were infected with either *R. microsporus* [27] or *F. graminearum* [28] resulted in substantial levels of JA and Et, which preceded kauralexin and zealexin accumulation, respectively. Key enzymes of the biosynthetic pathway of both phytohormones were upregulated after attack by *Ostrinia nubilalis*, followed by increased accumulation of phytohormones and kauralexins [92]. Transcriptome analysis of maize roots post-infection by *P. cinnamomi* also revealed over expression of several genes involved in the biosynthesis of JA and Et [69]. Furthermore, the expression of a copalyl diphosphate synthase (CPS), *An2*, was induced by *R. microsporus* and preceded kauralexin accumulation, which suggested that kauralexin production is dependent on *An2* activity [27]. *An2* expression has been shown in response to *Fusarium verticillioides*, *F. graminearum*, *P. cinnamomi* and *Ustilago maydis* [69,99–101]. Strikingly, kauralexins have also been shown to accumulate in maize roots as a result of abiotic stressors [102]. Kauralexins display antimicrobial activity against a variety of maize pathogens such as *R. microsporus*, *P. cinnamomi* and *C. graminicola* [27,69]. Likewise, zealexin production is preceded by increased expression of the biosynthetic genes *Tps6/11* [28]. Expression of both the *Tps6* and *Tps11* alleles have also been stimulated following *U. maydis* infection [18] and when silenced using VIGS, plants are more susceptible to *U. maydis* [103]. Taken together, these results propose that the plant hormones JA and Et mediate production of terpenoid phytoalexins and thereby defence in maize (Table 1).

3.3. Jasmonic acid-dependant and independent pathways in rice produce flavonoids and diterpenoids

Rice produces an assortment of phytoalexins. Many rice diterpenoids have been identified, and one flavonoid, sakuranetin, has been studied in detail [25]. The diterpenoids are clustered into oryzalexins, momilactones, and phytocassanes, based on their carbon skeleton [25]. *M. grisea* induces JA biosynthesis in rice and both sakuranetin and the diterpenoids are induced by JA as well as *M. grisea* infection (Fig. 1A), suggesting that phytoalexin accumulation results from increased JA (Table 1) [42,94,95]. Interestingly, JA-deficient mutants accumulated diterpenoid phytoalexins, but were deficient in the flavonoid sakuranetin, alluding to JA-independent pathways for pathogen-induced biosynthesis of diterpenoid phytoalexins in rice [104]. Accumulation of sakuranetin relies on *OsJAR1* (jasmonic acid resistant 1) to regulate JA-induced rice defence responses. OsJAR1 is responsible for catalysing the formation of JA-isoleucine, the active form of JA, which is indispensable for phytoalexin production [105]. Both sakuranetin and the diterpenoids have demonstrated activity against *Rhizoctonia solani* and *Xoo* [40–44]. Curiously, antifungal activity varies between rice phytoalexins, and it has been shown that sakuranetin is more potent against blast fungus than a momilactone [42].

3.4. Oat defences: salicylic acid induces avenanthramides in leaves, whereas jasmonates stimulate saponin production in roots

SA stimulates the production of phenolic phytoalexins termed avenanthramides in oats (Table 1) [90]. These compounds are substituted N-cinnamoylanthranilate derivatives synthesised in response to crown rust infection (*Puccinia coronata*) [106,107]. A benzothiadiazole derivative, BTH, is a commercially available, synthetic, immune-priming compound, which mimics a fungal infection and acts as a functional analogue of SA [108]. It has shown immune induction in oats resulting in avenanthramide accumulation [90]. Oat roots on the other hand, rely on phytoanticipins such as saponins – antimicrobial triterpenoid glycosides – to confer resistance to various pathogens in soil [9,30]. *Gaeumannomyces graminis* is a fungus commonly infecting the roots of wheat and barley. However, there are no documented cases of oats infected with *G. graminis*. Interestingly, saponin deficient mutants are highly susceptible to this fungus and therefore a logical conclusion was drawn that saponins are likely to confer resistance to *G. graminis* in oats [30]. Saponin accumulation is stimulated by application of jasmonate derivatives [22]. Two types of saponins are defined, avenacins and avenacosides [22]. Like benzoxazinoids, avenacosides are preformed and sequestered in the vacuole and activated by β-glucosidases once pathogens disrupt the cell membrane [22].

3.5. Phytoanticipins are stimulated by jasmonic acid in wheat and maize

Diterpenoid phytoalexins have not yet been reported in wheat despite a full complement of diterpenoid biosynthetic genes [109–111]. Instead, this crop relies heavily on phytoanticipins such as BXs for protection against infection [12]. The principal BX in maize and wheat is 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA) [13] whereas dicot plants only produce the DIMBOA precursor, DIBOA, and its glucoside DIBOA-glc [6]. JA regulates DIMBOA accumulation in maize leaves as well as both the roots and above ground parts of wheat (Table 1) [93,112]. DIMBOA furthermore accumulates after *O. nubilalis* herbivory in conjunction with build-up of JA and Et [92]. Treatment of maize leaves with exogenous JA and E resulted in a marked increase in DIMBOA, suggesting that JA/Et synergy plays a positive role in signal transduction leading to DIMBOA accumulation [92]. Additionally it has been shown that systemic ABA induces the production of DIMBOA in maize leaves after root herbivory [91]. It has been shown that DIMBOA confers resistance to several maize pests including *O. nubilalis* and maize plant louse (*Rhophalosiphum maydis*) [15,16]. Additionally, resistance to fungal infections such as northern and southern corn leaf blight (caused by *Helminthosporium turcicum* and *B. maydis* respectively) as well as *U. maydis* are well documented [15,17,18]. Ahmad et al. (2011) proposed that DIMBOA is also involved in penetration resistance, independent of tissue damage, which challenges the classical mode of action accepted for BXs in maize [5]. The authors showed evidence of enhanced apoplastic BX deposition during early infestation by *Setosphaeria turtica* before any significant host tissue damage, which coincided with substantial colonisation impediment of *S. turtica* [5]. Moreover, DIMBOA has been implicated in negative feedback inhibition [5]. The application of DIMBOA, as well as an indole precursor, suppressed expression of the BX biosynthetic pathway genes [5]. BXs are synthesised with a glucosyl moiety that renders the product inactive. β-glucosidases cleave the moiety upon tissue disruption and the toxic aglucone is released. The purpose behind negative feedback inhibition remains unclear but it is speculated that it is a conserved response triggered in order to preclude autotoxicity and detoxify excess aglucones [5].

4. Chromosomal organisation of genes governing biosynthesis of phytoanticipins and phytoalexins

Many secondary metabolite biosynthetic genes in cereals are scattered throughout the genome. However, lately it has become evident that operon-like gene clusters are predominantly responsible for encoding secondary metabolites in plants. It is hypothesised that their physical proximity might facilitate coordinated gene regulation and co-inheritance as well as provide a selective advantage [113–115].

