

**Title: Regulation of phytoalexin biosynthesis for agriculture and human health**

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1 Abstract

2 Phytoalexins are diverse secondary metabolites of plants that are biosynthesized transiently  
3 and in relatively low amounts in response to pathogens and certain abiotic stresses. They  
4 commonly have potent antimicrobial and medicinal activities. As such, scientists have  
5 attempted to increase their accessibility by inventing diverse *in vitro* and *in vivo* approaches.  
6 Among these approaches, bioengineering plant transcription factors that directly regulate  
7 phytoalexin biosynthesis genes may be the most promising. Recent research has identified  
8 conserved transcription factors that directly regulate distinct phytoalexin biosynthesis  
9 pathways in different plant species. The intriguing results provide new insight into how  
10 conserved defense signaling pathways in plants result in lineage-specific biochemical defenses.  
11 These recent findings also suggest that a common transcription factor network could be  
12 engineered to enhance the biosynthesis of different phytoalexins in plants. However, the picture  
13 is far from complete since one or more transcription factors required to fully activate  
14 phytoalexin biosynthesis remain unidentified, and the exact mechanism of how the conserved  
15 factors regulate diverse phytoalexin pathways remains to be clarified. Here we review the  
16 agricultural and medicinal importance of phytoalexins, recent approaches to increase their  
17 accessibility, and the mechanisms that plants employ to activate and limit their biosynthesis.  
18 This review contributes to providing a systems level understanding of the regulation of  
19 phytoalexin biosynthesis so that effective bioengineering strategies can be developed to  
20 enhance phytoalexin biosynthesis for medicine and agriculture.

21 Keywords

22 Bioengineering · Transcription factor · Defense · Secondary metabolite · Signaling

23 Abbreviations

<b>ABA</b>	<b>Abscisic acid</b>
<b>BLAST</b>	Basic local alignment search tool
<b>BLASTPs</b>	BLAST for proteins
<b>CHIP</b>	Chromatin immunoprecipitation
<b>CHIP-Seq</b>	Chromatin immunoprecipitation next generation sequencing
<b>COR</b>	Cornatine
<b>DNA</b>	Deoxyribonucleic acid
<b>ED</b>	Effective Dose
<b>H22 cells</b>	Hippocampal neuronal cell line
<b>HR</b>	Hypersensitive Response
<b>JA</b>	Jasmonic Acid
<b>PCR-</b>	Polymerase chain reaction
<b>pH</b>	Potential hydrogen
<b>PP2C</b>	Protein phosphatase 2C
<b>qRT-PCR</b>	Quantitative real-time reverse ranscription PCR
<b>RNA</b>	Ribonucleic acid
<b>RNAi</b>	RNA interference

<b>RNA-Seq</b>	RNA next generation sequencing
<b>ROS</b>	Reactive oxygen species
<b>RT-PCR</b>	Reverse transcription PCR
<b>TF</b>	Transcription factor
<b>UV</b>	Ultra violet irradiation
<b>WGE</b>	Wall glucan elicitor
<b>WT</b>	Wild type

24

25 Introduction

26       The concept of a *phytoalexin* was first introduced by Karl O. Müller who described that  
 27 potato inoculated with an incompatible race of *Phytophthora infestans* provoked the synthesis  
 28 of a putative defense metabolite that provided resistance to a compatible race of the pathogen  
 29 (Müller et al. 1939). Later in 1940 along with Hermann Borger, Müller defined phytoalexins  
 30 as metabolites specific to plants that are produced upon contact with microbes and that show  
 31 antimicrobial properties (Müller 1940). Due to their implicit role in plant defense, various  
 32 aspects of phytoalexins were intensively researched over the following decades, including their  
 33 chemical structures, biosynthetic genes and pathways, anti-pathogenic activities, and elicitors  
 34 (Jeandet et al. 2014; Großkinsky et al. 2012; Ahuja et al. 2012; Jeandet et al. 2013).

35       Over time, phytoalexins that were biosynthesized in different plant species were  
 36 discovered to be highly diverse in chemical structure. In fact, their chemical diversity may have  
 37 caused a decrease in research intensity on phytoalexins over the past decades in favor of

38 defense responses that are less diversified among plant species. Eventually evidence mounted  
39 that several phytoalexins had major roles in providing crop plants resistance to important  
40 microbial pathogens (Fig. 1) (Ahuja et al. 2012; Graham et al. 2007; Großkinsky et al. 2012).  
41 Yet, evidence also mounted that no single phytoalexin was effective against all microbial  
42 pathogens, despite their broad-spectrum antimicrobial activities. For example, the *pen3 pdr12*  
43 double mutant of *Arabidopsis* that is defective in the cellular secretion of camalexin and the  
44 *pad3* mutant of that is defective in the final step of camalexin synthesis exhibit enhanced  
45 susceptibility to *Botrytis cinerea* and a number of fungal and oomycete pathogens, respectively  
46 (He et al. 2019). However, camalexin is not required for the WRKY33-dependent resistance to  
47 the hemibiotrophic fungus *Colletotrichum higginsianum* (Schmidt et al. 2020). WRKY33 is a  
48 well-characterized transcription factor (TF) that activates camalexin biosynthetic genes  
49 (described below), and may also activate the synthesis of indole carbonyl nitriles (Schmidt et  
50 al. 2020), a recently discovered group of cyanogenic phytoalexins from *Arabidopsis* (Rajniak  
51 et al. 2015). The fact that phytoalexins continue to be discovered even in model plants that are  
52 intensively studied highlights how our knowledge of phytoalexins is still extremely limited.

53         Now 80 years after their discovery, evidence has mounted that not only do phytoalexins  
54 have important roles in plant protection against microbes, many of them have potent medicinal  
55 activities such as anticancer and neuroprotective properties, that render them highly attractive  
56 for pharmaceutical development (Pham et al. 2019; Sen 2017; Cheng et al. 2019). Plant  
57 biologists remain challenged with the difficult task of seeking-out these ‘hidden’ metabolites,  
58 describing their diversity, medicinal and plant protective activities, and understanding how to  
59 improve their accessibility for human health and agriculture.

## 60 Importance of phytoalexins in agriculture

61 Müller and Borger proposed phytoalexin theory in 1939 (Mueller and Börger 1939;  
62 Müller et al. 1939). They suggested that the rapidity and magnitude of phytoalexin  
63 accumulation, rather than the magnitude alone, were critical features required for providing  
64 potato tubers resistance against *Phytophthora infestans*. Prior inoculation of tubers with an  
65 incompatible race of *P. infestans* triggered *rapid* and high level resistance against a compatible  
66 race. Presently, there is a large body of experimental evidence supporting that phytoalexins  
67 play critical roles in plant defense against microbial pathogens when they are biosynthesized  
68 *rapidly* at concentrations that can provide toxicity [for reviews see (Ahuja et al. 2012;  
69 Großkinsky et al. 2012; Kuc 1995)]. For example, to mediate incompatibility with  
70 *Magnaporthe oryzae*, gene transcripts and metabolites of the diterpenoid phytoalexins of rice  
71 had to accumulate more rapidly and to a higher level than the susceptible genotype to restrict  
72 fungal growth prior to the HR-response (Hasegawa et al. 2010). Similarly, phytoalexin gene  
73 transcripts and metabolites accumulated ~100 h earlier during the incompatible interaction of  
74 a resistant common bean genotype compared to the compatible interaction of the the  
75 susceptible genotype (Lamb et al. 1992). In this example, the compatible genotype  
76 demonstrated water soaked anthracnose lesions by that time, whereas in the resistant genotype  
77 lesion spread of *Colletotrichum lindemuthianum* was arrested soon after peak phytoalexin  
78 accumulation. Similarly, the treatment of tobacco plants with cytokinins resulted in the rapid  
79 accumulation of scopoletin and capsidiol and provided resistance to *Pseudomonas syringae*  
80 (Großkinsky et al. 2012). Scopoletin and capsidiol were determined to be the cause of  
81 resistance by infiltrating them into tobacco leaves at physiological levels just prior to bacterial  
82 inoculation. The rapidity of scopoletin and capsidiol biosynthesis, rather than their absolute  
83 levels was concluded to be the decisive factor for providing incompatibility since their absolute  
84 levels were much higher in the susceptible, mock-treated control, at later stages of infection.

85           The glyceollins of soybean have long served as a model for understanding how  
86 phytoalexins mediate resistance to pathogens. Yoshikawa and colleagues were the first to  
87 suggest that the rapid elicitation of glyceollins was critical for mediating resistance to a  
88 agricultural pest of soybean, namely *Phytophthora sojae*. In these early studies, glyceollin  
89 concentrations were measured in localized infection sites of hypocotyls of the resistant variety  
90 Harosoy 63 and the susceptible nearly isogenic variety Harosoy at various times after  
91 inoculation with zoospores of *P. sojae* (Yoshikawa and Masago 1982; Yoshikawa et al. 1978).  
92 The extent of mycelium growth was then compared as a proxy of infection. In the resistant  
93 genotype Harosoy 63, the levels of glyceollins exceeded the ED90 values (*i.e* the effective dose  
94 required to inhibit growth of 90% of the population) at the site of infection within 8 h after  
95 inoculation and increased transiently thereafter. However, it took the susceptible variety 24 h  
96 to accumulate glyceollins to concentrations exceeding the ED90. *P. sojae* grew at similar rates  
97 in both the resistant and susceptible hypocotyls until 8 h after inoculation, but then growth was  
98 restricted in the resistant hypocotyls and no further growth occurred after 9 h. Bhattacharyya  
99 and Ward confirmed the delayed accumulation of glyceollins in susceptible Harosoy compared  
100 to resistant Harosoy 63 following treatment of hypocotyls with race 1 *P. sojae* zoospores  
101 (Bhattacharyya and Ward 1986). Plant genotypes are thought to be resistant due to their ability  
102 to rapidly increase the ratio of phytoalexins relative to the number of pathogen cells, rather  
103 than simply exceeding a specific threshold concentration (Großkinsky et al. 2012). Graham and  
104 colleagues observed that the inoculation of cotyledons of race 1-resistant variety Williams 79  
105 resulted in glyceollin levels peaking within 24 h after elicitation but they did not peak until 48  
106 h after the infection front had passed in the susceptible nearly-isogenic variety Williams  
107 (Graham et al. 1990). Similarly, the hypocotyls of Williams 79 exhibited delayed elicitation of  
108 glyceollins during the compatible interaction with Race 3 *P. sojae* compared to the  
109 incompatible interaction with Race 1 (Hahn et al. 1985). Purified glyceollins and several other

110 intermediates were confirmed to be toxic to *P. sojae*, and unlike several other pathogens, *P.*  
111 *sojae* was found to lack genes for glyceollin degradation (Bhattacharyya and Ward 1985;  
112 Fischer et al. 1990; Lygin et al. 2010). It was Graham and colleagues that provided compelling  
113 evidence that glyceollins and other similar metabolites were responsible for mediating  
114 resistance rather than other defense responses. They silenced soybean *ISOFLAVONE*  
115 *SYNTHASE* genes reducing the biosynthesis of glyceollins and several 5-deoxyisoflavonoids  
116 including daidzein, which resulted in susceptibility to *P. sojae* (Graham et al. 2007). Finally,  
117 by silencing the glyceollin TF *GmMYB29A2* in variety Williams 82 that is resistant to *Race I*  
118 *P. sojae*, and overexpressing the same gene in the susceptible variety Williams, we recently  
119 found that compatibility and incompatibility coincided with reduced and enhanced  
120 accumulations of glyceollin I specifically, and not the other glyceollins or 5-  
121 deoxyisoflavonoids (Jahan et al. 2020) Since *P. sojae* causes an annual yield loss of \$1-2 billion  
122 worldwide in soybean agriculture (Lin et al. 2014; Tyler 2007), bioengineering *GmMYB29A2*  
123 expressions should be field tested as an approach to enhance food security. Various reports  
124 have hinted that glyceollins may have roles in defending crop plants against insects (Huang  
125 and Barker 1991; Hohenstein et al. 2019; Chang and Hartman 2017; Liu et al. 1992), so the  
126 approach could have broader utility. Various studies have demonstrated potent medicinal  
127 properties of some phytoalexins, such as glyceollin I. Thus, engineering phytoalexin gene  
128 regulatory networks could also be an economical tool for producing phytoalexins for human  
129 health.

### 130 Value of phytoalexins for human health

131 Phytoalexins may inherently have bioactivities that render them useful as medicines  
132 due to their evolution as plant defense compounds. Yet, they have not been characterized from  
133 most plant species, and hence their medicinal properties remain largely unknown. It also



134 remains unknown how some phytoalexins show protective activities in some cell types, yet  
135 they inhibit the proliferation of others. The glyceollins are an example of phytoalexins that are  
136 toxic to cancer cells yet promote the survival of insulted neurons. Glyceollin I exhibits potent  
137 antiestrogenic activities that are distinct from the conventional therapeutic tamoxifen  
138 (Zimmermann et al. 2010). It directly binds to estrogen receptor (ER) alpha and inhibits breast  
139 tumor progression (Salvo et al. 2006). It also hinders the invasion and migration of breast  
140 cancer cells that are resistant to letrozole and it reverses the transition of epithelial to  
141 mesenchymal cells in part by inhibiting the expression of EGFR (Epidermal growth factor  
142 receptor) (Carriere et al. 2015). A mixture of glyceollins I, II, and III inhibits the survival and  
143 tumor proliferation of triple-negative breast cancer (TNBC) by an unknown mechanism that is  
144 ER-independent (Rhodes et al. 2012). TNBC is resistant to conventional chemotherapeutics  
145 and thus is a cancer subtype that is in need of new therapies. Glyceollins were also shown to  
146 inhibit cell proliferation by blocking the synthesis and destabilizing hypoxia inducible factor 1  
147 alpha (HIF-1 $\alpha$ ) in TNBC, non-small cell lung (NSCL) cancer, and in other chemo-recalcitrant  
148 cell types (Lee et al. 2015). In contrast, glyceollins protect primary cortical neurons derived  
149 from mice and hippocampal HT22 cells from glutamate-induced cytotoxicity (Seo et al. 2018).  
150 The medicinal properties of glyceollins and potential mechanisms have recently been reviewed  
151 (Pham et al. 2019; Bamji and Corbitt 2017).

152 Other phytoalexins with promising medicinal properties include the (ptero)stilbenes  
153 from grapevine and camalexin from Arabidopsis. Camalexin induces apoptosis in prostate  
154 cancer cells (Smith et al. 2014) with strong preference over non-cancerous cells (Pilatova et al.  
155 2013). Pterostilbene inhibits invasion, metastasis, and migration of human hepatoma cells (Pan  
156 et al. 2009) and suppresses the establishment of abnormal crypt foci in a rat colon cancer model  
157 (Suh et al. 2007). Resveratrol is a stilbene that has anticancer, anti-inflammatory, and cardio  
158 protective activities (Shukla and Singh 2011; Sen 2017). Recently, resveratrol was suggested

159 to reduce the viability and migration of melanoma cells by inhibiting the AKT/mTOR pathway  
160 (Gong and Xia 2020). Treatment with the autophagy inhibitor, 3-methyladenine, reversed the  
161 resveratrol-dependent effects (Gong and Xia 2020). Resveratrol also protects hippocampal  
162 neurons against cerebral ischemia, putatively by scavenging free radicals and elevating cerebral  
163 blood flow resulting from the release of nitric oxide (Lu et al. 2006). There has been widespread  
164 efforts to semi-synthesize methoxylated, hydroxylated, and halogenated derivatives of  
165 resveratrol that have improved bioavailability, anticancer, cardioprotective, neuroprotective or  
166 antioxidant properties (Nawaz et al. 2017). Synthetic chemistry approaches were also used to  
167 make 5-bromosubstituted derivatives of indole phytoalexins (e.g. brassinin) (Budovská et al.  
168 2020). Some of these had greater antiproliferative activities in cancer cells than cisplatin and  
169 lower toxicity to immortalized primary mouse embryonic fibroblasts (Budovská et al. 2020).  
170 Approaches that could be used for making novel derivatives of phytoalexins that have enhanced  
171 medicinal properties include combining semi-synthesis with genetically engineered microbes  
172 and plants in succession and in different combinations to (bio)synthesize novel molecules  
173 (Gary et al. 2018).

