

1 **Shoot-root interaction in control of camalexin exudation in Arabidopsis**

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14 Date of submission:

15 Number of figures:

16 Total word count:

17 Supplementary data:

18 Running title: Camalexin exudation

19

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21 **Abstract**

22 Plants exude secondary metabolites from the roots to shape the composition and  
23 function of their microbiome. Many of these compounds are known for their anti-  
24 microbial activity and are part of the plant immunity, such as the indole-derived  
25 phytoalexin camalexin. Here we studied the dynamics of camalexin synthesis and  
26 exudation upon induction of *Arabidopsis thaliana* with a plant growth promotion  
27 bacteria *Pseudomonas sp.* CH267 or a bacterial pathogen *Burkholderia glumae* PG1.  
28 We show that while the camalexin accumulation and exudation is more rapidly but  
29 transiently induced upon interaction with the growth promoting strain, the pathogen  
30 induces a higher and more stable camalexin levels. The concentration of camalexin in  
31 shoots, roots and exudates is well correlated, triggering a question on the origin of the  
32 exuded camalexin. By combination of experiments with cut shoots and roots and  
33 grafting of wild type plant with mutants in camalexin synthesis we showed that while  
34 camalexin can be produced and released by both organs, in intact plant the exuded  
35 camalexin originates in the shoots. We show that camalexin synthesis in response to  
36 *B. glumae* PG1 is dependent on cooperation of four CYP71 genes and a loss of function  
37 of any of them reduces camalexin synthesis. In conclusion, camalexin synthesis seems  
38 to be controlled on a whole plant level and coordinated between shoots and roots.

39

40 **Keywords**

41 bacterial pathogen, camalexin, grafting, plant defense, plant growth promoting  
42 rhizobacteria, root exudates

43

## 44 INTRODUCTION

45 Plants are cohabiting their natural environments with plethora of microorganisms  
46 some beneficial or commensal, some harmful (Bulgarelli *et al.*, 2013). Plants therefore  
47 evolved number of mechanisms that enable them to communicate with the microbiota,  
48 to attract the beneficial ones and defend themselves against the harmful ones. Number  
49 of these mechanisms are based on plant metabolites that can fulfil both of these  
50 functions (reviewed in (Jacoby *et al.*, 2020; Sasse *et al.*, 2018). Plants produce a  
51 number of secondary compounds that are directly involved in defense (Piasecka *et al.*,  
52 2015). Some of these compounds are synthesised only in response to the infection, and  
53 therefore they are classified as phytoalexins, whereas others are constitutive and  
54 activated upon tissue damage or pathogen triggered signalling; these are termed  
55 phytoanticipins (VanEtten *et al.*, 1994). Chemically, the metabolites used by plants for  
56 defense belong to all major classes of secondary compounds, terpenes, phenolic  
57 compounds, and alkaloids (Zaynab *et al.*, 2018).

58 One of the best characterised classes of phytoalexins are the sulfur containing indolic  
59 compounds, such as camalexin and brassinin in the crucifers (Pedras and Yaya, 2010).  
60 Camalexin, 3-thiazol-2'-yl-indole, accumulates upon infection with fungal pathogens,  
61 such as *Botrytis cinerea* or *Alternaria brassicicola* (Bednarek *et al.*, 2005;  
62 Kliebenstein *et al.*, 2005; Millet *et al.*, 2010; Thomma *et al.*, 1999). Variation in  
63 camalexin synthesis is associated with variation to susceptibility to *Botrytis* in  
64 *Arabidopsis* accessions (Rowe and Kliebenstein, 2008). Camalexin is synthesised  
65 from tryptophan, the first step in the pathway being the production of indole-3-  
66 acetaldoxime (IAOx), a common precursor for auxin, camalexin, and indole  
67 glucosinolate synthesis (Glawischnig *et al.*, 2004). The first dedicated step in  
68 camalexin synthesis is the conversion of IAOx into indole-3-acetonitrile (IAN) by  
69 CYP71A12 and CYP71A13 (Glawischnig *et al.*, 2004; Nafisi *et al.*, 2007). IAN is  
70 conjugated by glutathione, which introduces the sulfur into the chemical structure and  
71 camalexin is ultimately synthesised by CYP71B15 (Geu-Flores *et al.*, 2011;  
72 Schuegger *et al.*, 2006; Su *et al.*, 2011; Zhou *et al.*, 1999). The pathway may,  
73 however, be more complex, as two other P-450 enzymes, CYP71A27 and CYP71A28,  
74 were associated with camalexin accumulation in roots (Koprivova *et al.*, 2019). The  
75 role of the individual isoforms particularly in roots is thus not very clear.