The first gene cluster in plants was discovered in maize and shown to be involved in the biosynthesis of the BX, DIMBOA [10]. Thereafter numerous gene clusters in oat and rice were found [113–116]. The cluster of genes encoding the core enzymes of DIMBOA in maize are located on the short arm of chromosome 4 [10,117,118]. The first 5 genes in the pathway, *ZmBX1-ZmBX5*, are tightly clustered. The first committed step in the BX pathway is defined by the conversion of indole-3-glycerole phosphate into indole by BX1 in the plastid [10]. Thereafter, four successive oxygen atoms are introduced into the indole moiety within the microsomes by four distinct members of the CYP71 family of cytochrome P450 dependent monooxygenases (P450s) - BX2 to BX5 [119]. These four enzymes are purported to be substrate specific despite their apparent homology [10]. Subsequent reactions are catalysed by two serial UDP-glucosyltransferases, BX8 and BX9 (located on chromosome 1), a dioxygenase, BX6, and a methyltransferase, BX7, within the cytosol [117,118,120].

Interestingly, all BX biosynthetic genes are present in all three genomes of hexaploid wheat; however, unlike maize, the BX biosynthetic cluster is not intact [121]. Homeologs of *TaBx1* and *TaBx2* are positioned on chromosome 4 in all three genomes, while *TaBx3* to *TaBx5* homeologs are situated on chromosome 5 in all three genomes. Despite the physical separation, transcription of *TaBx1* to *TaBx5* is synchronised, though each genome contributes disproportionately to the detected BX load [12,121].

In oats, a trio of genes adjacent to each other within a larger cluster of biosynthetic genes are collectively necessary for the production of avenacins [113,115]. Notably, the rice genes for the biosynthesis of phytocassanes and momilactones are clustered on chromosomes 2 and 4, which effectively enables a coordinated induction in rice cells after elicitation [25,122]. A key regulator of this coordinated expression is OsTGAP1 (*O. sativa* TGA factor for phytoalexin production 1), which is a basic leucine zipper transcription factor [25]. In conclusion, it is clear that clustering of biosynthetic genes for defensive secondary metabolites enables coordinated expression through exposure of the gene cluster to the transcriptional apparatus, thus facilitating rapid accumulation of anti-microbial secondary metabolites [113–115].

5. Priming and the production of phytoanticipins, phytoalexins

Plants are constantly in contact with either beneficial or pathogenic microbes, as well as molecules derived from these organisms. This has led to the phenomenon of “priming”. Plants become “sensitised” or “primed” through these interactions and develop an enhanced capacity to activate their defence responses when subsequently challenged by pathogens [91,123,124]. Recent data has implicated phytoanticipins and phytoalexins in the priming process [83,91,98,125].

Priming is thought to be a form of systemic acquired resistance (SAR) [124,126]. SAR is a biological state wherein localised exposure to a “weaker” strain of a pathogen creates long lasting protection throughout the whole plant to a broad spectrum of pathogens [89,126]. On a molecular level, SAR results from what is thought to be a concerted effort of many antimicrobial PR proteins which accumulate locally and systemically [127]. Moreover, it is thought that long-distance signalling molecules such as SA, JA, and ROS partake to mobilise and propagate a signal from the site of infection throughout the distal regions of the plant [89,124,127]. In recent years, SAR has been manipulated by researchers through the application of artificial chemical substances such as BTH in oat, wheat and rice [108,128,129], but BTH has shown limited application in maize [91,130,131.].

Until recently, there was experimental data only implicating DIMBOA as an active participant of immune priming through symbiosis and herbivory [91,125]. However, recently emerging data has alluded to a role for phytoalexins [83,98]. For instance, DIMBOA accumulates in maize roots as a consequence of a mutualistic symbiotic relationship with arbuscular mycorrhizal fungi (AMF) (Fig. 1B) [125]. Researchers used *Glomus mosseae* to pre-inoculate two maize varieties before challenging the plants with *R. solani* [125]. One maize variety, normally susceptible to *R. solani*, responded favourably to the pre-inoculation and displayed reduced disease severity as well as slower disease development, implying that the interaction with AMF resulted in an augmented immune response. Their study suggested that sheath blight can be prevented by mycorrhiza-induced priming of defence responses [125]. AMF are an ideal bio-protection agent because they occur naturally in the soil and are therefore able to establish stable associations [125].

Root herbivory has likewise been shown to modulate the defence response in aerial leaves in maize (Fig. 1B) and is under hormonal control [91]. Infestation by a specialist root herbivore in maize, *Diabrotica virgifera virgifera*, resulted in resistance to the leaf herbivore, *Spodoptera littoralis*, and the necrotrophic fungus, *S. turcica*. The study demonstrated that the levels of DIMBOA increased in the systemic leaves after root herbivory [91]. They further indicated that JA and ABA accumulates locally in the roots, but only ABA is translocated systemically and thus inferred that high levels of ABA in the leaves restricts *S. turcica* growth through stimulation of DIMBOA biosynthetic genes [91]. Therefore, it can be reasoned that ABA plays a role in positively regulating the DIMBOA biosynthesis pathway in systemic tissue distal to the initial infestation (Table 1).

An interesting approach is to use non-pathogenic mutant strains of a pathogen to invoke a primed state, which has been demonstrated in wheat [98]. Researchers used avirulent strains of *F. graminearum* to treat wheat heads and attempted to sensitise the plant immune system. This resulted in systemic transcriptome alterations in genes specifically associated with the phenylpropanoid pathway producing flavonoid phytoalexins, ultimately leading the authors to argue that phytoalexins play a role in pathogen-induced priming [98]. Likewise, maize roots were infected with *C. graminicola* and when distal leaves were subsequently challenged with the same pathogen, significant growth retardation was observed [131]. Concomitant up-regulation of flavonoid biosynthetic genes as well as the key biosynthetic gene of DIMBOA, *bx1* [10], was reported in the maize roots [131]. Another study revealed that rice phytoalexins accumulates in response to BTH treatment, resulting in induced resistance against *M. grisea* [83]. Interestingly, a WRKY transcription factor, OsWRKY45, was shown to be a vital intermediary between BTH and accrual of diterpenoid phytoalexins [83,132].

6. Future perspectives

As described in this review, a good understanding of the signal transduction processes that lead from pathogen recognition to defensive secondary metabolite production is starting to emerge in

particular plant–pathogen interactions. However, numerous intriguing questions remain with regards to the signal transduction pathways and regulation of protective secondary metabolites in economically important cereal crops.

- Have the terms "phytoanticipins" and "phytoalexins" become outdated with current data from gene expression and metabolite profiling experiments?
- Plants lack a circulatory system like mammals that can deploy mobile defence cells such as macrophages: is secondary metabolite production in plants regulated at the single cell, organ or whole plant level?
- In addition to signalling events described above, are there differences in how secondary metabolite biosynthetic pathways are regulated – what underlies constitutive versus induced expression?
- Does induction of defence proteins, e.g pathogenesis-related proteins, occur in concert with defence metabolites or are separate pathways involved?
- Is there co-ordinated/co-regulated transcriptional activation of secondary metabolite biosynthesis and hormone biosynthesis genes, resulting in hormone-specific control of defence through secondary metabolites?
- Does secondary metabolite biosynthesis take place in localised enzymatic factories in a particular compartment of the plant cell?
- Does the suite of defence metabolites expressed mirror the strategy of the pathogen i.e. are certain types of compounds induced in response to biotrophic pathogens and a distinctly different group of compounds induced in response to nectrotrophic pathogens?
- Will the targeted activation of secondary metabolite pathways through pathogen-inducible or tissue-specific regulation lead to novel crop protection strategies?