174         While phytoalexins have been described from relatively few plant species, their  
175 impressive effects on human health and diverse chemical structures highlights why scientists  
176 have suggested ‘mining’ plants for these valuable molecules (Mead 2007). The fact that new  
177 classes of phytoalexins continue to be discovered from some of the best-characterized plant  
178 species (Huffaker et al. 2011; Rajniak et al. 2015; Ube et al. 2019) suggests that our  
179 understanding of the biochemical diversity of phytoalexins and their medicinal activities  
180 remains in its infancy. Large scale metabolomics screenings for phytoalexins could very much  
181 improve our understanding of their diversity and evolution in plants, and could uncover novel  
182 pharmaceuticals. Yet, accessibility to these molecules will likely remain a challenge since  
183 phytoalexins are biosynthesized in relatively transiently and in low amounts in plants.

184 Efforts to improve accessibility to phytoalexins

185           Since phytoalexins are biosynthesized in low amounts and only transiently upon  
186 elicitation, various research efforts worldwide have focused on enhancing their accessibility.  
187 These have mainly been focused on enhancing their elicitation in plants, to synthesizing them  
188 using chemistry methods, or when known, introducing their entire biosynthetic gene sets into  
189 microbes. For example, the traditional method to obtain glyceollins has been to elicit soybean  
190 seeds with microbial pathogens (Boue et al. 2000). Various chemical synthesis methods have  
191 been developed, yet even the most promising approach strategy remains highly uneconomical  
192 (Khupse et al. 2011; Luniwal et al. 2011; Malik et al. 2015). Efforts to improve the elicitation  
193 of glyceollins include fermentation of soybeans (Park et al. 2012), and treating seeds with  
194 pathogens and malting (Simons et al. 2011), pathogens and environmental stresses (Aisyah et  
195 al. 2013), and pathogens and chemicals (Farrell et al. 2017). Screening various environmental  
196 stresses for their effects on phytoalexin biosynthesis recently identified acidic growth medium  
197 (pH 3.0) as a potent elicitor of glyceollins (Jahan et al. 2019; Jahan and Kovinich 2019). Yet,  
198 bioengineering may be the most promising strategy to improve accessibility. Overexpressing  
199 the isoflavonoid synthesis gene *ISOFLAVONE REDUCTASE (IFR)* in soybean seedlings  
200 increased glyceollin amounts only 3-fold (Cheng et al. 2015), which notably provided greater  
201 increases in yield compared to other elicitor treatments. This example highlights that  
202 overexpressing a gene for one rate-limiting biosynthetic step indefinitely uncovers another. For  
203 this reason, it may be more effective to overexpress the transcription factors (TFs) or signaling  
204 proteins that positively regulate all genes that are required to biosynthesize a phytoalexin. We  
205 recently demonstrated that overexpressing the NAC-family TF *GmNAC42-1* in soybean hairy  
206 roots increased glyceollin II elicitation more than 10-fold (Jahan et al. 2019). Notably, bubble-  
207 type bioreactors can be used to further enhance the production of secondary metabolites from  
208 roots (Lee et al. 2020).

209 An alternative production method has been to transfer full biosynthetic gene sets into  
210 microbes that have modified primary metabolism. For example, an *E. coli* strain engineered to  
211 supply more L-tyrosine could produce 3.6-fold more pterostilbene than the wild-type strain  
212 (Heo et al. 2017). Yet, this approach is not feasible for biosynthetic pathways where all  
213 biosynthetic genes have not been identified, such as for the glyceollins. Thus, for most  
214 phytoalexins, the key to providing economical accessibility could be engineering phytoalexin  
215 gene regulatory networks and their feedback mechanisms in plants. Fortunately we are entering  
216 an era where non-species-specific genetic transformation methods, such as nanoparticle-based  
217 methods are making efficient genetic engineering possible in a multitude of plant species  
218 (Demirer et al. 2019; Doyle et al. 2019; Zhang et al. 2019).

219 Diverse phytoalexin biosynthetic pathways have common elicitors

220 Phytoalexin biosynthesis genes and their metabolite products are highly diverse among  
221 plant species (Ahuja et al. 2012; Jeandet et al. 2014). Species of the *Leguminosae* family  
222 biosynthesize glyceollins (soybean), medicarpin (alfalfa), pisatin (*Pisum sativum*), phaseollin  
223 (*Phaseolus vulgaris*), and Maackiain (*Maackia*, *Trifolium* and *Cicer* groups) (Ingham 1979;  
224 VanEtten et al. 1983; Strange et al. 1985). Plants of the Poaceae family biosynthesize  
225 diterpenoid momilactones, phytocassanes, oryzalexins (*Oryza sativa*), kauralexins and  
226 zealexins (*Zea mays*), the phenolic alkaloid avenanthramides (oat), and the flavonoids  
227 sakuranetin (*Oryza sativa*) and 3-deoxyanthocyanidins (*Sorghum bicolor*) (Poloni and  
228 Schirawski 2014; Huffaker et al. 2011; Schmelz et al. 2014; Yamamura et al. 2015; Koga et al.  
229 1995). The Solanaceae family is known to produce the terpenoid rishitin, and the  
230 phenylpropanoids chlorogenic acid and caffeic acid (Ohnishi et al. 1994). Grapevine (*Vitis*  
231 *vinifera*), from the Vitaceae family biosynthesizes stilbenes like resveratrol (Jeandet et al.  
232 2019b; Kiselev 2011). *Eschscholzia californica* (Papaveraceae) produces benzophenanthridine

233 alkaloids while *Catharanthus roseus* (Apocynaceae) biosynthesizes monoterpenoid indole  
234 alkaloids (Heinze et al. 2015). *Arabidopsis thaliana* (Arabidopsis) from the Brassicaceae  
235 family produces the indole alkaloid camalexin, the indoles brassinin, indole-3-carboxylic acid,  
236 and the hydroxyindole 4-hydroxy-indole-3-carbonyl nitrile (Pastorczyk et al. 2020; Stahl et al.  
237 2016; Rajniak et al. 2015). Thus, even closely related plant species biosynthesize phytoalexins  
238 from a wide range of metabolic pathways.

239         Despite their biosynthetic heterogeneity, phytoalexins share common elicitors. These  
240 have been described in several reviews (Ahuja et al. 2012; Großkinsky et al. 2012; Jeandet et  
241 al. 2010). Elicitors that commonly induce phytoalexin biosynthesis in different plant species  
242 include microbial pathogens, UV irradiation, and inorganic heavy metals. Common elicitors  
243 could suggest that the diverse phytoalexin biosynthesis pathways of different plant species are  
244 stimulated by the same conserved signaling pathway(s). Since phytoalexin biosynthesis genes  
245 are commonly regulated at the level of gene transcription (Jeandet et al. 2019a; Höll et al. 2013;  
246 Moy et al. 2004; Saga et al. 2012; Yamamura et al. 2015), it is possible that TFs that directly  
247 regulate phytoalexin biosynthesis genes are components of this conserved signaling network.  
248 Here we review the TFs and signaling proteins that regulate diverse phytoalexin biosynthesis  
249 pathways. We also highlight two conserved TFs that were recently identified to directly  
250 regulate distinct phytoalexin biosynthesis pathways in different plant species, thus providing  
251 initial evidence of the conserved TF network hypothesis.

252         Transcription factors that activate phytoalexin biosynthesis

253         Out of 58 families of TFs in plants, six have been found to be involved in regulating  
254 phytoalexin biosynthesis. These families are: WRKY (WRKYGQK motif), MYB  
255 (myeloblastosis related), NAC [no apical meristem (NAM) *Arabidopsis* transcription  
256 activation factor (ATAF1/2) cup-shaped cotyledon (CUC2)], bHLH (basic helix-loop-helix),

257 AP2/ERF (APETALA2/ethylene responsive factor), and bZIP (basic leucine zipper). The first  
258 TF identified to regulate the biosynthesis of a phytoalexin was a WRKY family protein from  
259 cotton (*Gossypium arboreum* L.). The term 'WRKY' denotes a zinc-finger TF that has a 60-70  
260 amino acid WRKY domain containing the WRKYGQK motif (Amorim et al. 2017; Song et al.  
261 2018; Jiang et al. 2017) *GaWRKY1* transcripts were elicited both spatially and temporally with  
262 gossypol biosynthesis genes by pathogen and inorganic elicitors (Xu et al. 2004). Further, the  
263 GaWRKY1 protein was able to directly bind the promoter of the gossypol biosynthesis gene  
264 (+)- $\delta$ -cadinene synthase (*CADI*) in the yeast one-hybrid (Y1H) system. The second WRKY-  
265 family TF that was identified to regulate phytoalexin biosynthesis was WRKY33 for camalexin  
266 in *Arabidopsis* (Qiu et al. 2008). It directly bound to the promoter of the camalexin biosynthesis  
267 gene *PHYTOALEXIN DEFICIENT 3 (PAD3)* by ChIP-PCR upon treatment with the fungal  
268 elicitor flg22. WRKY33 requires phosphorylation from the pathogen-responsive mitogen-  
269 activated protein kinases (MAPKs) MPK3 and MPK6 for full activity (Mao et al. 2011).  
270 Despite that WRKY33 is essential for the activation of camalexin biosynthesis, the  
271 overexpression of wild-type (WT) or a phospho-mimic forms of WRKY33 did not enhance  
272 camalexin levels in elicited tissues beyond the WT (Qiu et al. 2008). This suggests that  
273 WRKY33, while essential, was not a rate-limiting for camalexin gene regulation.

274 MYB-family TFs were also found to regulate phytoalexin biosynthesis. *VvMYB14* and  
275 *VvMYB15* (R2R3-type MYB TFs) from grapevine (*Vitis vinifera*) were co-induced with  
276 stilbene biosynthesis genes and could directly bind the promoter of *STILBENE SYNTHASE*  
277 (*STS*) in transient promoter-reporter assays (Höll et al. 2013). *VvMYB15* enhanced the  
278 accumulation of stilbenes 2- to 5-fold when overexpressed in grapevine hairy roots. Further,  
279 the overexpression of *MYB14* from Chinese wild grape, *Vitis quinquangularis*-Pingyi,  
280 increased stilbene biosynthesis and the expressions of *STS* genes (Luo et al. 2019; Duan et al.  
281 2016; Luo et al. 2020). Various promoter segments of *MYB14* and *MYB15* responsible for

282 induction by pathogens have been identified (Luo et al. 2020). Potential ROS-responsive  
283 regions of the MYB14 promoter from *Vitis labrusca* 'Concord' were also reported (Bai et al.  
284 2019). Yet, the transacting factors that bind those promoter segments have not been reported.

285 Other MYBs were implicated in regulating camalexin biosynthesis in *Arabidopsis*.  
286 Reduced camalexin levels were observed upon elicitation of double and triple mutants of  
287 *AtMYB34*, *AtMYB51*, and *AtMYB122* (Frerigmann et al. 2015). However, promoter-reporter  
288 assays found that these MYBs do not bind camalexin biosynthesis gene promoters directly,  
289 suggesting indirect roles in camalexin gene regulation.

290 bHLH-type TFs were found to regulate phytoalexin synthesis in rice and *Medicago*  
291 *truncatula*. The elicitation of sakuranetin in rice was almost completely eliminated by RNAi  
292 silencing of the bHLH gene *OsMYC2* (Ogawa et al., 2017). *OsMYC2* was involved in the direct  
293 binding of the sakuranetin biosynthesis gene promoters by promoter-reporter assays (Ogawa  
294 et al., 2017). *OsDPF* (DITERPENOID PHYTOALEXIN FACTOR), another bHLH-family TF  
295 from rice, directly binds the promoters of phytocassane and momilactone biosynthesis genes  
296 by promoter-reporter assays (Yamamura et al. 2015). Two other bHLH-family TFs, *TSAR1*  
297 and *TSAR2* (TRITERPENE SAPONIN BIOSYNTHESIS ACTIVATING REGULATOR),  
298 directly bound triterpene saponin biosynthesis in *Medicago truncatula* (Goossens et al. 2015).

299 bHLHs are often required by R2R3-type MYBs for the MYBs to bind DNA in order to  
300 regulate various branches of phenylpropanoid metabolism (Zimmermann et al. 2004; Xu et al.  
301 2015). However, the *VvMYB14* and *VvMYB15* from grapevine did not require a bHLH to  
302 bind the *STS* gene promoter (Höll et al. 2013). Likewise, in *Arabidopsis* no bHLH-family TF  
303 was found to be involved in regulating camalexin biosynthesis. In contrast, loss-of-function  
304 mutants of the NAC-family TF *ANAC042* revealed that it was required for WT levels of  
305 camalexin elicitation by pathogens and heavy metals (Saga et al., 2012). Chromatin

306 immunoprecipitation followed by high-throughput sequencing (ChIP-seq) found that  
307 ANAC042 (a.k.a. JUNGBRUNNEN1) directly binds key genes involved in gibberellin (GA)  
308 and brassinosteroid (BR) biosynthesis to suppress plant growth (Shahnejat-Bushehri et al.  
309 2016). It remains unclear whether ANAC042 directly binds camalexin gene promoters in the  
310 presence of an elicitor.

311         Importantly, no phytoalexin TFs were found to be homologous among plant species  
312 until recently. Technically, reciprocal BLASTPs of each phytoalexin TF did not identify  
313 another phytoalexin TF from other plant species in the top 10 most similar proteins. This has  
314 led to the concept that phytoalexin TFs are as diverse as the biosynthetic pathways that they  
315 regulate. Yet, in two recent studies we reported two TFs that regulate glyceollin biosynthesis  
316 in soybean that are orthologous to TFs that regulate different phytoalexin biosynthesis  
317 pathways in other plant species. By screening a panel of abiotic stresses, we identified acidity  
318 stress to be a novel elicitor and dehydration stress to be a suppressor of glyceollin biosynthesis,  
319 respectively (Jahan et al. 2019). RNA-seq of seedlings treated with those stresses found several  
320 TF genes that were up- and down-regulated with glyceollin biosynthesis gene transcripts and  
321 metabolites. *GmNAC42-1*, the soybean ortholog of the camalexin regulator *AtANAC042* from  
322 *Arabidopsis* (Saga et al. 2012) was among those TF genes. Silencing *GmNAC42-1* in soybean  
323 hairy roots elicited with *P. sojae* WGE decreased the expressions of glyceollin gene transcripts  
324 and metabolites, demonstrating that it was essential for the elicitation of glyceollin biosynthesis  
325 (Jahan et al. 2019). The *GmNAC42-1* protein interacted with the promoters of two glyceollin  
326 biosynthesis genes, namely *ISOFLAVONE SYNTHASE 2 (IFS2)* and *GLYCINOL 4-*  
327 *DIMETHYLALLYLTRANSFERASE (G4DT)* in the yeast one hybrid system, suggesting that  
328 *GmNAC42-1* is a direct regulator of glyceollin biosynthesis. Overexpressing *GmNAC42-1*  
329 increased the expressions of glyceollin gene transcripts and metabolites in soybean hairy roots  
330 treated with WGE. However, overexpression was not sufficient to upregulate all glyceollin



331 biosynthesis genes in the absence of an elicitor. Thus *GmNAC42-1* was not sufficient on its  
332 own to activate the entire glyceollin biosynthesis pathway. Since *GmNAC42-1* was the soybean  
333 ortholog of *AtANAC042* this strongly challenged the concept that phytoalexin TFs are  
334 pathway/lineage-specific. The results also provide a major focal point to address the long-  
335 standing question of how plants have evolved lineage-specific biochemical defenses that are  
336 regulated by conserved signaling networks. Yet, it remains unknown exactly how  
337 ANAC042/*GmNAC42-1* TFs have evolved to activate different biosynthetic pathways.