76 In the roots, camalexin was shown to have additional function to innate immunity, as  
77 a metabolite shaping the function of root associated microbiota (Koprivova *et al.*,

78 2019). Using sulfatase activity in rhizosphere soil from *Arabidopsis* accessions as a  
79 measure for microbiome activity, genome wide association analysis showed that  
80 variation in CYP71A27 affects this microbial function. Loss of CYP71A27 resulted in  
81 lower sulfatase activity in soil, which could be complemented by camalexin. In  
82 addition, the *cyp71A27* mutant did not benefit from plant growth promoting (PGP)  
83 effects of several rhizospheric bacteria, which again could be complemented by  
84 addition of camalexin (Koprivova *et al.*, 2019). Camalexin is exuded from the roots  
85 (Koprivova *et al.*, 2019; Millet *et al.*, 2010) and may represent an important player in  
86 the mechanisms by which plants control their microbiome (Jacoby *et al.*, 2020).  
87 However, camalexin exudation seems to be in conflict with its definition as  
88 phytoalexin, as phytoalexins act in the site of their synthesis (VanEtten *et al.*, 1994).  
89 Thus, it is important to discover more about the nature and control of camalexin  
90 exudation.

91 Here we show that camalexin exudation is triggered by both pathogenic and PGP  
92 bacteria and that camalexin accumulation in exudates, roots, and leaves is highly  
93 correlated. We also reveal that both leaves and roots are able to synthesise camalexin  
94 and used grafting to show that the camalexin exuded upon treatment of roots with  
95 *Burkholderia glumae* originates in the shoot.

96

## 97 **RESULTS**

### 98 *Both PGP and pathogenic bacteria trigger camalexin synthesis and exudation*

99 Previous work showed that camalexin can be exuded from plant roots incubated with  
100 PGP bacteria or the bacterial-derived peptide elicitor flagellin, which is the pre-  
101 requisite of camalexin function in shaping microbiome function (Koprivova *et al.*,  
102 2019; Millet *et al.*, 2010). PGP bacteria and flagellin trigger also camalexin  
103 accumulation in roots, as does infection with root fungal pathogen *Verticillium*  
104 *longisporum* (Iven *et al.*, 2012). To test, whether camalexin synthesis and exudation is  
105 triggered also by bacterial pathogens we incubated *Arabidopsis* growing in  
106 hydroculture with *Burkholderia glumae* PG1 (Gao *et al.*, 2015) or a PGP bacterium  
107 *Pseudomonas sp.* CH267 (Haney *et al.*, 2015; Koprivova *et al.*, 2019). To obtain a  
108 better picture of a control of camalexin synthesis we analysed its accumulation in roots  
109 and shoots and in the exudates. Both bacteria triggered camalexin synthesis in all three  
110 compartments, whereas minimal camalexin levels were detected in roots and shoots of  
111 mock treated plants and no camalexin was exuded without the bacterial trigger (Figure