Future research to address these questions is likely to involve precision phenotyping of each plant–pathogen interaction [133,134] combined with "omics" technologies that profile genetic and epigenetic differences between plant genotypes that can be correlated with differences in gene expression, global protein levels and quantitative metabolomics [101,135–138].

Competing interests

The authors declare no competing interests.

Acknowledgements

We acknowledge the financial assistance of the National Research Foundation (NRF) and the Genomics Research Institute at the University of Pretoria (UP), South Africa. Opinions expressed and conclusions arrived at, are those of the authors and are not necessarily to be attributed to UCT, CPGR, NRF or UP. We thank the anonymous reviewers for their comments and suggestions.

References

[1] R.A. Ingle, M. Carstens, K.J. Denby, PAMP recognition and the plant-pathogen arms race, BioEssays 28 (2006) 880–889, http://dx.doi.org/10.1002/bies.20457.
[2] J.D.G. Jones, J.L. Dangl, The plant immune system, Nature 444 (2006) 323–329, http://dx.doi.org/10.1038/nature05286.
[3] K. Müller, Studies on phytoalexins. I. The formation and the immunological significance of phytoalexin produced by *Phaseolus vulgaris* in response to infections with *Sclerotinia fructicola* and *Phytophthora* infestans, Aust. J. Biol. Sci. 11 (1958) 275–300.
[4] J. Degenhardt, I. Hiltpold, T.G. Köllner, M. Frey, A. Gierl, J. Gershenzon, et al., Restoring a maize root signal that attracts insect-killing nematodes to control a major pest, Proc. Natl. Acad. Sci. U. S. A. 106 (2009) 13213–13218, http://dx.doi.org/10.1073/pnas.0906365106.
[5] S. Ahmad, N. Veyrat, R. Gordon-Weeks, Y. Zhang, J. Martin, L. Smart, et al., Benzoxazinoid metabolites regulate innate immunity against aphids and fungi in maize, Plant Physiol. 157 (2011) 317–327, http://dx.doi.org/10.1104/pp.111.180224.
[6] M. Frey, K. Schullehner, R. Dick, A. Fiesselmann, A. Gierl, Benzoxazinoid biosynthesis, a model for evolution of secondary metabolic pathways in plants, Phytochemistry 70 (2009) 1645–1651, http://dx.doi.org/10.1016/j.phytochem.2009.05.012.
[7] H. VanEtten, J. Mansfield, J. Bailey, E. Farmer, Two classes of plant antibiotics: phytoalexins versus "Phytoanticipins", Plant Cell 6 (1994) 1191–1192, http://dx.doi.org/10.1105/tpc.6.9.1191.
[8] A.V. Morant, K. Jørgensen, C. Jørgensen, S.M. Paquette, R. Sánchez-Pérez, B.L. Møller, et al., β-glucosidases as detonators of plant chemical defense, Phytochemistry 69 (2008) 1795–1813, http://dx.doi.org/10.1016/j.phytochem.2008.03.006.
[9] A.E. Osbourn, Preformed antimicrobial compounds and plant defense against fungal attack, Plant Cell 8 (1996) 1821–1831, http://dx.doi.org/10.1105/tpc.8.10.1821.
[10] M. Frey, P. Chomet, E. Glawischnig, C. Stettner, S. Grun, A. Winklmair, et al., Analysis of a chemical plant defense mechanism in grasses, Science 277 (1997) 696–699, http://dx.doi.org/10.1126/science.277.5326.696.
[11] E. Glawischnig, S. Gru, M. Frey, A. Gierl, Cytochrome P450 monooxygenases of DIBOA biosynthesis: specificity and conservation among grasses, Phytochemistry 50 (1999) 925–930.
[12] T. Nomura, A. Ishihara, R.C. Yanagita, T.R. Endo, H. Iwamura, Three genomes differentially contribute to the biosynthesis of benzoxazinones in hexaploid wheat, Proc. Natl. Acad. Sci. U. S. A. 102 (2005) 16490–16495, http://dx.doi.org/10.1073/pnas.0505156102.
[13] H. Niemeyer, Hydroxamic acids derived from 2-Hydroxy-2H-1,4-Benzoxazin-3 (4H)-one: key defense chemicals of cereals, J. Agric. Food Chem. 57 (2009) 1677–1696.
[14] K. Schullehner, R. Dick, F. Vitzthum, W. Schwab, W. Brandt, M. Frey, et al., Benzoxazinoid biosynthesis in dicot plants, Phytochemistry 69 (2008) 2668–2677, http://dx.doi.org/10.1016/j.phytochem.2008.08.023.
[15] H. Niemeyer, Hydroxamic acids (4-Hydroxy-1, 4-Benzoxazin-3-ones), defence chemicals in the gramineae, Phytochemistry 27 (1988) 3349–3358.
[16] N.J. Dafoe, J.D. Thomas, P.D. Shirk, M.E. Legaspi, M.M. Vaughan, A. Huffaker, et al., European corn borer (*Ostrinia nubilalis*) induced responses enhance susceptibility in maize, PLoS One 8 (2013) e73394, http://dx.doi.org/10.1371/journal.pone.0073394.
[17] A. Oikawa, A. Ishihara, C. Tanaka, N. Mori, M. Tsuda, H. Iwamura, Accumulation of HDMBOA-Glc is induced by biotic stresses prior to the release of MBOA in maize leaves, Phytochemistry 65 (2004) 2995–3001, http://dx.doi.org/10.1016/j.phytochem.2004.09.006.
[18] C.W. Basse, Dissecting defense-related and developmental transcriptional responses of maize during *Ustilago maydis* infection and subsequent tumor formation, Plant Physiol. 138 (2005) 1774–1784, http://dx.doi.org/10.1104/pp.105.061200.fense.
[19] E.A. Schmelz, A. Huffaker, J.W. Sims, S. Christensen, X. Lu, K. Okada, et al., Biosynthesis, elicitation and roles of monocot terpenoid phytoalexins, Plant J. 79 (2014) 659–678, http://dx.doi.org/10.1111/tpj.12436.
[20] I. Ahuja, R. Kissen, A.M. Bones, Phytoalexins in defense against pathogens, Trends Plant Sci. 17 (2012) 73–90, http://dx.doi.org/10.1016/j.tplants.2011.11.002.
[21] A. Poloni, J. Schirawski, Red card for pathogens: phytoalexins in sorghum and maize, Molecules 19 (2014) 9114–9133, http://dx.doi.org/10.3390/molecules19079114.
[22] L.A. Du Fall, P.S. Solomon, Role of cereal secondary metabolites involved in mediating the outcome of plant-pathogen interactions, Metabolites 1 (2011) 64–78, http://dx.doi.org/10.3390/metabo1010064.
[23] M.L. Wise, H.K. Sreenath, R.W. Skadsen, H.F. Kaeppler, Biosynthesis of avenanthramides in suspension cultures of oat (*Avena sativa*), Plant Cell Tissue Organ Cult. 97 (2009) 81–90, http://dx.doi.org/10.1007/s11240-009-9501-6.
[24] H. Liu, Y. Du, H. Chu, C.H. Shih, Y.W. Wong, M. Wang, et al., Molecular dissection of the pathogen-inducible 3-deoxyanthocyanidin biosynthesis pathway in sorghum, Plant Cell Physiol. 51 (2010) 1173–1185, http://dx.doi.org/10.1093/pcp/pcq080.
[25] H. Yamane, Biosynthesis of phytoalexins and regulatory mechanisms of it in rice, Biosci. Biotechnol. Biochem. 77 (2013) 1141–1148, http://dx.doi.org/10.1271/bbb.130109.
[26] V. Lattanzio, V.M.T. Lattanzio, A. Cardinali, V. Amendola, Role of Phenolics in the Resistance Mechanisms of Plants against Fungal Pathogens and Insects, vol. 661, 2006.
[27] E.A. Schmelz, F. Kaplan, A. Huffaker, N.J. Dafoe, M.M. Vaughan, X. Ni, et al., Identity, regulation, and activity of inducible diterpenoid phytoalexins in maize, Proc. Natl. Acad. Sci. U. S. A. 108 (2011) 5455–5460, http://dx.doi.org/10.1104/pp.111.179457.
[28] A. Huffaker, F. Kaplan, M.M. Vaughan, N.J. Dafoe, X. Ni, J.R. Rocca, et al., Novel acidic sesquiterpenoids constitute a dominant class of pathogen-induced phytoalexins in maize, Plant Physiol. 156 (2011) 2082–2097, http://dx.doi.org/10.1104/pp.111.179457.