338         In a second study aimed at identifying glyceollin TFs, we conducted transcriptomics on  
339 two soybean varieties responding to *P. sojae* WGE and identified two soybean R2R3-type  
340 MYB TFs that had contrasting roles in regulating glyceollin biosynthesis (Jahan et al. 2020).  
341 *GmMYB29A2* was essential for eliciting glyceollin biosynthesis upon WGE treatment.  
342 Silencing *GmMYB29A2* in hairy roots decreased the expressions of *GmNAC42-1*, glyceollin  
343 biosynthesis gene transcripts and metabolites, demonstrating that it was essential for elicitation.  
344 Overexpressing *GmMYB29A2* increased the levels of those gene expressions and metabolites  
345 in the presence of WGE, but it failed to fully activate all glyceollin biosynthesis genes in the  
346 absence of an elicitor treatment, similar to *GmNAC42-1* (Jahan et al. 2019). The *GmMYB29A2*  
347 protein interacted with the promoters of two glyceollin biosynthesis genes in yeast one-hybrid  
348 and electrophoretic mobility shift assays (EMSAs), demonstrating that *GmMYB29A2* was a  
349 direct regulator of glyceollin biosynthesis genes. By contrast, overexpressing the second R2R3-  
350 type MYB, namely *GmMYB29A1*, reduced glyceollin accumulation despite that it encoded a  
351 protein that has only 10 amino acid differences compared to *GmMYB29A2* (Jahan et al. 2020).  
352 Overexpressing *GmMYB29A1* had little effect on the expression of glyceollin biosynthesis  
353 genes, suggesting that it reduced glyceollin levels by activating glyceollin turnover and/or  
354 competing biosynthetic genes.

355 Again supporting the concept that there exists a conserved network of phytoalexin TFs,  
356 we found that *GmMYB29A2* is the soybean ortholog of the stilbene biosynthesis regulator from  
357 grapevine *VvMYB14* (Höll et al. 2013; Bai et al. 2019; Duan et al. 2016; Luo et al. 2020) Again,  
358 how evolution resulted in conserved TFs regulating different phytoalexin biosynthetic  
359 pathways remains unclear. Perhaps the most parsimonious explanation is that, under  
360 evolutionary pressure, these TFs co-opted different biosynthetic genes through the evolution  
361 of recognition elements in their promoters. **Fig. 2** demonstrates our very limited knowledge of  
362 the lineage-specific roles of the conserved ANAC042/GmNAC42-1 and  
363 GmMYB29A2/VvMYB14 networks.

#### 364 Recognition elements of phytoalexin transcription factors

365 The recognition elements of phytoalexins transcription factors could inform on how  
366 conserved phytoalexin TFs regulate diverse biosynthetic genes. The *cis*-elements of the WRKY  
367 DNA binding domain (Brand et al. 2013) have a consensus sequence of TTGACT/C that is  
368 called the W-box (Rushton et al. 1996). WRKY33 binds to W-box elements in the promoters  
369 of *1-AMINOCYCLOPROPANE-1-CARBOXYLIC ACID SYNTHASE 2 (ACS2)/ACS6* to  
370 activate ethylene biosynthesis in response to *B. cinerea* (Li et al. 2012). The *Zea mays* WRKY  
371 ZmWRKY79 directly binds two W-box elements in the promoter of *ANTHER EAR 2 (ZmAn2)*,  
372 a gene involved in the biosynthesis of kauralexins (Fu et al. 2017). WRKY62 and WRKY45  
373 from rice form homodimers and heterodimers and bind the W-box-like sequences TTGACC  
374 and ATGACT of diterpenoid phytoalexin biosynthetic genes and their regulator *DPF*  
375 (Fukushima et al. 2016). Also in rice, mutation analysis of the kaurene synthesis gene *OsKSL4*  
376 revealed a *cis*-acting TGACG motif that is needed for elicitation (Okada et al. 2009). Further,  
377 the basic leucine zipper (bZIP) TF *OsTGAPI* is responsible for the JA-dependent activation of  
378 *OsKSL4* possibly by binding to TGACGT sequences (Yoshida et al. 2017).

379           The N termini of NAC transcription factors have ~150 amino acids in their NAC  
380 domain with DNA binding abilities (Puranik et al. 2012). However, the recognition sequences  
381 of individual NACs are still not clear due to limited data (Lindemose et al. 2014). Many NACs  
382 bind the core CGT[G/A] sequence with differences in the flanking bases (Xu et al. 2013b). The  
383 CGT[G/A] flanking sequences may dictate the binding specificity of different NACs  
384 (Lindemose et al. 2014). In the absence of an elicitor, the camalexin TF ANAC042 [a.k.a.  
385 JUNGBRUNNEN1 (JUB1)] binds to the consensus sequence RRYGCCGT in the promoter of  
386 *DEHYDRATION-RESPONSIVE ELEMENT BINDING PROTEIN2A (DREB2A)*, which is an  
387 important positive regulator of dehydration responses (Wu et al. 2012). The fact that  
388 dehydration and camalexin biosynthesis are opposing responses (Mewis et al. 2012), similar to  
389 dehydration and glyceollin biosynthesis (Jahan et al. 2019), could suggest that the homologous  
390 TFs ANAC042 and GmNAC42-1 have distinct (possibly opposite) roles in the presence or  
391 absence of a pathogen elicitor. Thus, it should be investigated whether their recognition  
392 elements and mechanism of gene regulation differ in the presence and absence of elicitation.

393           The MYB TF VvMYB14 was demonstrated to bind the Box-L5 element  
394 GAGTTGGTGAGA to regulate an *STS* gene in yeast one-hybrid and promoter reporter assays  
395 (Fang et al. 2014). Whether VvMYB14 binds similar elements in other genes, in the presence of  
396 an elicitor, or whether its homolog GmMYB29A2 from soybean binds similar sequences in  
397 glyceollin genes remains to be determined.

#### 398 Signaling pathways for the elicitation of phytoalexin biosynthesis

399           While elicitation ultimately converges on the cis-regulatory elements of phytoalexin  
400 biosynthetic genes and the TFs that regulate them, upstream signaling pathways have critical  
401 roles in activating those direct regulatory processes, and have been more intensively studied.  
402 Currently the plant immune system is viewed to consist of two branches [reviewed by (Jones

403 and Dangl 2006)]. In the first branch, plant trans-membrane pattern recognition receptors  
404 (PRRs) recognize and trigger a response to highly conserved, slowly evolving, pathogen-  
405 associated molecular patterns (PAMPs). This activates PAMP-triggered immunity (PTI) that  
406 stimulates broadly the defense responses of plants with no pathogen-specific prioritization.  
407 PRRs include receptor-like proteins (RLPs) and receptor-like kinases (RLKs). Examples of  
408 PRRs that are known to signal phytoalexin biosynthesis include the lysine motif receptor  
409 kinases (LYKs) AtLYK4/AtLYK5/AtCERK1 from Arabidopsis (Cao et al. 2014),  
410 FLAGELLIN SENSING 2 (FLS2) from Arabidopsis (Felix et al. 1999), EF-Tu receptor (EFR)  
411 from Arabidopsis (Kunze et al. 2004), and  $\beta$ -glucan-binding protein (GBP) from soybean  
412 (Fliegmann et al. 2004). Their corresponding PAMPs are chitin from fungi, the N-terminal  
413 portion of bacterial flagellin (flg22), elongation factor TU (EF-Tu) from bacteria, and WGE  
414 from oomycetes, respectively. Despite that PAMPs are highly conserved, a recent study has  
415 demonstrated that different plant species recognize different PAMPs, the example being long  
416 and short  $\beta$ -1,3-linked glucans (Wanke et al. 2020), raising the possibility that different PRR  
417 variants exist among plant species.

418         The second branch, known as effector triggered immunity (ETI), is triggered by effector  
419 proteins that are specific to certain species or races of a pathogen. ETI acts on PTI to prioritize  
420 (*i.e.* accelerate/increase in amplitude) particular defense responses over others. Effector  
421 proteins are numerous and encoded by variable, high-copy number genes. Effector proteins  
422 have generally evolved to suppress PTI, establishing effector triggered susceptibility (ETS).  
423 Plant resistance (R) genes have evolved to recognize specific effector proteins and prevent  
424 ETS. R genes commonly encode nucleotide binding-leucine rich repeat (NB-LRR) proteins  
425 (Gao et al. 2005). NB-LRR proteins function to block effector activities such as binding PRRs  
426 (the Guard Hypothesis) (Dangl and Jones 2001). R genes are numerous in plant genomes and  
427 their encoded proteins recognize effector proteins with gene-for-gene specificity (Flor 1942).

428 Yet, pathogen recognition generally involves the stimulation of a complex web of partially  
429 overlapping signaling networks stimulated by PAMPs and ETIs (Wu et al. 2018).

430 Following the perception of a biotic elicitor (*i.e.* a PAMP or effector) by plant cell  
431 receptor proteins, sequential events that typically follow include reversible phosphorylation  
432 and dephosphorylation of plasma membrane and cytosolic proteins, spiking of Ca<sup>2+</sup> and proton  
433 levels in the cytosol, mitogen-activated protein kinase (MAPK) activation, reactive oxygen  
434 species (ROS) production, early defense gene expression, ethylene/JA, oxylipin, or salicylic  
435 acid (SA) biosynthesis and signaling, and finally the activation or expression of TFs for defense  
436 gene expressions such as those for the biosynthesis of phytoalexins (Zhao et al. 2005).

437 Specifically which hormone signaling pathways control the elicitation of phytoalexins  
438 may differ depending on the combination of PAMPs and effectors presented by the pathogen.  
439 For example, camalexin biosynthesis was elicited by either SA-dependent or -independent  
440 pathways upon recognition of *Pseudomonas syringae*, *Peronospora parasitica*, and  
441 *Phytophthora porri* (Denby et al. 2005; Nawrath and Metraux 1999; Roetschi et al. 2001). SA  
442 and JA typically act antagonistically in plant defense signaling (Ahuja et al. 2012; Takahashi  
443 et al. 2004), yet JA also had a role in inducing camalexin in response to some pathogens. For  
444 example, the Arabidopsis JA biosynthesis mutant *aos* elicited only 14% of wild-type levels of  
445 camalexin in response to *Botrytis cinerea* (Rowe et al. 2010). By contrast the JA signaling  
446 mutant *coi1* did not exhibit a reduction in camalexin biosynthesis after infection with  
447 *Alternaria brassicicola* (Thomma et al. 1999). JA belongs to the oxylipin family of  
448 biomolecules. Treatment of soybean cell cultures with a biosynthetic precursor of JA, namely  
449 12-oxo phytodienoic acid (OPDA), dramatically induced the accumulation of glyceollins,  
450 whereas JA and methyl jasmonate (MeJA) did not (Fliegmann et al. 2003). Cytosolic Ca<sup>2+</sup>  
451 signaling was also found to have a role in activating glyceollin biosynthesis in response to *P.*  
452 *sojae* WGE (Ebel et al. 1995; Fliegmann et al. 2003; Stäb and Ebel 1987). Ca<sup>2+</sup> signaling and

453 the calcium-dependent protein kinase CPK5 function upstream of SA biosynthesis and  
454 signaling in the Arabidopsis response to *Pseudomonas syringae* (Guerra et al. 2020).

455         Phytoalexins are often a major output of R gene signaling. To begin to understand the  
456 signaling responses mediated by the *RESPONSE TO PHYTOPHTHORA SOJAE* (*Rps*) genes  
457 of soybean, Lin and colleagues conducted comparative transcriptomic analyses on 10 nearly-  
458 isogenic soybean lines (NILS) that were incompatible with race 1 *P. sojae* (Lin et al. 2014). A  
459 comparison of the 10 incompatible NILs identified 369 and 770 genes that were collectively  
460 up- and down-regulated compared to the susceptible variety Williams., respectively. All  
461 resistant lines shared the upregulation of the glyceollin biosynthesis gene *PAL*, and 13 other  
462 genes from ROS, SA, brassinosteroid (BR), ethylene and MAPK pathways.

463         Overall, a systems-level study of the responses of a wide range of plant species to  
464 PAMP/effector combinations is needed to understand how these hormone signaling pathways  
465 function to effect phytoalexin elicitation. Since PRRs and NB-LRRs directly affect the  
466 phosphorylation of proteins and kinase signaling is broadly involved in phytoalexin signaling,  
467 it is likely that hormones affect kinase signaling.

#### 468 Kinase signaling of phytoalexin biosynthesis

469         The elicitation of camalexin biosynthesis by ANAC042 in Arabidopsis is suppressed  
470 by K252a, which is a potent inhibitor of Ca<sup>2+</sup>/calmodulin-dependent protein kinases and  
471 serine/threonine protein kinases (Saga et al. 2012). However, most research on the involvement  
472 of kinases in signaling phytoalexin biosynthesis comes from studies of mitogen-activated  
473 protein kinases (MAPKs) in Arabidopsis. MAPK signaling is universal in eukaryotes. Upon  
474 recognition of a PAMP by PRRs, the signal is thought to be transmitted through sequential  
475 phosphorylations of MAPKKKs, MAPKKs, and then MAPKs. MAPKKKs phosphorylate the  
476 two serine/threonine residues that are in the conserved S/T-X33-5-S/T motif of specific

477 MAPKKs, which then phosphorylate the conserved tyrosine (Y) and threonine (T) residues of  
478 MAPKs (Huang et al. 2011; Zaïdi et al. 2010). The activated MAPKs then phosphorylate  
479 specific downstream proteins, such as TFs, to trigger cellular responses. In *Arabidopsis*,  
480 camalexin biosynthesis is regulated by the *AtMKK4/AtMKK5-AtMPK3/AtMPK6* cascade  
481 (Kishi-Kaboshi et al. 2010). In the absence of a pathogen, the activation of MPK3/MPK6 by  
482 MAPKK or MAPKKK is adequate to stimulate some camalexin biosynthesis (Ren et al. 2008).  
483 The activation of MPK3/MPK6 upregulates multiple tryptophan (Trp) and camalexin  
484 biosynthesis genes, such as *PAD3* (Ren et al. 2008). A careful molecular analysis demonstrated  
485 that MPK4 phosphorylates a nuclear localized protein complex of MKS1 and the camalexin TF  
486 WRKY33, releasing WRKY33 to bind the promoter of *PAD3* (Qiu et al. 2008). Further,  
487 WRKY33's transcription is regulated by the MPK3/MPK6 cascade and its phosphorylation by  
488 MPK3/MPK6 is required for full activity (Mao et al., 2011). These kinase signaling pathways  
489 leading to the activation of camalexin biosynthesis are illustrated in Fig. 3.

#### 490 Molecular mechanisms that limit phytoalexin accumulation by pathogens

491 In the final sections of this review, we focus on factors that limit or reduce phytoalexin  
492 accumulation. Prior to discussing mechanisms that exist in plant cells, we describe mechanisms  
493 used by microbes since 1) they are relevant to crop improvement for agriculture, and 2)  
494 potentially similar mechanisms could exist in plants. Phytoalexins are generally broadly toxic  
495 to microbial pathogens. Yet, some pathogens have evolved enzymes to catabolize, transform,  
496 or suppress phytoalexin synthesis to overcome their toxicity. The number of genes that are  
497 encoded by a pathogen to reduce the toxicity of any one phytoalexin remains unknown.  
498 Recently, using a transcriptomics approach, Wang and colleagues found that 187 genes were  
499 differentially expressed (DEGs) in *Bursaphelenchus xylophilus* in response to the terpenoid  
500 phytoalexin carvone (Wang et al. 2019). By RNAi silencing of a cathepsin protease gene, they

501 demonstrated that it was required for virulence. This could suggest that the degradation of  
502 phytoalexin biosynthesis or signaling proteins may be a mechanism used by some pathogens  
503 to reduce toxicity.

504 Several fungi detoxify phytoalexins directly using enzymes that have catabolic  
505 activities (Zeilinger et al. 2015). For instance, *A. brassicicola* catabolizes brassinin using the  
506 enzyme brassinin hydrolase (Pedras et al. 2011). Fungal catabolism of phytoalexins can involve  
507 oxidation (brassinins, a dithiocarbamate detoxified by *Leptosphaeria* sp), or reduction of the  
508 oxoindole ring double bonds (wasalexin A by detoxified by *L. maculans* (Pedras and Abdoli  
509 2017). Resveratrol from grapevine has antifungal activities against *Rhizopus stolonifer*  
510 *Plasmopara viticola*, and *B. cinerea* (Adrian et al. 1997). However, some races of *B. cinerea*  
511 can catabolize stilbenes by oxidation (Breuil et al. 1998; Bavaresco et al. 1997; Sbaghi et al.  
512 1996). Sakuranetin is catabolized into sternbin and naringenin by 3'-hydroxylation and 7-O-  
513 demethylation activities encoded by *Pyricularia oryzae* (Katsumata et al. 2017). Similarly, the  
514 fungus *Fusarium solani* encodes the enzyme kievitone hydratase that can efficiently catabolize  
515 kievitone into a less toxic product (Li et al. 1995).