112 1). The two bacteria elicited camalexin synthesis and exudation in a different way, but  
113 similar in all three compartments. The PGP strain *Pseudomonas sp.* CH267 triggered  
114 a rapid response but the accumulation of camalexin peaked between 2 and 4 days and  
115 decreased afterwards, whereas the synthesis and exudation reached a maximum after  
116 4 to 5 days and remained high upon treatment with *B. glumae* PG1. In the first days,  
117 the camalexin levels were higher upon treatment with *Pseudomonas sp.* CH267 but in  
118 later stages the pathogenic strain *B. glumae* PG1 triggered significantly higher levels  
119 of camalexin (Figure 1). Interestingly, even though the bacteria were in contact only  
120 with the roots, the camalexin concentrations were highly correlated between shoots  
121 and roots and also between both organs and the exudates. It is thus not possible to  
122 conclude whether the exuded camalexin is synthesised in the roots or the shoots.

123

#### 124 *Contribution of different isoforms of CYP71A family to camalexin synthesis*

125 We found previously that loss of two additional members of the CYP71A family of P-  
126 450 enzymes, CYP71A27 and CYP71A28, affected camalexin levels in roots  
127 (Koprivova *et al.*, 2019). We were therefore interested in their contribution to total  
128 camalexin synthesis and obtained all possible double and triple mutants of the four  
129 isoforms CYP71A12, CYP71A13, CYP71A27, and CYP71A28. *CYP71A12* and  
130 *CYP71A13* as well as *CYP71A27* and *CYP71A28* are two pairs of neighbouring genes,  
131 and while a double mutant *cyp71a12 cyp71a13* (*cyp12/13*) has been produced by  
132 TALEN mutagenesis (Muller *et al.*, 2015), double mutant of *CYP71A27* and  
133 *CYP71A28* is not available. These mutants were subjected to treatment with *B. glumae*  
134 PG1 for 3 days, leading to high synthesis of camalexin and allowing a good  
135 comparison of the individual genotypes. This analysis showed clearly that all four P-  
136 450 isoforms are important for camalexin synthesis (Figure 2A). Surprisingly, loss of  
137 CYP71A27 and CYP71A28 also led to a significant reduction of camalexin synthesis  
138 in the leaves, even if the corresponding genes are not expressed there. Camalexin  
139 levels in *cyp12/13* mutant is very low and in the range measured in mock treated plants,  
140 but still additional loss of either CYP71A27 or CYP71A28 lowers the camalexin  
141 accumulation further (Figure 2A). However, it needs to be seen, whether the effects of  
142 the mutations are due to loss of enzymatic activity or alteration of expression of other  
143 isoforms. Therefore, we determined the transcript levels of genes of camalexin  
144 synthesis pathway in all these mutants in roots. Inoculation with *B. glumae* PG1 led to  
145 increase of mRNA levels in roots of the genes for the enzymes of the canonical

146 camalexin synthesis pathway CYP71A12, CYP71A13, and CYP7B15, as well as of  
147 *CYP71A27* (Figure 2B). As expected, the expression of the camalexin synthesis genes  
148 have been affected in the various mutants. The induction of *CYP71A12* by *B. glumae*  
149 PG1 was attenuated in the single mutants of other P-450 isoforms and in the double  
150 mutant *cyp13/27*, but surprisingly, increased in *cyp13/28* (Figure 2B). Also the  
151 induction of *CYP71B15* was less pronounced in the mutants. On the other hand,  
152 *CYP71A13* transcript levels were significantly elevated in genotypes with disrupted  
153 *CYP71A12* already without bacterial trigger. Although the *CYP71A28* mRNA was not  
154 detectable, disruption of this gene resulted in increased transcript levels of *CYP71A27*  
155 both with and without inoculation (Figure 2B). Thus, the each of the four CYP71A  
156 isoforms seem to play some role in the camalexin network as loss of any of them  
157 affects at least one other member.