[29] J.P. Carter, J. Spink, P.F. Cannon, M.J. Daniels, A.E. Osbourn, Isolation, characterization, and avenacin sensitivity of a diverse collection of cereal-root-colonizing fungi, Appl. Environ. Microbiol. 65 (1999) 3364–3372.
[30] K. Papadopoulou, R. Melton, M. Leggett, M. Daniels, A. Osbourn, Compromised disease resistance in saponin-deficient plants, Proc. Natl. Acad. Sci. 96 (1999) 12923–12928, http://dx.doi.org/10.1073/pnas.96.22.12923.
[31] J.B. Harborne, The comparative biochemistry of phytoalexin induction in plants, Biochem. Syst. Ecol. 27 (1999) 335–367, http://dx.doi.org/10.1016/S0305-1978(98)00095-7.
[32] T. Köllner, C. Schnee, J. Gershenzon, J. Degenhardt, The variability of sesquiterpenes emitted from two Zea mays cultivars is controlled by allelic variation of two terpene synthase genes encoding stereoselective multiple product enzymes, Plant Cell 16 (2004) 1115–1131, http://dx.doi.org/10.1105/tpc.019877.tive.
[33] T.G. Köllner, C. Schnee, J. Gershenzon, J. Degenhardt, The sesquiterpene hydrocarbons of maize (*Zea mays*) form five groups with distinct developmental and organ-specific distributions, Phytochemistry 65 (2004) 1895–1902, http://dx.doi.org/10.1016/j.phytochem.2004.05.021.
[34] T.C. Turlings, J.H. Loughrin, P.J. Mccall, U.S. Rose, W.J. Lewis, J.H. Tumlinson, How caterpillar-damaged plants protect themselves by attracting parasitic wasps, Proc. Natl. Acad. Sci. 92 (1995) 4169–4174.
[35] R.A. Salzman, J.A. Brady, S.A. Finlayson, C.D. Buchanan, E.J. Summer, F. Sun, et al., Transcriptional profiling of sorghum induced by methyl jasmonate, salicylic acid, and aminocyclopropane carboxylic acid reveals cooperative regulation and novel gene responses, Plant Physiol. 138 (2005) 352–368, http://dx.doi.org/10.1104/pp.104.058206.et.
[36] P. Basavaraju, N.P. Shetty, H.S. Shetty, E. de Neergaard, H.J.L. Jørgensen, Infection biology and defence responses in sorghum against *Colletotrichum sublineolum*, J. Appl. Microbiol. 107 (2009) 404–415, http://dx.doi.org/10.1111/j.1365-2672.2009.04234.x.
[37] S. Lo, K. De Verdier, R. Nicholson, Accumulation of 3-deoxyanthocyanidin phytoalexins and resistance to *Colletotrichum sublineolum* in sorghum, Physiol. Mol. Plant Pathol. 55 (1999) 263–273.
[38] R.L. Nicholson, S.S. Kollipara, J.R. Vincent, P.C. Lyons, G. Cadena-gomez, Phytoalexin synthesis by the sorghum mesocotyl in response to infection by pathogenic and nonpathogenic fungi, Proc. Natl. Acad. Sci. 84 (1987) 5520–5524.
[39] R.J. Grayer, T. Kokubun, Plant–fungal interactions: the search for phytoalexins and other antifungal compounds from higher plants, Phytochemistry 56 (2001) 253–263, http://dx.doi.org/10.1016/S0031-9422(00)00450-7.
[40] T. Toyomasu, Recent advances regarding diterpene cyclase genes in higher plants and fungi, Biosci. Biotechnol. Biochem. 72 (2008) 1168–1175, http://dx.doi.org/10.1271/bbb.80044.
[41] R.J. Peters, Uncovering the complex metabolic network underlying diterpenoid phytoalexin biosynthesis in rice and other cereal crop plants, Phytochemistry 67 (2006) 2307–2317, http://dx.doi.org/10.1016/j.phytochem.2006.08.009.
[42] M. Hasegawa, I. Mitsuhara, S. Seo, K. Okada, H. Yamane, T. Iwai, et al., Analysis on blast fungus-responsive characters of a flavonoid phytoalexin sakuranetin; accumulation in infected rice leaves, antifungal activity and detoxification by fungus, Molecules 19 (2014) 11404–11418, http://dx.doi.org/10.3390/molecules190811404.
[43] W. Li, M. Shao, J. Yang, W. Zhong, K. Okada, H. Yamane, et al., *Oscyp71Z2* involves diterpenoid phytoalexin biosynthesis that contributes to bacterial blight resistance in rice, Plant Sci. 207 (2013) 98–107, http://dx.doi.org/10.1016/j.plantsci.2013.02.005.
[44] R. Rakwal, M. Hasegawa, O. Kodama, A methyltransferase for synthesis of the flavanone phytoalexin sakuranetin in rice leaves, Biochem. Biophys. Res. Commun. 222 (1996) 732–735, http://dx.doi.org/10.1006/bbrc.1996.0812.
[45] B.Y.P. Atkinson, J.P. Blakeman, Seasonal occurrance of an antimicrobial flavanone, sakuranetin, associated with glands on leaves of Ribes nigrum, New Phytol. 92 (1982) 63–74.
[46] C. Zipfel, Pattern-recognition receptors in plant innate immunity, Curr. Opin. Immunol. 20 (2008) 10–16, http://dx.doi.org/10.1016/j.coi.2007.11.003.
[47] T. Yamaguchi, A. Yamada, N. Hong, T. Ogawa, T. Ishii, N. Shibuya, Differences in the recognition of glucan elicitor signals between rice and soybean: B-glucan fragments from the rice blast disease fungus *Pyricularia oryzae* that elicit phytoalexin biosynthesis in suspension-cultured rice cells, Plant Cell 12 (2000) 817–826, http://dx.doi.org/10.1105/tpc.12.5.817.
[48] M. Kishi-Kaboshi, K. Okada, L. Kurimoto, S. Murakami, T. Umezawa, N. Shibuya, et al., A rice fungal MAMP-responsive MAPK cascade regulates metabolic flow to antimicrobial metabolite synthesis, Plant J. 63 (2010) 599–612, http://dx.doi.org/10.1111/j.1365-313X.2010.04264.x.
[49] K. Kishimoto, Y. Kouzai, H. Kaku, N. Shibuya, E. Minami, Y. Nishizawa, Perception of the chitin oligosaccharides contributes to disease resistance to blast fungus *Magnaporthe oryzae* in rice, Plant J. 64 (2010) 343–354, http://dx.doi.org/10.1111/j.1365-313X.2010.04328.x.
[50] A. Yamada, N. Shibuya, O. Kodama, T. Akatsuka, Induction of phytoalexin formation in suspension-cultured rice cells by N-acetylchitooligosaccharides, Biosci. Biotechnol. Biochem. 57 (1993) 405–409, http://dx.doi.org/10.1271/bbb.57.405.
[51] G.K. Agrawal, R. Rakwal, S. Tamogami, M. Yonekura, A. Kubo, H. Saji, Chitosan activates defense/stress response(s) in the leaves of *Oryza sativa* seedlings, Plant Physiol. Biochem. 40 (2002) 1061–1069, http://dx.doi.org/10.1016/S0981-9428(02)01471-7.