516 In addition to degradation, pathogens also biotransform phytoalexin molecules. The  
517 cruciferous phytoalexin rapalexin A undergoes the addition of a thiol group by *Colletotrichum*  
518 *higginsianum* and *Colletotrichum dematium* (Pedras and Thapa 2020) . Notably, this is the same  
519 metabolic transformation that is catalyzed by insects and mammals (Pedras and Thapa 2020).  
520 Camalexin can be bio-transformed by *Rhizoctonia solani* Kuhn to 5-hydroxycamalexin which  
521 is further hydroxylated into more polar metabolites that are less toxic (Pedras and Khan 2000).  
522 *P. sojae* was shown to lack the ability to biotransform glyceollins, as some other microbes  
523 could, which may be the reason why glyceollins are toxic to that pathogen (Lygin et al. 2013).  
524 *L. maculans* can biotransform a number of derivatives of camalexin, brassinin, and other  
525 phytoalexins, but it could not metabolize specifically camalexin and rapalexin, which were



526 toxic to the pathogen. Similarly, camalexin could not be detoxified by *A. brassicicola* (Pedras  
527 and Abdoli 2017). Thus, introducing new phytoalexins into plants could help broaden their  
528 pathogen resistance.

529 The successful biotransformation of benzoxazolinone by *Fusarium*  
530 *pseudograminearum* correlates with infection in wheat (Kettle et al. 2015). In maize, the  
531 biotransformation of benzoxazolinone by *Fusarium verticillioides* is facilitated by an enzyme  
532 called *N*-malonyltransferase, encoded by the gene *FUSARIUM DETOXIFICATION OF*  
533 *BENZOAZOLINONE 2 (FDB2)* (Kettle et al. 2015). Exogenous application of  
534 benzoxazolinone induced the expression of a nine gene cluster in *F. pseudograminearum*.  
535 Transgenic approaches including knocking out *FDB2* and gene complementation of mutants  
536 with homologous *FDB2* genes from *F. verticillioides* and *F. graminearum* showed that *N*-  
537 malonyltransferase is essential for benzoxazolinone detoxification (Kettle et al. 2015). The *N*-  
538 malonyltransferase enzyme from *F. verticillioides* also transforms 6-methoxy-benzoxazolin-2-  
539 one (MBOA) and benzoxazolin-2-one (BOA) (Glenn and Bacon 2009). These results raise the  
540 possibility that at least some pathogens have evolved promiscuous enzymes for the  
541 detoxification of *classes* of phytoalexins. *Sclerotinia sclerotiorum* (Lib.) encodes an inducible  
542 *N*-glucosyltransferase that converts brassinin to 1-b-D-glucopyranosylbrassinin (Pedras et al.  
543 2003). Similarly, virulent isolates of *Leptosphaeria maculans* transform brassinin into 3-  
544 indolecarboxaldehyde, however the enzyme remains unidentified (Pedras et al. 2003).

545 Interestingly, some phytoalexins can induce the detoxification of others. Camalexin  
546 induced BRASSININ GLUCOSYLTRANSFERASE (BGT) expression in *S. sclerotiorum*  
547 which then glucosylated brassinin and possibly camalexin (Pedras and Ahiahonu 2002).  
548 Similarly, the rate of brassinin detoxification in *L. maculans* cultures increased significantly  
549 with the addition of camalexin, whereas spirombrassinin remained unaffected (Pedras and  
550 Ahiahonu 2005). Since the presence of one phytoalexin may induce the detoxification of

551 another structurally similar molecule, engineering plants to biosynthesize a diversified  
552 repertoire of phytoalexin molecules could come at a cost in some instances.

### 553 Molecular mechanisms of plant cells that limit phytoalexin accumulation

554 In addition to signaling pathways that stimulate phytoalexin biosynthesis, there are also  
555 mechanisms in plant cells that limit the accumulation of those molecules. These include the  
556 inhibition of biosynthesis, metabolite sensing, conversion, and degradation. Phytoalexins are  
557 biosynthesized in relatively low amounts and only transiently upon elicitation. It remains  
558 unclear whether this is to limit any potential self-toxicity or to ensure sufficient cellular energy  
559 for other processes of the defense response program. In soybean, glyceollin degradation is  
560 constitutive. Supplying non-elicited seed tissues with glyceollins resulted in their rapid  
561 degradation (Farrell et al. 2017). Further, co-treatment with the heavy metal elicitor silver  
562 nitrate slowed the rate of glyceollin degradation and stimulated the hydrolysis of 6''-*O*-  
563 malonyldaidzin to daidzein, which is a biosynthetic intermediate of glyceollins. The putative  
564 enzyme or process responsible for catabolizing glyceollins remains unknown. Interestingly, the  
565 paralogue of the glyceollin activator *GmMYB29A2*, namely *GmMYB29A1*, reduced glyceollin  
566 metabolite accumulation when overexpressed in WGE-elicited soybean roots without affecting  
567 the transcript levels of most glyceollin biosynthesis genes (Jahan et al. 2020). Previous pulse-  
568 chase experiments using biosynthetic intermediates demonstrated that elicitation by *P. sojae*  
569 enhanced not only glyceollin I biosynthesis but also its turnover (Bhattacharyya and Ward  
570 1987). Thus, knocking out the enzyme or molecular process regulated by *GmMYB29A1* could  
571 be effective strategies for enhancing glyceollin accumulation.

572 A TF has been identified that limits phytoalexin biosynthesis. Both stable and transient  
573 overexpression of *VvWRKY8* in grapevine reduced expression levels of *VvMYB14*, the *STS*  
574 genes *VvSTS15/21*, and resveratrol metabolite levels (Jiang et al. 2018). *VvWRKY8* was found

575 to not bind the promoters of *VvMYB14* and *VvSTS15/21* by Y1H assays. Yet, using yeast two  
576 hybrid (Y2H), *VvWRKY8* was shown to physically interact with the *STS* regulator *VvMYB14*  
577 through their N-terminal domains. This interaction putatively blocks *VvMYB14* from binding  
578 the *VvSTS15/21* promoters to prevent the activation of resveratrol biosynthesis (Jiang et al.  
579 2018). In contrast, the WRKY TF *AsWRKY44* from the agarwood tree (*Aquilaria sinensis*) is  
580 released from the promoter of the *AGARWOOD SESQUITERPENE SYNTHASE 1 (ASS1)* gene  
581 in response to wounding or JA treatment (Sun et al. 2020), raising the possibility of similar  
582 regulation mechanisms for phytoalexins.

583         In addition to the degradation of phytoalexin molecules and the inhibition of their  
584 biosynthesis, metabolite sensing and subsequent signaling mechanisms have been shown to  
585 limit phytoalexin biosynthesis. The membrane associated signaling protein  
586 PHOSPHOLIPASE A2 (PLA2) is involved in initiating benzophenanthridin and monoterpene  
587 indole alkaloid phytoalexin biosynthesis in *Eschscholzia californica* and *Catharanthus roseus*,  
588 respectively (Heinze et al. 2015). However, each alkaloid inhibited the activity of their  
589 respective PLA2 only. 3D homology modeling predicted that the binding sites of each PLA2  
590 accommodated specifically the host alkaloid. It remains to be determined whether the binding  
591 pockets can be removed or whether introducing a PLA2 gene from one plant species into  
592 another can effectively remove this feedback inhibition mechanism.

593 ABA is a negative regulator of phytoalexins

594         The effects of plant hormones on the elicitation of phytoalexins are briefly described in  
595 the section entitled *Signaling pathways for the elicitation of phytoalexin biosynthesis* and have  
596 been reviewed in detail elsewhere (Erb et al. 2012; Mauch-Mani and Mauch 2005). However,  
597 abscisic acid (ABA) may warrant additional coverage due to relatively recent advances in  
598 understanding its role as a potent negative regulator of phytoalexin biosynthesis. ABA is a

599 plant hormone that is well known for its prominent role in signaling particular abiotic stresses,  
600 but comparatively little is known about its role in pathogen signaling (Mauch-Mani and Mauch  
601 2005). Early studies led to the speculation that ABA may inhibit phytoalexin responses since  
602 abiotic stresses that stimulate ABA biosynthesis strongly correlated with enhanced  
603 susceptibility. For example drought stress in *Arabidopsis* and cold stress in rice resulted in  
604 compatibility with *P. syringae* (Mohr and Cahill 2003) and *Magnaporthe grisea* (Koga et al.  
605 2004), respectively. Similarly, ABA signaling was shown to suppress the biosynthesis  
606 kievitone in common bean (Goossens and Vendrig 1982), and the rishitin in potato, causing  
607 compatibility with *Phytophthora infestans* (Henfling et al. 1980). More recently, ABA  
608 biosynthetic mutants exhibited enhanced resistance to bacterial and fungal pathogens  
609 (Asselbergh et al. 2007; de Torres Zabala et al. 2009), whereas ABA biosynthesis gene  
610 overexpressors had enhanced susceptibility (Fan et al. 2009). Further, effectors secreted by *P.*  
611 *syringae* stimulated the expression of ABA biosynthesis and signaling genes, rendering  
612 *Arabidopsis* compatible (de Torres-Zabala et al. 2007).

613 ABA signaling during pathogen interactions was often found to be antagonistic to  
614 ethylene, salicylic acid, and/or jasmonic acid pathways that induce plant defense gene  
615 expressions. This has been reported for tomato (Audenaert et al. 2002; Thaler and Bostock  
616 2004), *Arabidopsis* (de Torres-Zabala et al. 2007; Zabala et al. 2009; Anderson et al. 2004;  
617 Hillwig et al. 2016; Mohr and Cahill 2003; Kerchev et al. 2013), rice (Xu et al. 2013a; Jiang et  
618 al. 2010; Nahar et al. 2012) and tobacco (Kusajima et al. 2010). Transient increases in ABA  
619 were observed to accumulate at infection sites of compatible, but not incompatible interactions,  
620 in potato, sugar beet, and soybean (Cahill and Ward 1989; Henfling et al. 1980; Schmidt et al.  
621 2008). In contrast, ABA-deficient mutants of tobacco exhibited increases in the levels of  
622 capsidiol compared to wild-type plants when elicited with *B. cinerea* (Mialoundama et al.  
623 2009). Also, expressions of the ABA degradation gene *ABA 8'-HYDROXYLASE* increased with

624 capsidiol biosynthesis, suggesting that ABA degradation was a component of the capsidiol  
625 elicitation mechanism (Mialoundama et al. 2009).

626         During the interaction of incompatible soybean variety Harosoy 63 with race 1 *P. sojae*,  
627 ABA concentrations were rapidly reduced beginning 4 h after inoculation, whereas ABA  
628 concentrations were transiently increased at this time during a compatible interaction (Cahill  
629 and Ward 1989). Treatment with the ABA biosynthesis inhibitor norflurazon rendered the  
630 compatible genotype Harosoy incompatible and conversely, treatment of resistant genotype  
631 Harosoy 63 with ABA rendered it susceptible (Mohr and Cahill 2001; Ward et al. 1989;  
632 McDonald and Cahill 1999). ABA treatment was found to inhibit the expression of  
633 *PHENYLALANINE AMMONIA-LYASE (PAL)* gene that typically occurred early during an  
634 incompatible interaction (Ward et al. 1989). PAL isogenes are required for glyceollin and cell  
635 wall lignin biosynthesis. It was Mohr and Cahill that discovered that treating soybean with  
636 ABA affected specifically glyceollin biosynthesis and had no effect on lignin biosynthesis or  
637 HR during compatible interactions (Mohr and Cahill 2001).

638         Despite that ABA is an important negative regulator of phytoalexin biosynthesis in a  
639 broad range of plant species, its mechanisms of inhibiting phytoalexin signaling remain  
640 unclear. A recent study demonstrated that ABA induces the expression of the protein  
641 phosphatases 2Cs (PP2Cs), HAI1 , HAI2 , and HAI3, which directly dephosphorylate MPK3  
642 and MPK6 (Mine et al. 2017). As described in the section entitled *Kinase signaling of*  
643 *phytoalexin biosynthesis*, the phosphorylation of the TF WRKY33 by MPK3/MPK6 is required  
644 for WRKY33 to fully activate camalexin biosynthesis (Mao et al. 2011). Mine and colleagues  
645 also demonstrated that *P. syringae* induces the expression of *HAI1* through a coronatine (COR)-  
646 mediated pathway to promote compatibility with *P. syringae* (Mine et al. 2017). Thus, PP2C-  
647 mediated dephosphorylation of MPK3/MPK6 may be a mechanism by which ABA signaling  
648 is used by plant and exploited by pathogens to reduce phytoalexin biosynthesis (Fig. 3).

## 649 Conclusions

650 Phytoalexins have important roles in mediating the protection of crops against  
651 economically devastating pathogens in agriculture. Further, with increased accessibility, their  
652 potent medicinal activities could render them important pharmaceuticals for the treatment and  
653 prevention of debilitating diseases. However, more research is required to assess the  
654 bioactivities of phytoalexins and understand how they can be administered or chemically  
655 modified for particular clinical treatments. Some phytoalexins are not economical to synthesize  
656 using chemistry methods, making their biosynthesis in plants a major source for improving  
657 their accessibility. This has led to diverse efforts in understanding how to enhance their  
658 biosynthesis in plants. Recent studies have provided clues as to the existence of a conserved  
659 TF network that directly regulates diverse phytoalexin biosynthesis pathways in different plant  
660 species. Bioengineering this TF network could be the key to 'unlocking' plant metabolism to  
661 biosynthesize massive amounts of phytoalexin molecules. Recent evidence suggests that it  
662 could also be manipulated to engineer resistance to pathogens. A systems level re-engineering  
663 of plant cells, including upregulating key TFs, removing amino acid residues from feedback  
664 signaling proteins, and knocking out negative regulators could be achieved using recently  
665 discovered efficient plant transformation methods. A systems level understanding of  
666 phytoalexin gene regulation should be a major goal of modern plant science since it could lead  
667 to economical sources of pharmaceuticals and improved food security.

## 668 **Figure Captions**

669 **Fig. 1** Phytoalexins with well supported roles in mediating pathogen resistance in plants.

670 **Fig. 2** Conserved transcription factors regulate diverse phytoalexin biosynthesis pathways. The  
671 grapevine (TF) VvMYB14 and its soybean ortholog GmMYB29A2 directly regulate stilbene-  
672 and glyceollin-specific genes, respectively. The Arabidopsis TF ANAC042 and its soybean

673 ortholog GmNAC42-1 regulate camalexin and glyceollin biosynthesis pathways, respectively.  
674 Blue arrows indicate direct binding of a TF to the promoter of a biosynthesis gene. Most  
675 biosynthesis gene promoters have not been tested for binding by their putative TFs. ANAC042  
676 positively regulates camalexin biosynthesis; however it is not known whether it directly binds  
677 biosynthesis gene promoters. Solid and broken arrows indicate single and multiple enzymatic  
678 steps, respectively. CHR, chalcone reductase; CHI, chalcone isomerase; IFS, isoflavone  
679 synthase; G2'DT, genistein 2'-dimethylallyl transferase; I2'H, isoflavone 2'-hydroxylase;  
680 G4DT, glycinol 4-dimethylallyl transferase; G2DT, glycinol 2-dimethylallyl transferase; GLS,  
681 glyceollin synthase; TAL, tyrosine ammonia lyase; STS, stilbene synthase; ROMT, resveratrol  
682 O-methyltransferase; OMT, O-methyltransferase; CYP, cytochrome P450; PAD3, phytoalexin  
683 deficient 3 (a.k.a. CYP71B15).

684 **Fig. 3** Phytoalexin signaling networks in Arabidopsis. Signaling cascades that elicit camalexin  
685 biosynthesis begin with the recognition of pathogen-associated molecular patterns (PAMPs)  
686 and effectors by plant pattern recognition receptors (PRRs) and resistance (R) proteins,  
687 respectively. These initiate PAMP-triggered immunity (PTI) and effector-triggered immunity  
688 (ETI), respectively. Pathogens deliver effectors into the plant cell to suppress plant immunity,  
689 some plant genotypes encode the corresponding resistance (R) protein that recognize effectors,  
690 providing race-specific resistance. Among the earliest signaling events of PTI and ETI is  
691 mitogen-activated protein kinase (MAPK) cascades. MAPKs stimulate the activation of  
692 transcription of camalexin biosynthesis genes through the phosphorylation of WKRY33,  
693 complexed proteins (e.g. MKS1), and potentially other transcription factors (TFs). A strategy  
694 to enhance phytoalexin biosynthesis in plants includes overexpressing TFs, or phospho-  
695 mimicking mutants of TFs, to circumvent the MAPK cascade to partially (prime) or fully  
696 activate a phytoalexin biosynthesis pathway. The MYB orthologs GmMYB29A2 and  
697 VvMYB14 have conserved roles in activating glyceollin and stilbene biosynthesis pathways in

698 soybean and grapevine, respectively. Red arrows indicate phosphorylation steps, blue arrow  
699 dephosphorylation and dotted arrows indicate multiple steps. Question marks indicate  
700 unidentified signaling components. This figure was adapted from (Meng and Zhang 2013).