158

#### 159 *Dissection of tissue specificity of camalexin synthesis and exudation*

160 The coordinated accumulation of camalexin in shoots, roots, and exudates after  
161 exposure of roots led to a question, whether the bacteria trigger camalexin synthesis  
162 also in the leaves. We, therefore, grew *Arabidopsis* plants on agarose plates, inoculated  
163 either the leaves or the root tips with the two bacterial strains and measured camalexin  
164 after 3 days incubation. Both inoculations triggered accumulation of camalexin in  
165 shoots and roots, but to a different extent depending on the bacterial strains.  
166 *Pseudomonas sp.* CH267 induced only a small camalexin accumulation, which did not  
167 differ neither in the two organs nor in the two types of inoculation and was only slightly  
168 higher than the levels found in sterile plants (Figure 3A). *B. glumae* PG1 triggered a  
169 similarly low camalexin synthesis when inoculated from root tip, but resulted in a large  
170 accumulation in leaves and to some extent also roots when inoculated onto leaves.  
171 Camalexin synthesis in *Arabidopsis* leaves thus react to *B. glumae* PG1 in the same  
172 way as to the fungal pathogens. The induction of camalexin synthesis in roots might  
173 be due to camalexin transport or to movement of the bacteria in the plant. We therefore  
174 used qPCR to determine bacterial titre in the plant material. No amplification was  
175 possible using primers for *Pseudomonas sp.* CH267, probably due to a low titre in our  
176 inoculations. Using primers for *B. glumae* PG1, however, bacteria were clearly  
177 detected in both roots and shoots, irrespective of the inoculated tissue, which reveals  
178 the mobility of this strain within the plant, both root-to-shoot and shoot-to-root  
179 directions (Figure 3B). Interestingly, while in plants inoculated from the root tip the

180 amount of camalexin approximately correlates to the bacterial titre, in the plants  
181 inoculated from the leaves, the leaf camalexin concentration was almost 20-fold higher  
182 in leaf than in roots, despite a similar bacterial titre (Figure 3).

183 We therefore asked, whether a communication between shoot and root affects  
184 camalexin synthesis in response to *B. glumae* PG1. We used the hydroponics system  
185 with plants growing on a nylon membrane, cut the shoots, placed them and the  
186 corresponding remaining roots separately in the wells of the 12 well plates, and  
187 inoculated with *B. glumae* PG1. Camalexin was then determined in the tissues and the  
188 exudates, as well as in shoot, root and exudates of intact plants analysed as controls  
189 (Figure 4). Both cut roots and shoots were able to exude camalexin to the solution, to  
190 levels higher than intact plants. Interestingly, whereas cut shoots accumulated more  
191 camalexin than shoots of intact plants, cut roots possessed only very low camalexin  
192 concentration compared to the intact controls. Total camalexin production in cut shoots  
193 was with  $24 \pm 4$  nmol g<sup>-1</sup> FW higher than in intact plants ( $17 \pm 1$  nmol g<sup>-1</sup> FW) and cut  
194 roots ( $12 \pm 2$  nmol g<sup>-1</sup> FW). Thus, clearly, both roots and shoots are able to synthesise  
195 camalexin and its synthesis and exudation undergoes a control dependent on root-shoot  
196 communication.

197 While the experiments with cut roots and shoots were informative, they do not  
198 correspond to the *in vivo* situation. In order to determine where the camalexin exuded  
199 by root inoculation with *B. glumae* PG1 is synthesised, we performed grafting  
200 experiments with two mutants unable to synthesis camalexin, *pad3* and *cyp79b2*  
201 *cyp79b3* (*b2/b3*) (Hull *et al.*, 2000; Zhou *et al.*, 1999). Inoculation of roots with *B.*  
202 *glumae* PG1 resulted in accumulation of camalexin in roots, shoots and exudates of  
203 Col-0 wild type (WT) homografts but not in homografts of the two mutants (Figure  
204 5A). Even with a large variation due to analysis of individual seedlings, it can be  
205 clearly seen that in comparison with WT homografts, similarly high camalexin  
206 accumulation was found only in heterografted shoots originating from WT. Shoots of  
207 *b2/b3* mutant grafted on WT roots did not contain any camalexin higher than the  
208 background, whereas the shoots of *pad3* contained a low level of camalexin.  
209 Interestingly, WT roots grafted with *b2/b3* shoots contained same level of camalexin  
210 as roots of WT homografts, while WT roots grafted with *pad3* shoots did not contain  
211 any camalexin above the background. *pad3* roots grafted with WT shoots accumulated  
212 camalexin but *b2/b3* roots did not (Figure 5A). Importantly, camalexin was found only