[52] T. Shimizu, T. Nakano, D. Takamizawa, Y. Desaki, N. Ishii-Minami, Y. Nishizawa, et al., Two LysM receptor molecules, CEBiP and OsCERK1, cooperatively regulate chitin elicitor signaling in rice, Plant J. 64 (2010) 204–214, http://dx.doi.org/10.1111/j.1365-313X.2010.04324.x.
[53] H. Kaku, Y. Nishizawa, N. Ishii-Minami, C. Akimoto-Tomiyama, N. Dohmae, K. Takio, et al., Plant cells recognize chitin fragments for defense signaling through a plasma membrane receptor, Proc. Natl. Acad. Sci. U. S. A. 103 (2006) 11086–11091, http://dx.doi.org/10.1073/pnas.0508882103.
[54] E. Oliveira-Garcia, H.B. Deising, Infection structure – specific expression of b-1,3-glucan synthase is essential for pathogenicity of *Colletotrichum graminicola* and evasion of b-glucan–triggered immunity in maize, Plant Cell 25 (2013) 2356–2378, http://dx.doi.org/10.1105/tpc.112.103499.
[55] D. Berger, D. Oelofse, M. Arendse, E. Du Plessis, I. Dubery, Bean polygalacturonase inhibitor protein-1 (PGIP-1) inhibits polygalacturonases from *Stenocarpella maydis*, Physiol. Mol. Plant Pathol. 57 (2000) 5–14, http://dx.doi.org/10.1006/pmpp.2000.0274.
[56] K. Sawada, M. Hasegawa, L. Tokuda, J. Kameyama, O. Kodama, T. Kohchi, et al., Enhanced resistance to blast fungus and bacterial blight in transgenic rice constitutively expressing *OsSBP*, a rice homologue of mammalian selenium-binding proteins, Biosci. Biotechnol. Biochem. 68 (2004) 873–880.
[57] A. Huffaker, N.J. Dafoe, E.A. Schmelz, ZmPep1, an ortholog of arabidopsis elicitor peptide 1, regulates maize innate immunity and enhances disease resistance, Plant Physiol. 155 (2011) 1325–1338, http://dx.doi.org/10.1104/pp.110.166710.
[58] B. Randoux, D. Renard-merlier, G. Mulard, S. Rossard, F. Duyme, J. Sanssené, et al., Distinct defenses induced in wheat against powdery mildew by acetylated and nonacetylated oligogalacturonides, Phytopathology 100 (2010) 1352–1363.
[59] P. Reignault, A. Cogan, J. Muchembled, A. Lounes-Hadj Sahraoui, R. Durand, M. Sancholle, Trehalose induces resistance to powdery mildew in wheat, New Phytol. 149 (2002) 519–529, http://dx.doi.org/10.1046/j.1469-8137.2001.00035.x.
[60] D. Renard-Merlier, B. Randoux, E. Nowak, F. Farcy, R. Durand, P. Reignault, Iodus 40, salicylic acid, heptanoyl salicylic acid and trehalose exhibit different efficacies and defence targets during a wheat/powdery mildew interaction, Phytochemistry 68 (2007) 1156–1164, http://dx.doi.org/10.1016/j.phytochem.2007.02.011.
[61] J.D. Faris, Z. Zhang, H. Lu, S. Lu, L. Reddy, S. Cloutier, et al., A unique wheat disease resistance-like gene governs effector-triggered susceptibility to necrotrophic pathogens, Proc. Natl. Acad. Sci. U. S. A. 107 (2010) 13544–13549, http://dx.doi.org/10.1073/pnas.1004090107.
[62] L.A. Du Fall, P.S. Solomon, The necrotrophic effector SnToxA induces the synthesis of a novel phytoalexin in wheat, New Phytol. 200 (2013) 185–200, http://dx.doi.org/10.1111/nph.12356.
[63] S. Mayama, A. Bordin, T. Morikawa, H. Tanpo, H. Kato, Association of avenalumin accumulation with co-segregation of victorin sensitivity and crown rust resistance in oat lines carrying the *Pc-2* gene, Physiol. Mol. Plant Pathol. 46 (1995) 263–274.
[64] J. Zhao, L.C. Davis, R. Verpoorte, Elicitor signal transduction leading to production of plant secondary metabolites, Biotechnol. Adv. 23 (2005) 283–333, http://dx.doi.org/10.1016/j.biotechadv.2005.01.003.
[65] T. Yazawa, H. Kawahigashi, T. Matsumoto, H. Mizuno, Simultaneous transcriptome analysis of Sorghum and *Bipolaris sorghicola* by using RNA-seq in combination with de novo transcriptome assembly, PLoS One 8 (2013) e62460, http://dx.doi.org/10.1371/journal.pone.0062460.
[66] Q. Yang, H.X. Trinh, S. Imai, A. Ishihara, L. Zhang, H. Nakayashiki, et al., Analysis of the involvement of hydroxyanthranilate hydroxycinnamoyltransferase and caffeoyl-CoA 3-O-methyltransferase in phytoalexin biosynthesis in oat, MPMI 17 (2004) 81–89.
[67] T. Kurusu, J. Hamada, H. Nokajima, Y. Kitagawa, M. Kiyoduka, A. Takahashi, et al., Regulation of microbe-associated molecular pattern-induced hypersensitive cell death, phytoalexin production, and defense gene expression by calcineurin B-like protein-interacting protein kinases, OsCIPK14/15, in rice cultured cells, Plant Physiol. 153 (2010) 678–692, http://dx.doi.org/10.1104/pp.109.151852.
[68] I.M. Moller, Plant mitochondria and oxidative stress: electron transport, NADPH turnover, and metabolism of reactive oxygen species, Annu Rev. Plant Physiol. Plant Mol. Biol. 52 (2001) 561–591, http://dx.doi.org/10.1146/annurev.arplant.52.1.561.
[69] J.A. Allardyce, J.E. Rookes, H.I. Hussain, D.M. Cahill, Transcriptional profiling of *Zea mays* roots reveals roles for jasmonic acid and terpenoids in resistance against *Phytophthora cinnamomi*, Funct. Integr. Genomics 13 (2013) 217–228, http://dx.doi.org/10.1007/s10142-013-0314-7.
[70] W.A. Vargas, J.M.S. Martín, G.E. Rech, L.P. Rivera, E.P. Benito, J.M. Díaz-Mínguez, et al., Plant defense mechanisms are activated during biotrophic and necrotrophic development of *Colletotricum graminicola* in maize, Plant Physiol. 158 (2012) 1342–1358, http://dx.doi.org/10.1104/pp.111.190397.
[71] E. Ono, H.L. Wong, T. Kawasaki, M. Hasegawa, O. Kodama, K. Shimamoto, Essential role of the small GTPase Rac in disease resistance of rice, Proc. Natl. Acad. Sci. 98 (2001) 759–764.
[72] P. Bagnaresi, C. Biselli, L. Orrù, S. Urso, L. Crispino, P. Abbruscato, et al., Comparative transcriptome profiling of the early response to *Magnaporthe oryzae* in durable resistant vs susceptible rice (*Oryza sativa* L.) genotypes, PLoS One 7 (2012) e51609, http://dx.doi.org/10.1371/journal.pone.0051609.
[73] J. Mao, A.J. Burt, A.-I. Ramputh, J. Simmonds, L. Cass, K. Hubbard, et al.,