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## 702 References

- 703 Adrian M, Jeandet P, Veneau J, Weston LA, Bessis R (1997) Biological activity of resveratrol, a stilbenic  
704 compound from grapevines, against *Botrytis cinerea*, the causal agent for gray mold. *Journal*  
705 *of Chemical Ecology* 23 (7):1689-1702
- 706 Ahuja I, Kissen R, Bones AM (2012) Phytoalexins in defense against pathogens. *Trends in plant science*  
707 17 (2):73-90
- 708 Aisyah S, Gruppen H, Madzora B, Vincken JP (2013) Modulation of isoflavonoid composition of  
709 *Rhizopus oryzae* elicited soybean (*Glycine max*) seedlings by light and wounding. *Journal of*  
710 *agricultural and food chemistry* 61 (36):8657-8667. doi:10.1021/jf4020203
- 711 Amorim A, Lidiane L, da Fonseca dos Santos R, Pacifico Bezerra Neto J, Guida-Santos M, Crovella S,  
712 Maria Benko-Iseppon A (2017) Transcription factors involved in plant resistance to pathogens.  
713 *Current Protein and Peptide Science* 18 (4):335-351
- 714 Anderson JP, Badruzsafari E, Schenk PM, Manners JM, Desmond OJ, Ehlert C, Maclean DJ, Ebert PR,  
715 Kazan K (2004) Antagonistic interaction between abscisic acid and jasmonate-ethylene  
716 signaling pathways modulates defense gene expression and disease resistance in *Arabidopsis*.  
717 *Plant Cell* 16 (12):3460-3479. doi:10.1105/tpc.104.025833
- 718 Asselbergh B, Curvers K, França SC, Audenaert K, Vuylsteke M, Van Breusegem F, Höfte M (2007)  
719 Resistance to *Botrytis cinerea* in *sitiens*, an abscisic acid-deficient tomato mutant, involves



720 timely production of hydrogen peroxide and cell wall modifications in the epidermis. *Plant*  
721 *physiology* 144 (4):1863-1877

722 Audenaert K, De Meyer GB, Hofte MM (2002) Abscisic acid determines basal susceptibility of tomato  
723 to *Botrytis cinerea* and suppresses salicylic acid-dependent signaling mechanisms. *Plant*  
724 *Physiology* 128 (2):491-501. doi:10.1104/pp.010605

725 Bai R, Luo Y, Wang L, Li J, Wu K, Zhao G, Duan D (2019) A specific allele of MYB14 in grapevine  
726 correlates with high stilbene inducibility triggered by Al<sup>3+</sup> and UV-C radiation. *Plant cell*  
727 *reports* 38 (1):37-49

728 Bamji SF, Corbitt C (2017) Glyceollins: Soybean phytoalexins that exhibit a wide range of health-  
729 promoting effects. *Journal of Functional Foods* 34:98-105

730 Bavaresco L, Petegolli D, Canto E, Fregoni M, Chiusa G, Trevisan M (1997) Elicitation and accumulation  
731 of stilbene phytoalexins in grapevine berries infected by *Botrytis cinerea*. *VITIS-*  
732 *GEILWEILERHOF-* 36:77-84

733 Bhattacharyya M, Ward E (1985) Differential sensitivity of *Phytophthora megasperma* f. sp. *glycinea*  
734 isolates to glyceollin isomers. *Physiological Plant Pathology* 27 (3):299-310

735 Bhattacharyya M, Ward E (1986) Resistance, susceptibility and accumulation of glyceollins I–III in  
736 soybean organs inoculated with *Phytophthora megasperma* f. sp. *glycinea*. *Physiological and*  
737 *molecular plant pathology* 29 (2):227-237

738 Bhattacharyya M, Ward E (1987) Biosynthesis and metabolism of glyceollin I in soybean hypocotyls  
739 following wounding or inoculation with *Phytophthora megasperma* f. sp. *glycinea*.  
740 *Physiological and molecular plant pathology* 31 (3):387-405

741 Boue SM, Carter CH, Ehrlich KC, Cleveland TE (2000) Induction of the soybean phytoalexins coumestrol  
742 and glyceollin by *Aspergillus*. *Journal of agricultural and food chemistry* 48 (6):2167-2172

743 Brand LH, Fischer NM, Harter K, Kohlbacher O, Wanke D (2013) Elucidating the evolutionary conserved  
744 DNA-binding specificities of WRKY transcription factors by molecular dynamics and in vitro  
745 binding assays. *Nucleic acids research* 41 (21):9764-9778

746 Breuil A-C, Adrian M, Pirio N, Meunier P, Bessis R, Jeandet P (1998) Metabolism of stilbene  
747 phytoalexins by *Botrytis cinerea*: 1. Characterization of a resveratrol dehydrodimer.  
748 *Tetrahedron Letters* 39 (7):537-540

749 Budovská M, Selešová I, Tischlerová V, Michalková R, Mojžiš J (2020) Design, synthesis and biological  
750 evaluation of novel 5-bromo derivatives of indole phytoalexins. *Beilstein Archives* 2020 (1):23

751 Cahill D, Ward E (1989) Rapid localized changes in abscisic acid concentrations in soybean in  
752 interactions with *Phytophthora megasperma* f. sp. *glycinea* or after treatment with elicitors.  
753 *Physiological and molecular plant pathology* 35 (6):483-493

754 Cao Y, Liang Y, Tanaka K, Nguyen CT, Jedrzejczak RP, Joachimiak A, Stacey G (2014) The kinase LYK5 is  
755 a major chitin receptor in *Arabidopsis* and forms a chitin-induced complex with related kinase  
756 CERK1. *Elife* 3:e03766

757 Carriere PP, Llopis SD, Naiki AC, Nguyen G, Phan T, Nguyen MM, Preyan LC, Yearby L, Pratt J, Burks H  
758 (2015) Glyceollin I reverses epithelial to mesenchymal transition in letrozole resistant breast  
759 cancer through ZEB1. *International journal of environmental research and public health* 13  
760 (1):10

761 Chang H-X, Hartman GL (2017) Characterization of insect resistance loci in the USDA soybean  
762 germplasm collection using genome-wide association studies. *Frontiers in plant science* 8:670

763 Cheng CK, Luo JY, Lau CW, Chen ZY, Tian XY, Huang Y (2019) Pharmacological basis and new insights  
764 of resveratrol action in the cardiovascular system. *British journal of pharmacology*

765 Cheng Q, Li N, Dong L, Zhang D, Fan S, Jiang L, Wang X, Xu P, Zhang S (2015) Overexpression of Soybean  
766 Isoflavone Reductase (GmIFR) Enhances Resistance to *Phytophthora sojae* in Soybean.  
767 *Frontiers in plant science* 6:1024. doi:10.3389/fpls.2015.01024

768 Cristina MS, Petersen M, Mundy J (2010) Mitogen-activated protein kinase signaling in plants. *Annual*  
769 *review of plant biology* 61:621-649

770 Dangl JL, Jones JD (2001) Plant pathogens and integrated defence responses to infection. *Nature* 411  
771 (6839):826-833. doi:10.1038/35081161

772 de Torres-Zabala M, Truman W, Bennett MH, Lafforgue G, Mansfield JW, Egea PR, Bogre L, Grant M  
773 (2007) *Pseudomonas syringae* pv. tomato hijacks the Arabidopsis abscisic acid signalling  
774 pathway to cause disease. *Embo J* 26 (5):1434-1443. doi:10.1038/sj.emboj.7601575

775 de Torres-Zabala M, Truman W, Bennett MH, Lafforgue G, Mansfield JW, Egea PR, Bögre L, Grant M  
776 (2007) *Pseudomonas syringae* pv. tomato hijacks the Arabidopsis abscisic acid signalling  
777 pathway to cause disease. *The EMBO Journal* 26 (5):1434-1443

778 de Torres Zabala M, Bennett MH, Truman WH, Grant MR (2009) Antagonism between salicylic and  
779 abscisic acid reflects early host–pathogen conflict and moulds plant defence responses. *The*  
780 *Plant Journal* 59 (3):375-386

781 Deavours BE, Dixon RA (2005) Metabolic engineering of isoflavonoid biosynthesis in alfalfa. *Plant*  
782 *Physiology* 138 (4):2245-2259

783 Demirer GS, Zhang H, Matos JL, Goh NS, Cunningham FJ, Sung Y, Chang R, Aditham AJ, Chio L, Cho M-  
784 J (2019) High aspect ratio nanomaterials enable delivery of functional genetic material without  
785 DNA integration in mature plants. *Nat Nanotechnol* 10

786 Denby KJ, Jason LJ, Murray SL, Last RL (2005) *ups1*, an Arabidopsis thaliana camalexin accumulation  
787 mutant defective in multiple defence signalling pathways. *The Plant Journal* 41 (5):673-684

788 Doyle C, Higginbottom K, Swift TA, Winfield M, Bellas C, Benito-Alifonso D, Fletcher T, Galan CM,  
789 Edwards K, Whitney HM (2019) A simple method for spray-on gene editing in planta.  
790 bioRxiv:805036

791 Duan D, Fischer S, Merz P, Bogs J, Riemann M, Nick P (2016) An ancestral allele of grapevine  
792 transcription factor MYB14 promotes plant defence. *Journal of experimental botany* 67  
793 (6):1795-1804

794 Ebel J, Feger M, Kissel U, Mithöfer A, Waldmüller T, Bhagwat AA, Cosio EG (1995) Elicitor-binding  
795 proteins and signal transduction in the activation of a phytoalexin defense response. *Canadian*  
796 *journal of botany* 73 (S1):506-510

797 Erb M, Meldau S, Howe GA (2012) Role of phytohormones in insect-specific plant reactions. *Trends in*  
798 *plant science* 17 (5):250-259

799 Fan J, Hill L, Crooks C, Doerner P, Lamb C (2009) Abscisic acid has a key role in modulating diverse  
800 plant-pathogen interactions. *Plant physiology* 150 (4):1750-1761

801 Fang L, Hou Y, Wang L, Xin H, Wang N, Li S (2014) Myb14, a direct activator of STS, is associated with  
802 resveratrol content variation in berry skin in two grape cultivars. *Plant cell reports* 33  
803 (10):1629-1640

804 Farrell KC, Jahan MA, Kovinich N (2017) Distinct Mechanisms of Biotic and Chemical Elicitors enable  
805 Additive Elicitation of the Anticancer Phytoalexin Glyceollin I. *Molecules* 22 (8):1261-1273

806 Felix G, Duran JD, Volko S, Boller T (1999) Plants have a sensitive perception system for the most  
807 conserved domain of bacterial flagellin. *The Plant Journal* 18 (3):265-276

808 Fischer DC, Kogan M, Paxton J (1990) Effect of glyceollin, a soybean phytoalexin, on feeding by three  
809 phytophagous beetles (Coleoptera: Coccinellidae and Chrysomelidae): dose versus response.  
810 *Environmental Entomology* 19 (5):1278-1282

811 Fliegmann J, Mithofer A, Wanner G, Ebel J (2004) An ancient enzyme domain hidden in the putative  
812 beta-glucan elicitor receptor of soybean may play an active part in the perception of  
813 pathogen-associated molecular patterns during broad host resistance. *J Biol Chem* 279  
814 (2):1132-1140. doi:10.1074/jbc.M308552200

815 Fliegmann J, SCHÜLER G, Boland W, Ebel J, Mithöfer A (2003) The role of octadecanoids and functional  
816 mimics in soybean defense responses. *Biological chemistry* 384 (3):437-446

817 Flor H (1942) Inheritance of pathogenicity in *Melampsora lini*. *Phytopathology* 32:653-669

818 Frerigmann H, Glawischnig E, Gigolashvili T (2015) The role of MYB34, MYB51 and MYB122 in the  
819 regulation of camalexin biosynthesis in Arabidopsis thaliana. *Frontiers in plant science* 6

820 Fu J, Liu Q, Wang C, Liang J, Liu L, Wang Q (2017) ZmWRKY79 positively regulates maize phytoalexin  
821 biosynthetic gene expression and is involved in stress response. *Journal of experimental*  
822 *botany* 69 (3):497-510

823 Fukushima S, Mori M, Sugano S, Takatsuji H (2016) Transcription factor WRKY62 plays a role in  
824 pathogen defense and hypoxia-responsive gene expression in rice. *Plant and Cell Physiology*  
825 57 (12):2541-2551

826 Gao H, Narayanan NN, Ellison L, Bhattacharyya MK (2005) Two classes of highly similar coiled coil-  
827 nucleotide binding-leucine rich repeat genes isolated from the Rps1-k locus encode  
828 *Phytophthora* resistance in soybean. *Mol Plant Microbe Interact* 18 (10):1035-1045.  
829 doi:10.1094/MPMI-18-1035

830 Gary S, Adegboye J, Popp B, Cocuron J-C, Woodrum B, Kovinich N (2018) Combining Semi-Synthesis  
831 with Plant and Microbial Biocatalysis: New Frontiers in Producing a Chemical Arsenal Against  
832 Cancer. *RSC Advances* 8 (38):21332-21339

833 Glenn A, Bacon C (2009) FDB2 encodes a member of the arylamine N-acetyltransferase family and is  
834 necessary for biotransformation of benzoxazolinones by *Fusarium verticillioides*. *Journal of*  
835 *applied microbiology* 107 (2):657-671

836 Gong C, Xia H (2020) Resveratrol suppresses melanoma growth by promoting autophagy through  
837 inhibiting the PI3K/AKT/mTOR signaling pathway. *Experimental and Therapeutic Medicine* 19  
838 (3):1878-1886

839 Goossens A, Mertens J, Pollier J, Bossche RV, López-Vidriero I, Franco-Zorrilla JM (2015) The bHLH  
840 transcription factors TSAR1 and TSAR2 regulate triterpene saponin biosynthesis in *Medicago*  
841 *truncatula*. *Plant physiology*:pp. 01645.02015

842 Goossens J, Vendrig J (1982) Effects of abscisic acid, cytokinins, and light on isoflavonoid phytoalexin  
843 accumulation in *Phaseolus vulgaris* L. *Planta* 154 (5):441-446

844 Graham T, Kim J, Graham M (1990) Role of constitutive isoflavone conjugates in the accumulation of  
845 glyceollin in soybean infected with *Phytophthora megasperma*. *Mol Plant Microbe Interact*  
846 3:157-166

847 Graham TL, Graham MY, Subramanian S, Yu O (2007) RNAi silencing of genes for elicitation or  
848 biosynthesis of 5-deoxyisoflavonoids suppresses race-specific resistance and hypersensitive  
849 cell death in *Phytophthora sojae* infected tissues. *Plant physiology* 144 (2):728-740

850 Großkinsky DK, van der Graaff E, Roitsch T (2012) Phytoalexin transgenics in crop protection—fairly  
851 tale with a happy end? *Plant science* 195:54-70

852 Guerra T, Schilling S, Hake K, Gorzolka K, Sylvester FP, Conrads B, Westermann B, Romeis T (2020)  
853 Calcium-dependent protein kinase 5 links calcium signaling with N-hydroxy-L-pipecolic acid-  
854 and SARD 1-dependent immune memory in systemic acquired resistance. *New Phytologist*  
855 225 (1):310-325

856 Hahn MG, Bonhoff A, Grisebach H (1985) Quantitative localization of the phytoalexin glyceollin I in  
857 relation to fungal hyphae in soybean roots infected with *Phytophthora megasperma* f. sp.  
858 *glycinea*. *Plant physiology* 77 (3):591-601

859 Hasegawa M, Mitsuhara I, Seo S, Imai T, Koga J, Okada K, Yamane H, Ohashi Y (2010) Phytoalexin  
860 accumulation in the interaction between rice and the blast fungus. *Mol Plant Microbe In* 23  
861 (8):1000-1011