213 on exudates of grafted plants with WT shoots. Thus, the camalexin exuded upon  
214 inoculation of the roots by *B. glumae* PG1 must originate in the shoots.

215 When the grafted plants were inoculated with *B. glumae* PG1 on the leaves, camalexin  
216 was found mainly in shoots of WT homografts with much lower levels in roots and  
217 concentration not different to background in the exudates (Figure 5B). In the  
218 heterografts with *b2/b3*, only the tissues originating from WT accumulated camalexin,  
219 and none was exuded, whereas in grafts with *pad3* high level of camalexin was found  
220 in the shoots originating from WT, and low levels in both types of roots. Thus in whole  
221 plants camalexin synthesis seems to be tightly controlled and primarily occurring in  
222 the shoots.

223

## 224 **DISCUSSION**

225 Camalexin is a relatively new addition to the list of plant metabolites that shape the  
226 root associated microbiome (Jacoby *et al.*, 2020; Koprivova *et al.*, 2019). Similar to  
227 other such compounds, e.g. coumarins or benzoxazinoids (de Bruijn *et al.*, 2018;  
228 Stringlis *et al.*, 2018), camalexin has first been characterised for its antimicrobial  
229 properties (Rogers *et al.*, 1996). However, in the rhizosphere these compounds affect  
230 the microbiota in a way that they support plant fitness and performance. For example,  
231 coumarins exuded from plants were shown to affect the communities to improve plant  
232 iron nutrition (Harbort *et al.*, 2020). Camalexin was shown to affect the microbial  
233 sulfatase activity in rhizosphere soil that mineralises organic sulfur and so the bacteria  
234 help plants to access this sulfur pool (Kertesz and Mirleau, 2004; Koprivova *et al.*,  
235 2019). Thus, camalexin has to be exuded to fulfil this function, however, unlike the  
236 coumarins or benzoxazinoids, camalexin has not been found in roots exudates unless  
237 elicited (Millet *et al.*, 2010; Monchgesang *et al.*, 2016; Neal *et al.*, 2012). In addition,  
238 while the other metabolites characterised so far change the taxonomic assembly of the  
239 microbial community, this still needs to be tested for camalexin.

240 Since camalexin has always been a prime example of a phytoalexin, i.e. acting locally  
241 at the site of pathogen attack, it is not obvious how camalexin exudation from the roots  
242 is regulated. Previous work focused on camalexin synthesis in the leaves as a reaction  
243 to leaf pathogens (Glazebrook and Ausubel, 1994; Kliebenstein *et al.*, 2005; Thomma  
244 *et al.*, 1999). It was shown previously that the exudation could be elicited by flagellin  
245 or by PGP bacteria (Koprivova *et al.*, 2019; Millet *et al.*, 2010), but to relatively low  
246 levels. It was therefore important that high camalexin exudation can be triggered by