Diverted secondary metabolism and improved resistance to European corn borer (*Ostrinia nubilalis*) in maize (*Zea mays* L.) transformed with wheat oxalate oxidase, J. Agric. Food Chem. 55 (2007) 2582–2589, http://dx.doi.org/10.1021/jf063030f.
[74] E.M. Govrin, A. Levine, The hypersensitive response facilitates plant infection by the necrotrophic pathogen *Botrytis cinerea*, Curr. Biol. 10 (2000) 751–757, http://dx.doi.org/10.1016/S0960-9822(00)00560-1.
[75] W. Li, M. Shao, W. Zhong, J. Yang, K. Okada, H. Yamane, et al., Ectopic expression of *Hrf1* enhances bacterial resistance via regulation of diterpene phytoalexins, silicon and reactive oxygen species burst in rice, PLoS One 7 (2012) 1–10, http://dx.doi.org/10.1371/journal.pone.0043914.
[76] T. Kawasaki, K. Henmi, E. Ono, S. Hatakeyama, M. Iwano, H. Satoh, et al., The small GTP-binding protein rac is a regulator of cell death in plants, Proc. Natl. Acad. Sci. U. S. A. 96 (1999) 10922–10926, http://dx.doi.org/10.1073/pnas.96.19.10922.
[77] A. Akamatsu, H.L. Wong, M. Fujiwara, J. Okuda, K. Nishide, K. Uno, et al., An OsCEBiP/OsCERK1-OsRacGEF1-OsRac1 module is an essential early component of chitin-induced rice immunity, Cell Host Microbe 13 (2013) 465–476, http://dx.doi.org/10.1016/j.chom.2013.03.007.
[78] H. Shen, C. Liu, Y. Zhang, X. Meng, X. Zhou, C. Chu, et al., OsWRKY30 is activated by MAP kinases to confer drought tolerance in rice, Plant Mol. Biol. 80 (2012) 241–253, http://dx.doi.org/10.1007/s11103-012-9941-y.
[79] T. Chujo, K. Miyamoto, S. Ogawa, Y. Masuda, T. Shimizu, M. Kishi-Kaboshi, et al., Overexpression of phosphomimic mutated OsWRKY53 leads to enhanced blast resistance in rice, PLoS One 9 (2014), http://dx.doi.org/10.1371/journal.pone.0098737.
[80] S.P. Pandey, I.E. Somssich, The role of WRKY transcription factors in plant immunity, Plant Physiol. 150 (2009) 1648–1655, http://dx.doi.org/10.1104/pp.109.138990.
[81] C. Ross, Y. Liu, Q. Shen, The WRKY gene family in rice (Oryza sativa), J. Integr. Plant Biol. 49 (2007) 827, http://dx.doi.org/10.1111/j.1672-9072.2007.00504.x.
[82] K.-F. Wei, J. Chen, Y.-F. Chen, L.-J. Wu, D.-X. Xie, Molecular phylogenetic and expression analysis of the complete WRKY transcription factor family in maize, DNA Res. 19 (2012) 153–164, http://dx.doi.org/10.1093/dnares/dsr048.
[83] A. Akagi, S. Fukushima, K. Okada, C.J. Jiang, R. Yoshida, A. Nakayama, et al., WRKY45-dependent priming of diterpenoid phytoalexin biosynthesis in rice and the role of cytokinin in triggering the reaction, Plant Mol. Biol. (2014) 171–183, http://dx.doi.org/10.1007/s11103-014-0221-x.
[84] N. Yokotani, Y. Sato, S. Tanabe, T. Chujo, T. Shimizu, K. Okada, et al., WRKY76 is a rice transcriptional repressor playing opposite roles in blast disease resistance and cold stress tolerance, J. Exp. Bot. 64 (2013) 5085–5097, http://dx.doi.org/10.1093/jxb/ert298.
[85] T. Nemoto, A. Okada, K. Okada, N. Shibuya, T. Toyomasu, H. Nojiri, et al., Promoter analysis of the rice stemar-13-ene synthase gene *OsDTC2*, which is involved in the biosynthesis of the phytoalexin oryzalexin S, Biochim. Biophys. Acta Gene Struct. Expr. 1769 (2007) 678–683, http://dx.doi.org/10.1016/j.bbaexp.2007.08.007.
[86] Z. Tao, H. Liu, D. Qiu, Y. Zhou, X. Li, C. Xu, et al., A pair of allelic WRKY genes play opposite roles in rice-bacteria interactions, Plant Physiol. 151 (2009) 936–948, http://dx.doi.org/10.1104/pp.109.145623.
[87] F. Ibraheem, I. Gaffoor, S. Chopra, Flavonoid phytoalexin-dependent resistance to anthracnose leaf blight requires a functional *yellow seed1* in Sorghum bicolor, Genetics 184 (2010) 915–926, http://dx.doi.org/10.1534/genetics.109.111831.
[88] F. Ibraheem, I. Gaffoor, Q. Tan, C.-R. Shyu, S. Chopra, A Sorghum MYB transcription factor induces 3-deoxyanthocyanidins and enhances resistance against leaf blights in maize, Molecules 20 (2015) 2388–2404, http://dx.doi.org/10.3390/molecules20022388.
[89] W.E. Durrant, X. Dong, Systemic acquired resistance, Annu Rev. Phytopathol. 42 (2004) 185–209, http://dx.doi.org/10.1146/annurev.phyto.42.040803.140421.
[90] M.L. Wise, Effect of chemical systemic acquired resistance elicitors on avenanthramide biosynthesis in oat (*Avena sativa*), J. Agric. Food Chem. 59 (2011) 7028–7038, http://dx.doi.org/10.1021/jf2008869.
[91] M. Erb, R. Gordon-Weeks, V. Flors, G. Camañes, T.C.J. Turlings, J. Ton, Belowground ABA boosts aboveground production of DIMBOA and primes induction of chlorogenic acid in maize, Plant Signal Behav. 4 (2009) 636–638, http://dx.doi.org/10.1111/j.1365-313X.2009.03868.x.
[92] N.J. Dafoe, A. Huffaker, M.M. Vaughan, A.J. Duehl, P.E. Teal, E.A. Schmelz, Rapidly induced chemical defenses in maize stems and their effects on short-term growth of *Ostrinia nubilalis*, J. Chem. Ecol. 37 (2011) 984–991, http://dx.doi.org/10.1007/s10886-011-0002-9.
[93] M.C.B. Moraes, M.A. Birkett, R. Gordon-Weeks, L.E. Smart, J.L. Martin, B.J. Pye, et al., cis-Jasmone induces accumulation of defence compounds in wheat, *Triticum aestivum*, Phytochemistry 69 (2008) 9–17, http://dx.doi.org/10.1016/j.phytochem.2007.06.020.
[94] S. Tamogami, R. Rakwal, O. Kodama, Phytoalexin production by amino acid conjugates of jasmonic acid through induction of naringenin-7-O-methyltransferase, a key enzyme on phytoalexin biosynthesis in rice (*Oryza sativa* L, FEBS Lett. 401 (1997) 239–242.
[95] R. Rakwal, G. Kumar, M. Yonekura, O. Kodama, Naringenin 7-O-methyltransferase involved in the biosynthesis of the flavanone phytoalexin sakuranetin from rice (*Oryza sativa* L, Plant Sci. 155 (2000) 213–221.