862 He Y, Xu J, Wang X, He X, Wang Y, Zhou J, Zhang S, Meng X (2019) The *Arabidopsis* pleiotropic drug  
863 resistance transporters PEN3 and PDR12 mediate camalexin secretion for resistance to  
864 *Botrytis cinerea*. *The Plant Cell* 31 (9):2206-2222

865 Heinze M, Brandt W, Marillonnet S, Roos W (2015) “Self” and “non-self” in the control of phytoalexin  
866 biosynthesis: plant phospholipases A2 with alkaloid-specific molecular fingerprints. *The Plant*  
867 *Cell* 27 (2):448-462

868 Henfling J, Bostock R, Kuc J (1980) Effect of abscisic acid on rishitin and lubimin accumulation and  
869 resistance to *Phytophthora infestans* and *Cladosporium cucumerinum* in potato tuber tissue  
870 slices. *Phytopathology* 70 (11):1074-1078

871 Heo KT, Kang S-Y, Hong Y-S (2017) De novo biosynthesis of pterostilbene in an *Escherichia coli* strain  
872 using a new resveratrol O-methyltransferase from *Arabidopsis*. *Microbial cell factories* 16  
873 (1):30

874 Hillwig MS, Chiozza M, Casteel CL, Lau ST, Hohenstein J, Hernandez E, Jander G, MacIntosh GC (2016)  
875 Abscisic acid deficiency increases defence responses against *Myzus persicae* in *Arabidopsis*.  
876 *Molecular Plant Pathology* 17 (1):225-235. doi:10.1111/mpp.12274

877 Hohenstein JD, Studham ME, Klein A, Kovinich N, Barry K, Lee YJ, MacIntosh GC (2019) Transcriptional  
878 and chemical changes in soybean leaves in response to long-term Aphid colonization.  
879 *Frontiers in Plant Science* 10:310

880 Höll J, Vannozzi A, Czemplin S, D'Onofrio C, Walker AR, Rausch T, Lucchin M, Boss PK, Dry IB, Bogs J  
881 (2013) The R2R3-MYB transcription factors MYB14 and MYB15 regulate stilbene biosynthesis  
882 in *Vitis vinifera*. *The Plant Cell* 25 (10):4135-4149

883 Huang J-S, Barker KR (1991) Glyceollin I in soybean-cyst nematode interactions: spatial and temporal  
884 distribution in roots of resistant and susceptible soybeans. *Plant Physiology* 96 (4):1302-1307

885 Huang X-S, Luo T, Fu X-Z, Fan Q-J, Liu J-H (2011) Cloning and molecular characterization of a mitogen-  
886 activated protein kinase gene from *Poncirus trifoliata* whose ectopic expression confers  
887 dehydration/drought tolerance in transgenic tobacco. *Journal of experimental botany* 62  
888 (14):5191-5206

889 Huffaker A, Kaplan F, Vaughan MM, Dafoe NJ, Ni X, Rocca JR, Alborn HT, Teal PE, Schmelz EA (2011)  
890 Novel acidic sesquiterpenoids constitute a dominant class of pathogen-induced phytoalexins  
891 in maize. *Plant physiology* 156 (4):2082-2097

892 Ingham JL (1979) Phytoalexin production by flowers of garden pea (*Pisum sativum*). *Zeitschrift für*  
893 *Naturforschung C* 34 (3-4):296-298

894 Jahan MA, Harris B, Lowery M, Coburn K, Infante AM, Percifield RJ, Ammer AG, Kovinich N (2019) The  
895 NAC family transcription factor GmNAC42-1 regulates biosynthesis of the anticancer and  
896 neuroprotective glyceollins in soybean. *BMC genomics* 20 (1):149

897 Jahan MA, Harris B, Lowery M, Infante AM, Percifield RJ, Kovinich N (2020) Glyceollin Transcription  
898 Factor GmMYB29A2 Regulates Soybean Resistance to *Phytophthora sojae*. *Plant Physiology*  
899 183 (2). doi:DOI:10.1104/pp.19.01293

900 Jahan MA, Harris B, Lowery M, Infante AM, Percifield RJ, Kovinich N (Under Revision) Glyceollin  
901 Transcription Factor GmMYB29A2 is a Regulator of Soybean Resistance to *Phytophthora*  
902 *sojae*. *Plant physiology*

903 Jahan MA, Kovinich N (2019) Acidity stress for the systemic elicitation of glyceollin phytoalexins in  
904 soybean plants. *Plant Signaling & Behavior* (Accepted March 2019) *Plant signaling & behavior*

905 Jeandet P, Clément C, Cordelier S (2019a) Regulation of resveratrol biosynthesis in grapevine: new  
906 approaches for disease resistance? *Journal of experimental botany* 70 (2):375-378

907 Jeandet P, Clément C, Courot E, Cordelier S (2013) Modulation of phytoalexin biosynthesis in  
908 engineered plants for disease resistance. *International journal of molecular sciences* 14  
909 (7):14136-14170

910 Jeandet P, Delaunoy B, Conreux A, Donneux D, Nuzzo V, Cordelier S, Clément C, Courot E (2010)  
911 Biosynthesis, metabolism, molecular engineering, and biological functions of stilbene  
912 phytoalexins in plants. *Biofactors* 36 (5):331-341

913 Jeandet P, Hébrard C, Deville M-A, Cordelier S, Dorey S, Aziz A, Crouzet J (2014) Deciphering the role  
914 of phytoalexins in plant-microorganism interactions and human health. *Molecules* 19  
915 (11):18033-18056

916 Jeandet P, Sobarzo-Sánchez E, Silva AS, Clément C, Nabavi SF, Battino M, Rasekhian M, Belwal T,  
917 Habtemariam S, Koffas M (2019b) Whole-cell biocatalytic, enzymatic and green chemistry  
918 methods for the production of resveratrol and its derivatives. *Biotechnology advances*:107461

919 Jiang C-J, Shimono M, Sugano S, Kojima M, Yazawa K, Yoshida R, Inoue H, Hayashi N, Sakakibara H,  
920 Takatsuji H (2010) Abscisic Acid Interacts Antagonistically with Salicylic Acid Signaling Pathway

921 in Rice-Magnaporthe grisea Interaction. *Molecular Plant-Microbe Interactions* 23 (6):791-798.  
922 doi:10.1094/mpmi-23-6-0791

923 Jiang J, Ma S, Ye N, Jiang M, Cao J, Zhang J (2017) WRKY transcription factors in plant responses to  
924 stresses. *Journal of integrative plant biology* 59 (2):86-101

925 Jiang J, Xi H, Dai Z, Lecourieux F, Yuan L, Liu X, Patra B, Wei Y, Li S, Wang L (2018) VvWRKY8 represses  
926 stilbene synthase genes through direct interaction with VvMYB14 to control resveratrol  
927 biosynthesis in grapevine. *Journal of experimental botany* 70 (2):715-729

928 Jones JD, Dangl JL (2006) The plant immune system. *Nature* 444 (7117):323-329.  
929 doi:10.1038/nature05286

930 Katsumata S, Hamana K, Horie K, Toshima H, Hasegawa M (2017) Identification of sternbin and  
931 naringenin as detoxified metabolites from the rice flavanone phytoalexin sakuranetin by  
932 *Pyricularia oryzae*. *Chemistry & biodiversity* 14 (2):e1600240

933 Kerchev PI, Karpinska B, Morris JA, Hussain A, Verrall SR, Hedley PE, Fenton B, Foyer CH, Hancock RD  
934 (2013) Vitamin C and the Abscisic Acid-Insensitive 4 Transcription Factor Are Important  
935 Determinants of Aphid Resistance in Arabidopsis. *Antioxid Redox Signal* 18 (16):2091-2105.  
936 doi:10.1089/ars.2012.5097

937 Kettle AJ, Batley J, Benfield AH, Manners JM, Kazan K, Gardiner DM (2015) Degradation of the  
938 benzoxazolinone class of phytoalexins is important for virulence of *Fusarium*  
939 *pseudograminearum* towards wheat. *Molecular plant pathology* 16 (9):946-962

940 Khupse RS, Sarver JG, Trendel JA, Bearss NR, Reese MD, Wiese TE, Boue SM, Burow ME, Cleveland TE,  
941 Bhatnagar D, Erhardt PW (2011) Biomimetic syntheses and antiproliferative activities of  
942 racemic, natural (-), and unnatural (+) glyceollin I. *Journal of medicinal chemistry* 54  
943 (10):3506-3523. doi:10.1021/jm101619e

944 Kiselev KV (2011) Perspectives for production and application of resveratrol. *Applied microbiology and*  
945 *biotechnology* 90 (2):417-425

946 Kishi-Kaboshi M, Takahashi A, Hirochika H (2010) MAMP-responsive MAPK cascades regulate  
947 phytoalexin biosynthesis. *Plant signaling & behavior* 5 (12):1653-1656

948 Koga H, Dohi K, Mori M (2004) Abscisic acid and low temperatures suppress the whole plant-specific  
949 resistance reaction of rice plants to the infection of *Magnaporthe grisea*. *Physiological and*  
950 *molecular plant pathology* 65 (1):3-9

951 Koga J, Shimura M, Oshima K, Ogawa N, Yamauchi T, Ogasawara N (1995) Phytocassanes A, B, C and  
952 D, novel diterpene phytoalexins from rice, *Oryza sativa* L. *Tetrahedron* 51 (29):7907-7918

953 Kuc J (1995) Phytoalexins, stress metabolism, and disease resistance in plants. *Annual review of*  
954 *phytopathology* 33 (1):275-297

955 Kunze G, Zipfel C, Robatzek S, Niehaus K, Boller T, Felix G (2004) The N terminus of bacterial elongation  
956 factor Tu elicits innate immunity in Arabidopsis plants. *The Plant Cell* 16 (12):3496-3507

957 Kusajima M, Yasuda M, Kawashima A, Nojiri H, Yamane H, Nakajima M, Akutsu K, Nakashita H (2010)  
958 Suppressive effect of abscisic acid on systemic acquired resistance in tobacco plants. *Journal*  
959 *of General Plant Pathology* 76 (2):161-167. doi:10.1007/s10327-010-0218-5

960 Lamb CJ, Ryals JA, Ward ER, Dixon RA (1992) Emerging strategies for enhancing crop resistance to  
961 microbial pathogens. *Nature Biotechnology* 10 (11):1436-1445

962 Lee EJ, Jiménez Z, Seo K-H, Nam GB, Kang Y-G, Lee TR, Kim D, Yang DC (2020) Mass production of  
963 coumestrol from soybean (*Glycine max*) adventitious roots through bioreactor: effect on  
964 collagen production. *Plant Biotechnology Reports* 14 (1):99-110

965 Lee SH, Jee JG, Bae JS, Liu KH, Lee YM (2015) A Group of Novel HIF-1 $\alpha$  Inhibitors, Glyceollins, Blocks  
966 HIF-1 $\alpha$  Synthesis and Decreases Its Stability via Inhibition of the PI3K/AKT/mTOR Pathway and  
967 Hsp90 Binding. *Journal of cellular physiology* 230 (4):853-862

968 Li D, Chung K-R, Smith DA, Schardl CL (1995) The *Fusarium solani* gene encoding kievitone hydratase,  
969 a secreted enzyme that catalyzes detoxification of a bean phytoalexin. *MPMI-Molecular Plant*  
970 *Microbe Interactions* 8 (3):388-397

971 Li G, Meng X, Wang R, Mao G, Han L, Liu Y, Zhang S (2012) Dual-level regulation of ACC synthase activity  
972 by MPK3/MPK6 cascade and its downstream WRKY transcription factor during ethylene  
973 induction in Arabidopsis. *PLoS genetics* 8 (6):e1002767

974 Lin F, Zhao M, Baumann DD, Ping J, Sun L, Liu Y, Zhang B, Tang Z, Hughes E, Doerge RW (2014)  
975 Molecular response to the pathogen *Phytophthora sojae* among ten soybean near isogenic  
976 lines revealed by comparative transcriptomics. *BMC genomics* 15 (1):18

977 Lindemose S, Jensen MK, de Velde JV, O'Shea C, Heyndrickx KS, Workman CT, Vandepoele K, Skriver  
978 K, Masi FD (2014) A DNA-binding-site landscape and regulatory network analysis for NAC  
979 transcription factors in *Arabidopsis thaliana*. *Nucleic acids research* 42 (12):7681-7693

980 Liu S, Norris DM, Hartwig EE, Xu M (1992) Inducible phytoalexins in juvenile soybean genotypes predict  
981 soybean looper resistance in the fully developed plants. *Plant physiology* 100 (3):1479-1485

982 Lu KT, Chiou RY, Chen LG, Chen MH, Tseng WT, Hsieh HT, Yang YL (2006) Neuroprotective effects of  
983 resveratrol on cerebral ischemia-induced neuron loss mediated by free radical scavenging and  
984 cerebral blood flow elevation. *Journal of agricultural and food chemistry* 54 (8):3126-3131

985 Luniwal A, Khupse R, Reese M, Liu J, El-Dakdouki M, Malik N, Fang L, Erhardt P (2011) Multigram  
986 synthesis of glyceollin I. *Organic Process Research & Development* 15 (5):1149-1162

987 Luo Y, Bai R, Li J, Yang W, Li R, Wang Q, Zhao G, Duan D (2019) The transcription factor MYB15 is  
988 essential for basal immunity (PTI) in Chinese wild grape. *Planta* 249 (6):1889-1902

989 Luo Y, Wang Q, Bai R, Li R, Chen L, Xu Y, Zhang M, Duan D (2020) The effect of transcription factor  
990 MYB14 on defense mechanisms in *Vitis quinquangularis*-Pingyi. *International journal of*  
991 *molecular sciences* 21 (3):706

992 Lygin AV, Hill CB, Zernova OV, Crull L, Widholm JM, Hartman GL, Lozovaya VV (2010) Response of  
993 soybean pathogens to glyceollin. *Phytopathology* 100 (9):897-903. doi:10.1094/PHYTO-100-  
994 9-0897

995 Lygin AV, Zernova OV, Hill CB, Kholina NA, Widholm JM, Hartman GL, Lozovaya VV (2013) Glyceollin is  
996 an important component of soybean plant defense against *Phytophthora sojae* and  
997 *Macrophomina phaseolina*. *Phytopathology* 103 (10):984-994

998 Malik N, Zhang Z, Erhardt P (2015) Total synthesis of (±)-glyceollin II and a dihydro derivative. *Journal*  
999 *of natural products* 78 (12):2940-2947

1000 Mao G, Meng X, Liu Y, Zheng Z, Chen Z, Zhang S (2011) Phosphorylation of a WRKY transcription factor  
1001 by two pathogen-responsive MAPKs drives phytoalexin biosynthesis in *Arabidopsis*. *The Plant*  
1002 *Cell* 23 (4):1639-1653

1003 Mauch-Mani B, Mauch F (2005) The role of abscisic acid in plant-pathogen interactions. *Current*  
1004 *opinion in plant biology* 8 (4):409-414

1005 McDonald KL, Cahill DM (1999) Influence of abscisic acid and the abscisic acid biosynthesis inhibitor,  
1006 norflurazon, on interactions between *Phytophthora sojae* and soybean (*Glycine max*).  
1007 *European Journal of Plant Pathology* 105 (7):651-658

1008 Mead MN (2007) Women's health: mining for glyceollins. *Environmental Health Perspectives* 115  
1009 (4):A189

1010 Meng X, Zhang S (2013) MAPK cascades in plant disease resistance signaling. *Annual review of*  
1011 *phytopathology* 51:245-266

1012 Mewis I, Khan MA, Glawischnig E, Schreiner M, Ulrichs C (2012) Water stress and aphid feeding  
1013 differentially influence metabolite composition in *Arabidopsis thaliana* (L.). *PLoS One* 7  
1014 (11):e48661

1015 Mialoundama AS, Heintz D, Debayle D, Rahier A, Camara B, Bouvier F (2009) Abscisic acid negatively  
1016 regulates elicitor-induced synthesis of capsidiol in wild tobacco. *Plant physiology* 150 (3):1556-  
1017 1566