247 the pathogenic bacteria *B. glumae* PG1 as a robust high exudation is needed to dissect  
248 the regulation. Interestingly, the dynamics of camalexin synthesis and exudation  
249 responds differentially to pathogenic and commensal or beneficial bacteria. While  
250 the PGP strain seemed to trigger the camalexin synthesis quicker, the response was  
251 only transient. The pathogenic bacterial strain seemed to be slower in initiation of the  
252 synthesis, but this was stronger and remained active for longer and did not diminish.  
253 The levels of camalexin in roots of *B. glumae* PG1 treated Arabidopsis plants in our  
254 system were similar to those in roots treated with *V. longisporum* (Iven et al., 2012).  
255 It seems therefore, that while the camalexin synthesis is initiated by both types of  
256 microorganisms, only upon interaction with pathogens the synthesis and exudation is  
257 sustained long-term. Similarly, when the plants were grown on agar plates and  
258 inoculated with from the leaves, *B. glumae* PG1 induced much higher camalexin levels  
259 than *Pseudomonas sp.* CH267 (Figure 3). *B. glumae* PG1 can thus be used as a tool to  
260 study the control of camalexin synthesis and exudation.

261 The first question addressed using *B. glumae* PG1 was the contribution of the “old”  
262 (CYP71A12 and A13) and “new” (A27 and A28) CYP71A isoforms to camalexin  
263 synthesis and exudation. All mutants clearly showed reduced total accumulation of  
264 camalexin, when the concentrations in shoots, roots, and exudates were summed  
265 (Figure 2A). This was particularly true for the concentration in the roots, which  
266 contributed most to the total camalexin; not surprisingly, as the pathogen was  
267 inoculated by the roots. However, with exception of *cyp71a28* all other mutants  
268 showed also reduced accumulation in the shoots. This result is in contrast to previous  
269 experiments with the “old” *cyp71a12* and *cyp71a13*, as in the former, upon abiotic  
270 elicitation with UV light or AgNO<sub>3</sub> camalexin in leaves was not affected and upon  
271 treatment with spores of fungal pathogen *Plectosphaerella cucumerina* BMM even  
272 increased (Muller *et al.*, 2015; Pastorczyk *et al.*, 2020). On the other hand it agrees  
273 with the measurements of camalexin in roots of soil grown plants, where all four single  
274 mutants showed lower concentrations (Koprivova *et al.*, 2019). The data also clearly  
275 demonstrate the very high contribution of CYP71A12 and CYP71A13 to camalexin  
276 synthesis in all compartments, despite previous conclusion that CYP71A12 is  
277 responsible for root synthesis and exudation upon elicitation with flagellin (Millet *et*  
278 *al.*, 2010). However, the data also show that even in the absence of these two enzymes,  
279 some camalexin is produced and this production is dependent on CYP71A27.  
280 Interestingly, the loss of the individual genes affects also transcript levels of the other

281 members of the biosynthesis network (Figure 2B). With one notable exception, an  
282 induction of *CYP71A13* in the *cyp71a12* background, the level of induction of the  
283 genes by *B. glumae* PG1 was attenuated in the mutants. Since the enzymes of  
284 camalexin synthesis form a metabolon (Mucha *et al.*, 2019) this might be a mechanism  
285 to prevent accumulation of proteins that cannot be part of this structure.

286 The analysis of camalexin in the 3 compartments of different mutants showed that its  
287 concentrations correlate well between all three of them. The genes for camalexin  
288 synthesis are also expressed in both shoots and roots. Thus, the camalexin in the  
289 exudates might originate in the roots as well as in the leaves.