[96] R. Naidoo, L. Ferreira, D. Berger, A. Myburg, S. Naidoo, The identification and differential expression of *Eucalyptus grandis* pathogenesis-related genes in response to salicylic acid and methyl jasmonate, Front. Plant Sci. 4 (2013), http://dx.doi.org/10.3389/fpls.2013.00043.
[97] R.M. Bostock, Signal crosstalk and induced resistance: straddling the line between cost and benefit, Annu. Rev. Phytopathol. 43 (2005) 545–580, http://dx.doi.org/10.1146/annurev.phyto.41.052002.095505.
[98] M. Ravensdale, H. Rocheleau, L. Wang, C. Nasmith, T. Ouellet, R. Subramaniam, Components of priming-induced resistance to Fusarium head blight in wheat revealed by two distinct mutants of *Fusarium graminearum*, Mol. Plant Pathol. 15 (2014) 948–956, http://dx.doi.org/10.1111/mpp.12145.
[99] G. Doehlemann, R. Wahl, R.J. Horst, L.M. Voll, B. Usadel, F. Poree, et al., Reprogramming a maize plant: transcriptional and metabolic changes induced by the fungal biotroph *Ustilago maydis*, Plant J. 56 (2008) 181–195, http://dx.doi.org/10.1111/j.1365-313X.2008.03590.x.
[100] L. Harris, A. Saparno, A. Johnston, S. Prisic, M. Xu, S. Allard, et al., The maize *An2* gene is induced by *Fusarium* attack and encodes an *ent* -copalyl diphosphate synthase, Plant Mol. Biol. 59 (2005) 881–894, http://dx.doi.org/10.1007/s11103-005-1674-8.
[101] A. Lanubile, A. Ferrarini, V. Maschietto, M. Delledonne, A. Marocco, D. Bellin, Functional genomic analysis of constitutive and inducible defense responses to *Fusarium verticillioides* infection in maize genotypes with contrasting ear rot resistance, BMC Genomics 15 (2014) 710, http://dx.doi.org/10.1186/1471-2164-15-710.
[102] M.M. Vaughan, S. Christensen, E.A. Schmelz, A. Huffaker, H.J. Mcauslane, H.T. Alborn, et al., Accumulation of terpenoid phytoalexins in maize roots is associated with drought tolerance, Plant Cell Environ. (2015), http://dx.doi.org/10.1111/pce.12482.
[103] K. Van der Linde, C. Kastner, J. Kumlehn, R. Kahmann, G. Doehlemann, Systemic virus-induced gene silencing allows functional characterization of maize genes during biotrophic interaction with *Ustilago maydis*, New Phytol. 189 (2011) 471–483, http://dx.doi.org/10.1111/j.1469-8137.2010.03474.x.
[104] M. Riemann, A. Muller, A. Korte, M. Furuya, E.W. Weiler, P. Nick, Impaired induction of the jasmonate pathway in the rice mutant *hebiba*, Plant Physiol. 133 (2003) 1820–1830, http://dx.doi.org/10.1104/pp.103.027490.
[105] T. Shimizu, K. Miyamoto, K. Miyamoto, E. Minami, Y. Nishizawa, M. Iino, et al., OsJAR1 contributes mainly to biosynthesis of the stress-induced jasmonoyl-isoleucine involved in defense responses in rice, Biosci. Biotechnol. Biochem. 77 (2013) 1556–1564, http://dx.doi.org/10.1271/bbb.130272.
[106] L. Dimberg, O. Theander, H. Lingnert, Avenanthramides – a group of phenolic antioxidants in oats, Cereal Chem. 70 (1993) 637–641.
[107] H. Miyagawa, A. Ishihara, T. Nishimoto, T. Ueno, S. Mayama, Induction of avenanthramides in oat leaves inoculated with crown rust fungus, *Puccinia coronata* f. sp. *avenae*, Biosci. Biotechnol. Biochem. 59 (1995) 2305–2306, http://dx.doi.org/10.1271/bbb.59.2305.
[108] J. Gorlach, S. Volrath, G.K.G. Hengy, U. Beckhove, K. Kogel, M. Oostendorp, et al., Benzothiadiazole, a novel class of inducers of systemic acquired resistance, activates gene expression and disease resistance in wheat, Plant Cell 8 (1996) 629–643.
[109] Y. Wu, K. Zhou, T. Toyomasu, C. Sugawara, M. Oku, Functional characterization of wheat copalyl diphosphate synthases sheds light on the early evolution of labdane-related diterpenoid metabolism in the cereals, Phytochemistry 84C (2012) 40–46, http://dx.doi.org/10.1016/j.phytochem.2012.08.022.Functional.
[110] K. Zhou, M. Xu, M. Tiernan, Q. Xie, T. Toyomasu, C. Sugawara, et al., Functional characterization of wheat ent-kaurene(-like) synthases indicates continuing evolution of labdane-related diterpenoid metabolism in the cereals, Phytochemistry 84 (2012) 47–55, http://dx.doi.org/10.1016/j.phytochem.2012.08.021.
[111] W. Spielmeyer, M. Ellis, M. Robertson, S. Ali, J.R. Lenton, P.M. Chandler, Isolation of gibberellin metabolic pathway genes from barley and comparative mapping in barley, wheat and rice, Theor. Appl. Genet. 109 (2004) 847–855, http://dx.doi.org/10.1007/s00122-004-1689-6.
[112] A. Oikawa, A. Ishihara, M. Hasegawa, O. Kodama, Induced accumulation of 2-hydroxy-4, 7-dimethoxy-1, 4-benzoxazin-3-one glucoside (HDMBOA-Glc) in maize leaves, Phytochemistry 56 (2001) 669–675.
[113] S.T. Mugford, T. Louveau, R. Melton, X. Qi, S. Bakht, L. Hill, et al., Modularity of plant metabolic gene clusters: a trio of linked genes that are collectively required for acylation of triterpenes in oat, Plant Cell 25 (2013) 1078–1092, http://dx.doi.org/10.1105/tpc.113.110551.
[114] K. Shimura, A. Okada, K. Okada, Y. Jikumaru, K.W. Ko, T. Toyomasu, et al., Identification of a biosynthetic gene cluster in rice for momilactones, J. Biol. Chem. 282 (2007) 34013–34018, http://dx.doi.org/10.1074/jbc.M703344200.
[115] X. Qi, S. Bakht, M. Leggett, C. Maxwell, R. Melton, A. Osbourn, A gene cluster for secondary metabolism in oat: implications for the evolution of metabolic diversity in plants, Proc. Natl. Acad. Sci. U. S. A. 101 (2004) 8233–8238, http://dx.doi.org/10.1073/pnas.0401301101.
[116] S. Swaminathan, D. Morrone, Q. Wang, D.B. Fulton, R.J. Peters, CYP76M7 is an *ent* -cassadiene C11-alpha-hydroxylase defining a second multifunctional diterpenoid biosynthetic gene cluster in rice, Plant Cell 21 (2009) 3315–3325, http://dx.doi.org/10.1105/tpc.108.063677.
[117] U. Von Rad, R. Hüttl, F. Lottspeich, A. Gierl, M. Frey, Two glucosyltransferases are involved in detoxification of benzoxazinoids in maize, Plant J. 28 (2001)