1018 Mine A, Berens ML, Nobori T, Anver S, Fukumoto K, Winkelmüller TM, Takeda A, Becker D, Tsuda K  
1019 (2017) Pathogen exploitation of an abscisic acid-and jasmonate-inducible MAPK phosphatase  
1020 and its interception by *Arabidopsis* immunity. *Proceedings of the National Academy of*  
1021 *Sciences* 114 (28):7456-7461

- 1022 Mohr PG, Cahill DM (2001) Relative roles of glyceollin, lignin and the hypersensitive response and the  
 1023 influence of ABA in compatible and incompatible interactions of soybeans with *Phytophthora*  
 1024 *sojae*. *Physiological and molecular plant pathology* 58 (1):31-41
- 1025 Mohr PG, Cahill DM (2003) Abscisic acid influences the susceptibility of *Arabidopsis thaliana* to  
 1026 *Pseudomonas syringae* pv. *tomato* and *Peronospora parasitica*. *Functional Plant Biology* 30  
 1027 (4):461-469
- 1028 Moy P, Qutob D, Chapman BP, Atkinson I, Gijzen M (2004) Patterns of gene expression upon infection  
 1029 of soybean plants by *Phytophthora sojae*. *Molecular Plant-Microbe Interactions* 17 (10):1051-  
 1030 1062
- 1031 Mueller KO, Börger H (1939) Experimentelle Untersuchungen über die *Phytophthora*-Resistenz der  
 1032 Kartoffel.
- 1033 Müller K (1940) Experimentelle untersuchungcn uber die *Phytophthora*-resistem der kartoffel.  
 1034 *Reichsanstalt Landw Forstw Berlin Arb Biol* 23:189-231
- 1035 Müller KO, Meyer G, Klinkowski M (1939) Physiologisch-genetische Untersuchungen über die  
 1036 Resistenz der Kartoffel gegenüber *Phytophthora infestans*. *Naturwissenschaften* 27 (46):765-  
 1037 768
- 1038 Nahar K, Kyndt T, Nzogela YB, Gheysen G (2012) Abscisic acid interacts antagonistically with classical  
 1039 defense pathways in rice-migratory nematode interaction. *New Phytologist* 196 (3):901-913.  
 1040 doi:10.1111/j.1469-8137.2012.04310.x
- 1041 Nawaz W, Zhou Z, Deng S, Ma X, Ma X, Li C, Shu X (2017) Therapeutic versatility of resveratrol  
 1042 derivatives. *Nutrients* 9 (11):1188
- 1043 Nawrath C, Metraux J-P (1999) Salicylic acid induction-deficient mutants of *Arabidopsis* express *PR-2*  
 1044 and *PR-5* and accumulate high levels of camalexin after pathogen inoculation. *The Plant Cell*  
 1045 11 (8):1393-1404
- 1046 Ohnishi M, Morishita H, Iwahashi H, Toda S, Shirataki Y, Kimura M, Kido R (1994) Inhibitory effects of  
 1047 chlorogenic acids on linoleic acid peroxidation and haemolysis. *phytochemistry* 36 (3):579-583
- 1048 Okada A, Okada K, Miyamoto K, Koga J, Shibuya N, Nojiri H, Yamane H (2009) OsTGAP1, a bZIP  
 1049 transcription factor, coordinately regulates the inductive production of diterpenoid  
 1050 phytoalexins in rice. *Journal of Biological Chemistry* 284 (39):26510-26518
- 1051 Pan M-H, Chiou Y-S, Chen W-J, Wang J-M, Badmaev V, Ho C-T (2009) Pterostilbene inhibited tumor  
 1052 invasion via suppressing multiple signal transduction pathways in human hepatocellular  
 1053 carcinoma cells. *Carcinogenesis* 30 (7):1234-1242
- 1054 Park S, Kim da S, Kim JH, Kim JS, Kim HJ (2012) Glyceollin-containing fermented soybeans improve  
 1055 glucose homeostasis in diabetic mice. *Nutrition* 28 (2):204-211.  
 1056 doi:10.1016/j.nut.2011.05.016
- 1057 Pastorczyk M, Kosaka A, Piślewska-Bednarek M, López G, Frerigmann H, Kułak K, Glawischmig E, Molina  
 1058 A, Takano Y, Bednarek P (2020) The role of CYP 71A12 monooxygenase in pathogen-triggered  
 1059 tryptophan metabolism and *Arabidopsis* immunity. *New Phytologist* 225 (1):400-412
- 1060 Pedras M, Jha M, Ahiahonu P (2003) The synthesis and biosynthesis of phytoalexins produced by  
 1061 cruciferous plants. *Current Organic Chemistry* 7 (16):1635-1647
- 1062 Pedras MSC, Abdoli A (2017) Pathogen inactivation of cruciferous phytoalexins: Detoxification  
 1063 reactions, enzymes and inhibitors. *RSC Advances* 7 (38):23633-23646
- 1064 Pedras MSC, Ahiahonu PW (2002) Probing the phytopathogenic stem rot fungus with phytoalexins and  
 1065 analogues: unprecedented glucosylation of camalexin and 6-methoxycamalexin. *Bioorganic &*  
 1066 *medicinal chemistry* 10 (10):3307-3312
- 1067 Pedras MSC, Ahiahonu PW (2005) Metabolism and detoxification of phytoalexins and analogs by  
 1068 phytopathogenic fungi. *Phytochemistry* 66 (4):391-411
- 1069 Pedras MSC, Khan AQ (2000) Biotransformation of the phytoalexin camalexin by the phytopathogen  
 1070 *Rhizoctonia solani*. *Phytochemistry* 53 (1):59-69

1071 Pedras MSC, Thapa C (2020) Unveiling fungal detoxification pathways of the cruciferous phytoalexin  
1072 rapalexin A: Sequential L-cysteine conjugation, acetylation and oxidative cyclization mediated  
1073 by *Colletotrichum* spp. *Phytochemistry* 169:112188

1074 Pedras MSC, Yaya EE, Glawischnig E (2011) The phytoalexins from cultivated and wild crucifers:  
1075 chemistry and biology. *Natural product reports* 28 (8):1381-1405

1076 Pham TH, Lecomte S, Efstathiou T, Ferriere F, Pakdel F (2019) An update on the effects of glyceollins  
1077 on human health: possible anticancer effects and underlying mechanisms. *Nutrients* 11 (1):79

1078 Pilatova M, Ivanova L, Kutschy P, Varinska L, Saxunova L, Repovska M, Sarissky M, Seliga R, Mirossay  
1079 L, Mojzis J (2013) In vitro toxicity of camalexin derivatives in human cancer and non-cancer  
1080 cells. *Toxicology in Vitro* 27 (2):939-944

1081 Poloni A, Schirawski J (2014) Red card for pathogens: phytoalexins in sorghum and maize. *Molecules*  
1082 19 (7):9114-9133

1083 Puranik S, Sahu PP, Srivastava PS, Prasad M (2012) NAC proteins: regulation and role in stress  
1084 tolerance. *Trends in plant science* 17 (6):369-381

1085 Qiu JL, Fiil BK, Petersen K, Nielsen HB, Botanga CJ, Thorgrimsen S, Palma K, Suarez-Rodriguez MC,  
1086 Sandbech-Clausen S, Lichota J (2008) Arabidopsis MAP kinase 4 regulates gene expression  
1087 through transcription factor release in the nucleus. *The EMBO journal* 27 (16):2214-2221

1088 Rajniak J, Barco B, Clay NK, Sattely ES (2015) A new cyanogenic metabolite in Arabidopsis required for  
1089 inducible pathogen defence. *Nature* 525 (7569):376-379

1090 Ren D, Liu Y, Yang K-Y, Han L, Mao G, Glazebrook J, Zhang S (2008) A fungal-responsive MAPK cascade  
1091 regulates phytoalexin biosynthesis in Arabidopsis. *Proceedings of the National Academy of  
1092 Sciences* 105 (14):5638-5643

1093 Rhodes LV, Tilghman SL, Boue SM, Wang S, Khalili H, Muir SE, Bratton MR, Zhang Q, Wang G, Burow  
1094 ME (2012) Glyceollins as novel targeted therapeutic for the treatment of triple-negative breast  
1095 cancer. *Oncology letters* 3 (1):163-171

1096 Roetschi A, Si-Ammour A, Belbahri L, Mauch F, Mauch-Mani B (2001) Characterization of an  
1097 Arabidopsis–*Phytophthora* pathosystem: resistance requires a functional PAD2 gene and is  
1098 independent of salicylic acid, ethylene and jasmonic acid signalling. *The Plant Journal* 28  
1099 (3):293-305

1100 Rowe HC, Walley JW, Corwin J, Chan EK-F, Dehesh K, Kliebenstein DJ (2010) Deficiencies in jasmonate-  
1101 mediated plant defense reveal quantitative variation in *Botrytis cinerea* pathogenesis. *PLoS  
1102 pathogens* 6 (4):e1000861

1103 Rushton PJ, Torres JT, Parniske M, Wernert P, Hahlbrock K, Somssich I (1996) Interaction of elicitor-  
1104 induced DNA-binding proteins with elicitor response elements in the promoters of parsley PR1  
1105 genes. *The EMBO journal* 15 (20):5690-5700

1106 Saga H, Ogawa T, Kai K, Suzuki H, Ogata Y, Sakurai N, Shibata D, Ohta D (2012) Identification and  
1107 characterization of ANAC042, a transcription factor family gene involved in the regulation of  
1108 camalexin biosynthesis in Arabidopsis. *Molecular plant-microbe interactions* 25 (5):684-696

1109 Salvo VA, Boue SM, Fonseca JP, Elliott S, Corbitt C, Collins-Burow BM, Curiel TJ, Srivastav SK, Shih BY,  
1110 Carter-Wientjes C, Wood CE, Erhardt PW, Beckman BS, McLachlan JA, Cleveland TE, Burow ME  
1111 (2006) Antiestrogenic glyceollins suppress human breast and ovarian carcinoma  
1112 tumorigenesis. *Clinical cancer research : an official journal of the American Association for  
1113 Cancer Research* 12 (23):7159-7164. doi:10.1158/1078-0432.CCR-06-1426

1114 Sbaghi M, Jeandet P, Bessis R, Leroux P (1996) Degradation of stilbene-type phytoalexins in relation  
1115 to the pathogenicity of *Botrytis cinerea* to grapevines. *Plant Pathology* 45 (1):139-144

1116 Schmelz EA, Huffaker A, Sims JW, Christensen SA, Lu X, Okada K, Peters RJ (2014) Biosynthesis,  
1117 elicitation and roles of monocot terpenoid phytoalexins. *The Plant Journal* 79 (4):659-678

1118 Schmidt A, Mächtel R, Ammon A, Engelsdorf T, Schmitz J, Maurino VG, Voll LM (2020) Reactive oxygen  
1119 species dosage in Arabidopsis chloroplasts can improve resistance towards *Colletotrichum  
1120 higginsianum* by the induction of WRKY33. *New Phytologist* 226 (1):189-204



1121 Schmidt K, Pflugmacher M, Klages S, Maeser A, Mock A, Stahl DJ (2008) Accumulation of the hormone  
1122 abscisic acid (ABA) at the infection site of the fungus *Cercospora beticola* supports the role of  
1123 ABA as a repressor of plant defence in sugar beet. *Mol Plant Pathol* 9 (5):661-673  
1124 Sen S (2017) Role of Phytoalexins in Plant-Microbe Interactions and Human Health. *International*  
1125 *Journal of Scientific Research and Management* 5 (7):6215-6225  
1126 Seo E, Choi D (2015) Functional studies of transcription factors involved in plant defenses in the  
1127 genomics era. *Briefings in functional genomics* 14 (4):260-267  
1128 Seo J, Kim B, Oh J, Kim J-S (2018) Soybean-derived phytoalexins improve cognitive function through  
1129 activation of Nrf2/HO-1 signaling pathway. *International journal of molecular sciences* 19  
1130 (1):268  
1131 Shahnejat-Bushehri S, Tarkowska D, Sakuraba Y, Balazadeh S (2016) Arabidopsis NAC transcription  
1132 factor JUB1 regulates GA/BR metabolism and signalling. *Nature plants* 2:16013  
1133 Shukla Y, Singh R (2011) Resveratrol and cellular mechanisms of cancer prevention. *Annals of the New*  
1134 *York Academy of Sciences* 1215 (1):1-8  
1135 Simons R, Vincken JP, Roidos N, Bovee TFH, van Iersel M, Verbruggen MA, Gruppen H (2011) Increasing  
1136 Soy Isoflavonoid Content and Diversity by Simultaneous Malting and Challenging by a Fungus  
1137 to Modulate Estrogenicity. *Journal of agricultural and food chemistry* 59 (12):6748-6758.  
1138 doi:10.1021/jf2010707  
1139 Singh KB, Foley RC, Oñate-Sánchez L (2002) Transcription factors in plant defense and stress  
1140 responses. *Current opinion in plant biology* 5 (5):430-436  
1141 Smith B, Randle D, Mezencev R, Thomas L, Hinton C, Odero-Marah V (2014) Camalexin-induced  
1142 apoptosis in prostate cancer cells involves alterations of expression and activity of lysosomal  
1143 protease cathepsin D. *Molecules* 19 (4):3988-4005  
1144 Song H, Sun W, Yang G, Sun J (2018) WRKY transcription factors in legumes. *BMC plant biology* 18  
1145 (1):243  
1146 Stäb MR, Ebel J (1987) Effects of Ca<sup>2+</sup> on phytoalexin induction by fungal elicitor in soybean cells.  
1147 *Archives of biochemistry and biophysics* 257 (2):416-423  
1148 Stahl E, Bellwon P, Huber S, Schlaeppi K, Bernsdorff F, Vallat-Michel A, Mauch F, Zeier J (2016)  
1149 Regulatory and functional aspects of indolic metabolism in plant systemic acquired resistance.  
1150 *Molecular plant* 9 (5):662-681  
1151 Strange R, Ingham J, Cole D, Cavill M, Edwards C, Cooksey C, Garrattd P (1985) Isolation of the  
1152 phytoalexin medicarpin from leaflets of *Arachis hypogaea* and related species of the tribe  
1153 Aeschynomeneae. *Zeitschrift für Naturforschung C* 40 (5-6):313-316  
1154 Suh N, Paul S, Hao X, Simi B, Xiao H, Rimando AM, Reddy BS (2007) Pterostilbene, an active constituent  
1155 of blueberries, suppresses aberrant crypt foci formation in the azoxymethane-induced colon  
1156 carcinogenesis model in rats. *Clinical Cancer Research* 13 (1):350-355  
1157 Sun P-W, Xu Y-H, Yu C-C, Lv F-F, Tang X-L, Gao Z-H, Zhang Z, Wang H, Liu Y, Wei J-H (2020) WRKY44  
1158 represses expression of the wound-induced sesquiterpene biosynthetic gene *ASS1* in *Aquilaria*  
1159 *sinensis*. *Journal of Experimental Botany* 71 (3):1128-1138  
1160 Takahashi H, Kanayama Y, Zheng MS, Kusano T, Hase S, Ikegami M, Shah J (2004) Antagonistic  
1161 interactions between the SA and JA signaling pathways in Arabidopsis modulate expression of  
1162 defense genes and gene-for-gene resistance to cucumber mosaic virus. *Plant and Cell*  
1163 *Physiology* 45 (6):803-809  
1164 Thaler JS, Bostock RM (2004) Interactions between abscisic-acid-mediated responses and plant  
1165 resistance to pathogens and insects. *Ecology* 85 (1):48-58. doi:10.1890/02-0710  
1166 Thomma BP, Nelissen I, Eggermont K, Broekaert WF (1999) Deficiency in phytoalexin production  
1167 causes enhanced susceptibility of Arabidopsis thaliana to the fungus *Alternaria brassicicola*.  
1168 *The Plant Journal* 19 (2):163-171  
1169 Tyler BM (2007) *Phytophthora sojae*: root rot pathogen of soybean and model oomycete. *Mol Plant*  
1170 *Pathol* 8 (1):1-8. doi:10.1111/j.1364-3703.2006.00373.x

1171 Ube N, Harada D, Katsuyama Y, Osaki-Oka K, Tonooka T, Ueno K, Taketa S, Ishihara A (2019)  
1172 Identification of phenylamide phytoalexins and characterization of inducible phenylamide  
1173 metabolism in wheat. *Phytochemistry* 167:112098

1174 VanEtten H, Matthews P, Mercer E (1983) (+)-Maackiain and (+)-medicarpin as phytoalexins in  
1175 *Sophora Japonica* and identification of the (–) isomers by biotransformation. *Phytochemistry*  
1176 22 (10):2291-2295

1177 Wang F, Chen Q, Zhang R, Li D, Ling Y, Song R (2019) The anti-phytoalexin gene Bx-cathepsin W  
1178 supports the survival of *Bursaphelenchus xylophilus* under *Pinus massoniana* phytoalexin  
1179 stress. *BMC genomics* 20 (1):779

1180 Wanke A, Rovenich H, Schwanke F, Velte S, Becker S, Hehemann JH, Wawra S, Zuccaro A (2020) Plant  
1181 species-specific recognition of long and short  $\beta$ -1, 3-linked glucans is mediated by different  
1182 receptor systems. *The Plant Journal*

1183 Ward EW, Cahill DM, Bhattacharyya MK (1989) Abscisic Acid Suppression of Phenylalanine Ammonia-  
1184 Lyase Activity and mRNA, and Resistance of Soybeans to *Phytophthora megasperma* f.sp.  
1185 *glycinea*. *Plant physiology* 91 (1):23-27

1186 Wu A, Allu AD, Garapati P, Siddiqui H, Dortay H, Zanol M-I, Asensi-Fabado MA, Munné-Bosch S,  
1187 Antonio C, Tohge T (2012) JUNGBRUNNEN1, a reactive oxygen species-responsive NAC  
1188 transcription factor, regulates longevity in *Arabidopsis*. *The Plant Cell* 24 (2):482-506

1189 Wu C-H, Derevnina L, Kamoun S (2018) Receptor networks underpin plant immunity. *Science* 360  
1190 (6395):1300-1301

1191 Xu J, Audenaert K, Hofte M, Vleesschauwer Dd, Xu J, de Vleesschauwer D (2013a) Abscisic acid  
1192 promotes susceptibility to the rice leaf blight pathogen *Xanthomonas oryzae* pv *oryzae* by  
1193 suppressing salicylic acid-mediated defenses. *PLoS ONE* 8 (6):e67413-e67413

1194 Xu W, Dubos C, Lepiniec L (2015) Transcriptional control of flavonoid biosynthesis by MYB–bHLH–WDR  
1195 complexes. *Trends in plant science* 20 (3):176-185

1196 Xu Y-H, Wang J-W, Wang S, Wang J-Y, Chen X-Y (2004) Characterization of GaWRKY1, a cotton  
1197 transcription factor that regulates the sesquiterpene synthase gene (+)- $\delta$ -cadinene synthase-  
1198 A. *Plant physiology* 135 (1):507-515

1199 Xu Z-Y, Kim SY, Kim DH, Dong T, Park Y, Jin JB, Joo S-H, Kim S-K, Hong JC, Hwang D (2013b) The  
1200 *Arabidopsis* NAC transcription factor ANAC096 cooperates with bZIP-type transcription  
1201 factors in dehydration and osmotic stress responses. *The Plant Cell* 25 (11):4708-4724

1202 Yamamura C, Mizutani E, Okada K, Nakagawa H, Fukushima S, Tanaka A, Maeda S, Kamakura T,  
1203 Yamane H, Takatsuji H (2015) Diterpenoid phytoalexin factor, a bHLH transcription factor,  
1204 plays a central role in the biosynthesis of diterpenoid phytoalexins in rice. *The Plant Journal*  
1205 84 (6):1100-1113

1206 Yi J, Derynck MR, Li X, Telmer P, Marsolais F, Dhaubhadel S (2010) A single-repeat MYB transcription  
1207 factor, GmMYB176, regulates CHS8 gene expression and affects isoflavonoid biosynthesis in  
1208 soybean. *The Plant Journal* 62 (6):1019-1034

1209 Yoshida Y, Miyamoto K, Yamane H, Nishizawa Y, Minami E, Nojiri H, Okada K (2017) OsTGAP1 is  
1210 responsible for JA-inducible diterpenoid phytoalexin biosynthesis in rice roots with biological  
1211 impacts on allelopathic interaction. *Physiologia plantarum* 161 (4):532-544

1212 Yoshikawa M, Masago H (1982) Biochemical mechanism of glyceollin accumulation in soybean. *Plant*  
1213 *infection: the physiological and biochemical basis*/edited by Yasuji Asada[et al]

1214 Yoshikawa M, Yamauchi K, Masago H (1978) Glyceollin: its role in restricting fungal growth in resistant  
1215 soybean hypocotyls infected with *Phytophthora megasperma* var. *sojae*. *Physiological Plant*  
1216 *Pathology* 12 (1):73-82

1217 Zabala MD, Bennett MH, Truman WH, Grant MR (2009) Antagonism between salicylic and abscisic acid  
1218 reflects early host-pathogen conflict and moulds plant defence responses. *Plant J* 59 (3):375-  
1219 386. doi:10.1111/j.1365-313X.2009.03875.x

1220 Zaïdi I, Ebel C, Touzri M, Herzog E, Evrard J-L, Schmit AC, Masmoudi K, Hanin M (2010) TMKP1 is a  
1221 novel wheat stress responsive MAP kinase phosphatase localized in the nucleus. *Plant*  
1222 *molecular biology* 73 (3):325-338

1223 Zeilinger S, Gupta VK, Dahms TE, Silva RN, Singh HB, Upadhyay RS, Gomes EV, Tsui CK-M, Nayak S C  
1224 (2015) Friends or foes? Emerging insights from fungal interactions with plants. *FEMS*  
1225 *microbiology reviews* 40 (2):182-207

1226 Zhang H, Demirer GS, Zhang H, Ye T, Goh NS, Aditham AJ, Cunningham FJ, Fan C, Landry MP (2019)  
1227 DNA nanostructures coordinate gene silencing in mature plants. *Proceedings of the National*  
1228 *Academy of Sciences* 116 (15):7543-7548

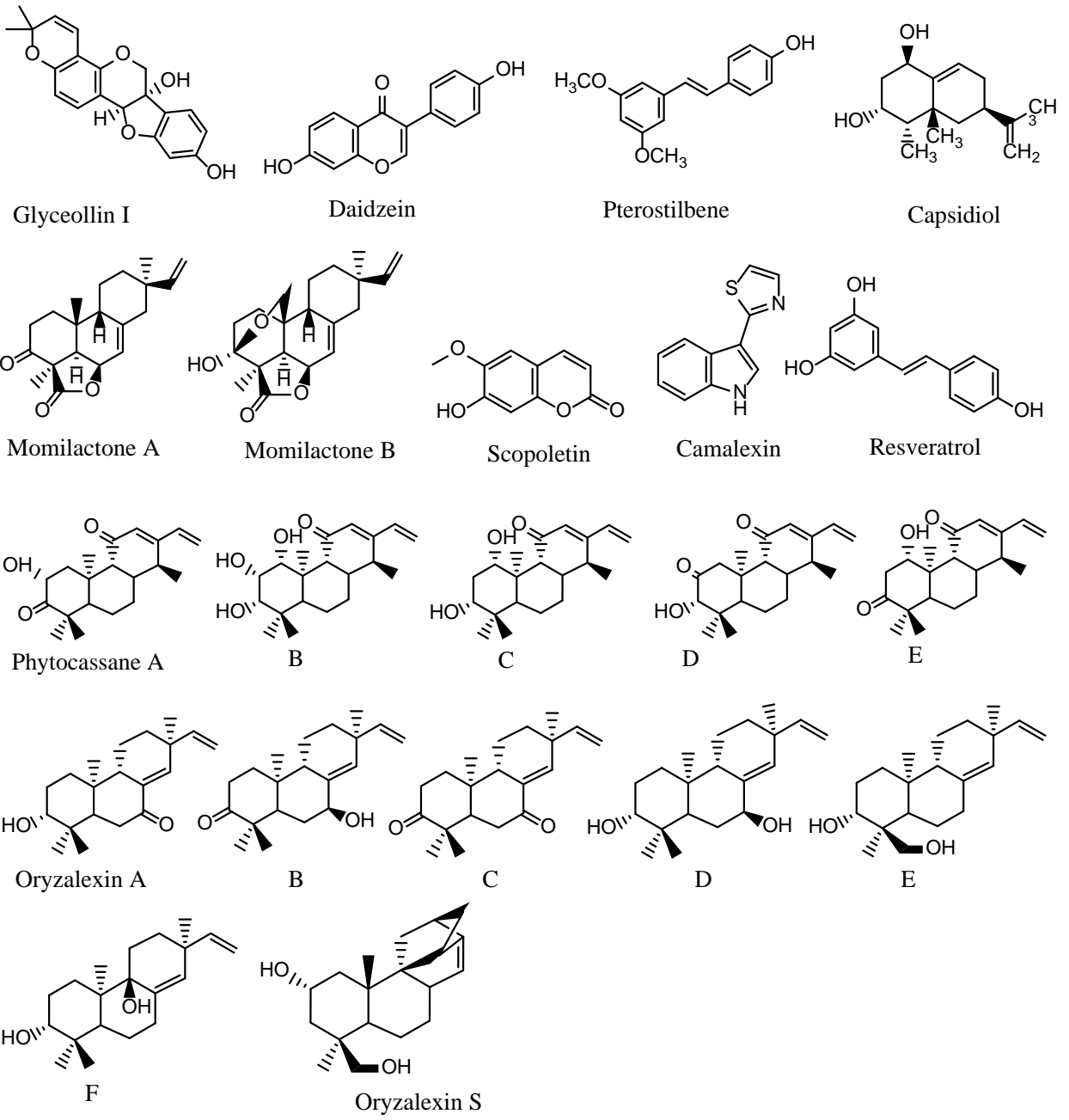
1229 Zhao J, Davis LC, Verpoorte R (2005) Elicitor signal transduction leading to production of plant  
1230 secondary metabolites. *Biotechnol Adv* 23 (4):283-333

1231 Zimmermann IM, Heim MA, Weisshaar B, Uhrig JF (2004) Comprehensive identification of *Arabidopsis*  
1232 *thaliana* MYB transcription factors interacting with R/B-like BHLH proteins. *The Plant Journal*  
1233 40 (1):22-34

1234 Zimmermann MC, Tilghman SL, Boue SM, Salvo VA, Elliott S, Williams KY, Skripnikova EV, Ashe H,  
1235 Payton-Stewart F, Vanhoy-Rhodes L, Fonseca JP, Corbitt C, Collins-Burow BM, Howell MH,  
1236 Lacey M, Shih BY, Carter-Wientjes C, Cleveland TE, McLachlan JA, Wiese TE, Beckman BS,  
1237 Burow ME (2010) Glyceollin I, a novel antiestrogenic phytoalexin isolated from activated soy.  
1238 *The Journal of pharmacology and experimental therapeutics* 332 (1):35-45.  
1239 doi:10.1124/jpet.109.160382

1240

1241

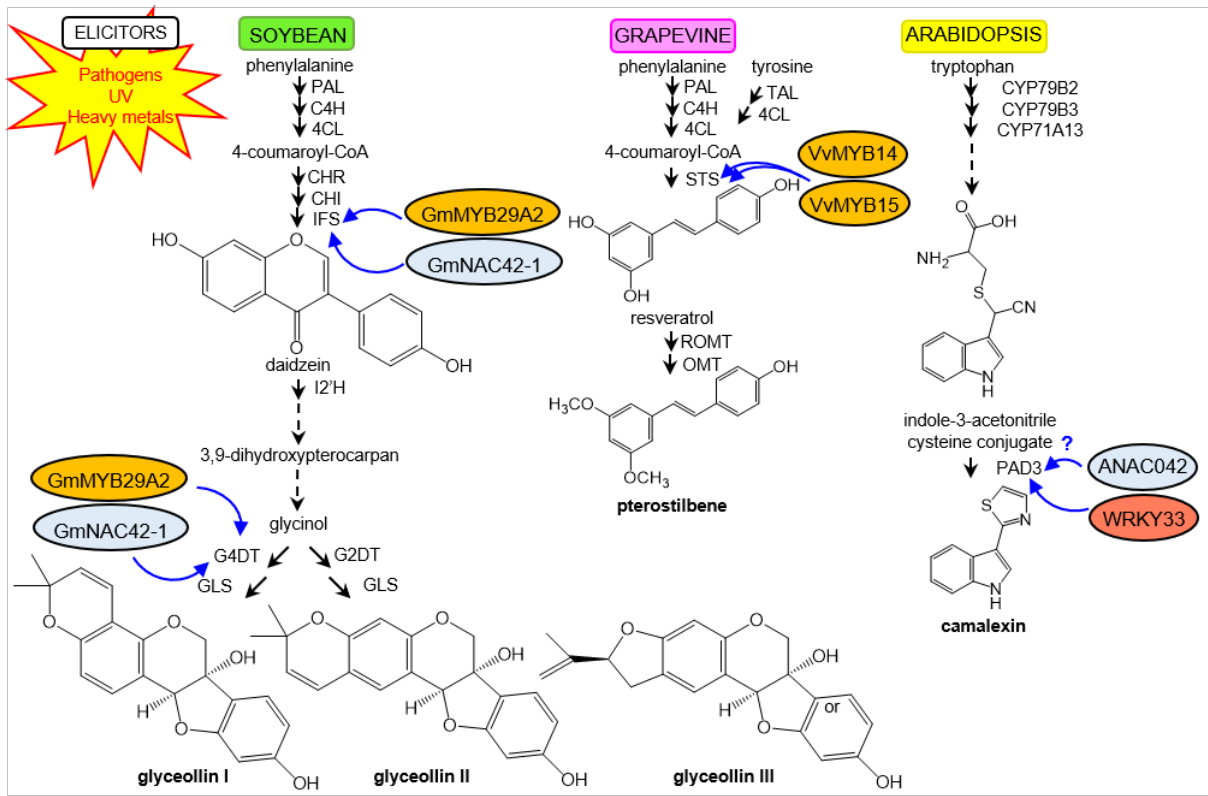


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1243 Fig. 1

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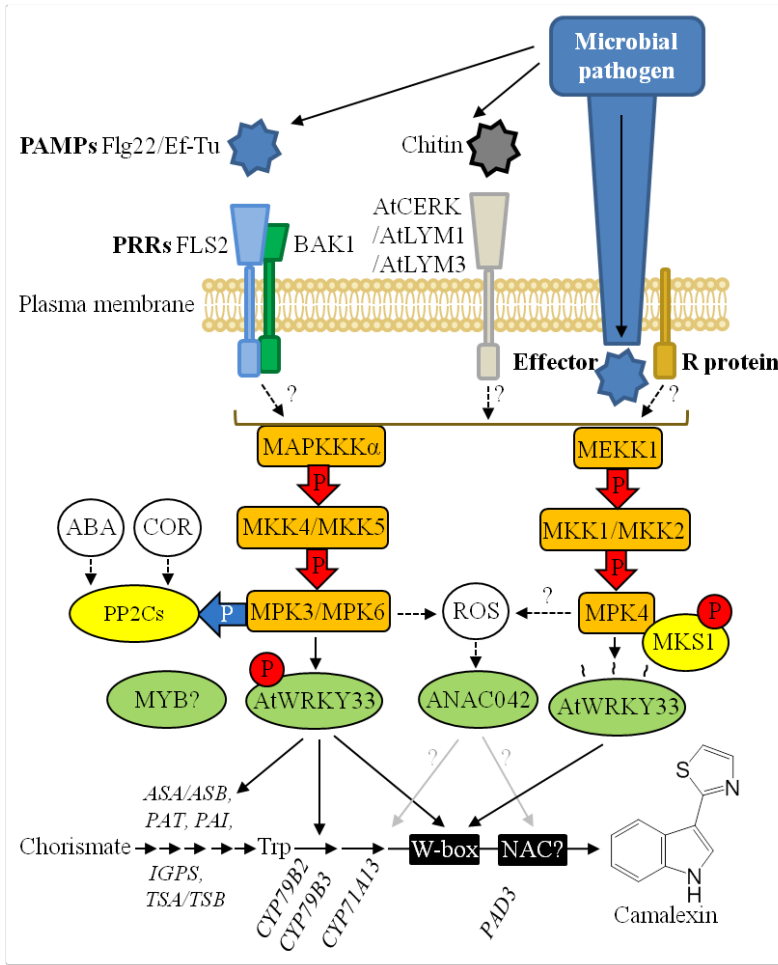


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1247 Fig. 2

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1251 Fig. 3