633–642.
[118] M. Frey, K. Huber, W. June, D. Sicker, P. Lindberg, R.B. Meeley, et al., A 2-oxoglutarate-dependent dioxygenase is integrated in DIMBOA-biosynthesis, Phytochemistry 62 (2003) 371–376.
[119] M. Frey, R. Kliem, H. Saedler, A. Gierl, Expression of a cytochrome P450 gene family in maize, Mol. Gen. Genet. 246 (1995) 100–109.
[120] R. Jonczyk, H. Schmidt, A. Osterrieder, A. Fiesselmann, K. Schullehner, M. Haslbeck, et al., Elucidation of the final reactions of DIMBOA-glucoside biosynthesis in maize: characterization of Bx6, Plant Physiol. 146 (2008) 1053–1063, http://dx.doi.org/10.1104/pp.107.111237.
[121] M. Sue, C. Nakamura, T. Nomura, Dispersed benzoxazinone gene cluster: molecular characterization and chromosomal localization of glucosyltransferase and glucosidase genes in wheat and rye, Plant Physiol. 157 (2011) 985–997, http://dx.doi.org/10.1104/pp.111.182378.
[122] K. Otomo, Y. Kanno, A. Motegi, H. Kenmoku, H. Yamane, W. Mitsuhashi, et al., Diterpene cyclases responsible for the biosynthesis of phytoalexins, momilactones A, B, and oryzalexins A–F in rice, Biosci. Biotechnol. Biochem. 68 (2006) 2001–2006.
[123] J. Ton, M. D'Alessandro, V. Jourdie, G. Jakab, D. Karlen, M. Held, et al., Priming by airborne signals boosts direct and indirect resistance in maize, Plant J. 49 (2007) 16–26, http://dx.doi.org/10.1111/j.1365-313X.2006.02935.x.
[124] U. Conrath, Priming of induced plant defense responses plant, Adv. Bot. Res. 51 (2009) 361–395, http://dx.doi.org/10.1016/S0065-2296(09)51009-9.
[125] Y.Y. Song, M. Cao, L.J. Xie, X.T. Liang, Zeng R. Sen, Y.J. Su, et al., Induction of DIMBOA accumulation and systemic defense responses as a mechanism of enhanced resistance of mycorrhizal corn (*Zea mays* L.) to sheath blight, Mycorrhiza 21 (2011) 721–731, http://dx.doi.org/10.1007/s00572-011-0380-4.
[126] J.A. Ryals, U.H. Neuenschwander, M.G. Willits, A. Molina, H. Steiner, M.D. Hunt, Systemic acquired resistance, Plant Cell 8 (1996) 1809–1819.
[127] L.C. Van Loon, M. Rep, C. Pieterse, Significance of inducible defense-related proteins in infected plants, Annu. Rev. Phytopathol. 44 (2006) 135–162, http://dx.doi.org/10.1146/annurev.phyto.44.070505.143425.
[128] M. Oostendorp, W. Kunz, B. Dietrich, T. Staub, Induced disease resistance in plants by chemicals, Eur. J. Plant Pathol. (2001) 19–28.
[129] L. Vechet, L. Burketova, M. Sindelarova, A comparative study of the efficiency of several sources of induced resistance to powdery mildew (*Blumeria graminis* f. sp. *tritici*) in wheat under field conditions, Crop Prot. 28 (2009) 151–154, http://dx.doi.org/10.1016/j.cropro.2008.09.009.
[130] P. Gautam, J. Stein, Induction of systemic acquired resistance to *Puccina sorghi* in corn, Int. J. Plant Pathol. 2 (2011) 43–50.
[131] D. Balmer, D.V. De Papajewski, C. Planchamp, G. Glauser, B. Mauch-Mani, Induced resistance in maize is based on organ-specific defence responses, Plant J. 74 (2013) 213–225, http://dx.doi.org/10.1111/tpj.12114.
[132] M. Shimono, S. Sugano, A. Nakayama, C.-J. Jiang, K. Ono, S. Toki, et al., Rice WRKY45 plays a crucial role in benzothiadiazole-inducible blast resistance, Plant Cell 19 (2007) 2064–2076, http://dx.doi.org/10.1105/tpc.106.046250.
[133] J. Korsman, B. Meisel, F.J. Kloppers, B.G. Crampton, D.K. Berger, Quantitative phenotyping of grey leaf spot disease in maize using real-time PCR, Eur. J. Plant Pathol. 133 (2012) 461–471, http://dx.doi.org/10.1007/s10658-011-9920-1.
[134] A.M. Mutka, R.S. Bart, Image-based phenotyping of plant disease symptoms, Front. Plant Sci. 5 (2015) 1–8, http://dx.doi.org/10.3389/fpls.2014.00734.
[135] D. Balmer, V. Flors, G. Glauser, B. Mauch-Mani, Metabolomics of cereals under biotic stress: current knowledge and techniques, Front. Plant Sci. 4 (2013) 82, http://dx.doi.org/10.3389/fpls.2013.00082.
[136] E. Becker, C. Herrfurth, S. Irmisch, T.G. Ko, I. Feussner, P. Karlovsky, et al., Infection of corn ears by Fusarium spp. induces the emission of volatile sesquiterpenes, J. Agric. Food Chem. (2014), http://dx.doi.org/10.1021/jf500560f.
[137] A.L. Heuberger, F.M. Robison, S.M.A. Lyons, C.D. Broeckling, J.E. Prenni, Evaluating plant immunity using mass spectrometry-based metabolomics workflows, Front. Plant Sci. 5 (2014) 291, http://dx.doi.org/10.3389/fpls.2014.00291.
[138] N. Coetzer, A.A. Myburg, D.K. Berger, Maize microarray annotation database, Plant Methods 7 (2011) 31, http://dx.doi.org/10.1186/1746-4811-7-31.

Web references

[139] www.fao.org/worldfoodsituation/csdb/en/.
[140] www.statistics.amis-outlook.org/data/index.html.