290 To find out which organ is responsible for the synthesis of camalexin found in exudates  
291 we designed two experiments. In a simple approach we cut shoots and roots and  
292 incubated them separately with the bacterial pathogen (Figure 4). This experiment  
293 revealed that both shoots and roots are autonomous in camalexin synthesis. Shoots  
294 alone even produced more camalexin than the whole plants, that can be explained by  
295 the direct leakage of the synthesised camalexin and also by a more rapid contact of the  
296 shoots with the bacteria. The inoculation of leaves with *B. glumae* PG1 (Figure 3)  
297 resulted in a much higher camalexin accumulation in leaves compared to the  
298 hydroculture setup (Figures 1 and 2), which is consistent with the high camalexin in  
299 cut shoots. The high camalexin production in the cut roots, on the other hand, was  
300 unexpected, since in previous experiments with inoculation from the leaves the same  
301 bacterial titre that triggered accumulation of ca. 40 nmol mg<sup>-1</sup> FW in leaves induced  
302 only 1 nmol mg<sup>-1</sup> FW in the roots. This means that a coordination between shoots and  
303 roots is necessary to prevent camalexin overproduction. There are number of examples  
304 how roots and shoots communicate in defense, from the resistance against leaf  
305 pathogens induced by the rhizobacterium *Pseudomonas fluorescens* SS101, which also  
306 involves camalexin (van de Mortel *et al.*, 2012), to the coordination in jasmonate  
307 signalling for resistance to nematodes (Wang *et al.*, 2019). Camalexin synthesis can  
308 be affected by auxin and by miRNA393, both known long-distance signals (Robert-  
309 Seilaniantz *et al.*, 2011). The nature of the signal controlling root camalexin synthesis  
310 in response to *B. glumae* PG1, however, still needs to be determined.

311 The existence of such coordination was clearly demonstrated in the second approach,  
312 using grafting with mutants that do not synthesise camalexin, *pad3* and *cyp79b2*  
313 *cyp79b3*. Any camalexin found in the grafted tissues originating from the mutants must  
314 be transported from WT and thus evidence for a long-distance transport. Indeed, the

315 grafting experiment showed unequivocally that camalexin exuded from the roots  
316 originates in the shoots. Camalexin was found in amounts over background only in  
317 exudates from heterografts with WT shoots, but not roots. Interestingly, when the  
318 plants were inoculated onto the leaves, camalexin synthesis in the leaves was induced  
319 to the same degree, but none of this camalexin was exuded. Thus the plants seem to  
320 recognise where the infection originates and steer the camalexin synthesis there. This  
321 process requires a sophisticated coordination between the roots and the shoots, and  
322 cannot rely solely on the actual perception of the bacteria, as seen also in Figure 3,  
323 where the same bacterial titres triggered different camalexin levels in shoots and roots.  
324 It remains to be seen whether camalexin exudation in response to PGP bacteria  
325 undergoes the same whole plant regulation or whether the camalexin is produced  
326 locally, in the root, as might be indicated by the lower production and different  
327 dynamics.

328 In conclusion, here we show that inoculation of *Arabidopsis* root with a bacterial  
329 pathogen *B. glumae* PG1 triggers camalexin synthesis in shoots and roots and its  
330 exudation. The camalexin can be produced and released by both organs, but in intact  
331 plant the exuded camalexin originates in the shoots. We show that the camalexin  
332 synthesis genes are tightly regulated and loss of function of any of them affects total  
333 camalexin synthesis. Finally, we conclude that camalexin synthesis is controlled by a  
334 whole plant regulation with a need for shoot root communication.

335

## 336 **MATERIALS AND METHODS**

### 337 **Plant material and growth conditions**

338 *Arabidopsis thaliana* L. accession Col-0 was used as wild type alongside mutants in  
339 camalexin synthesis *cyp71a12* (GABI\_127H03), *cyp71a13* (SALK\_105136), *pad3*  
340 (SALK\_026585), *cyp71a27* (SALK\_053817) and *cyp71a28* (SALK\_064792). The  
341 double mutants *cyp71a12 cyp71a13* and *cyp79b2 cyp79b3* were obtained from H.  
342 Frerigmann and T. Gigolashvili, University of Cologne, respectively.

343 For camalexin and expression analyses plants were surface sterilized with chlorine gas.  
344 Seeds were suspended in 0.1 % agarose, distributed onto square 1 cm x 1 cm sterile  
345 nylon membranes (about 30 seeds per sample) and placed in 12 well plates on top of  
346 1 ml of ½ Murashige Skoog (MS) medium with 0.5 % sucrose. After stratification for  
347 2 days in dark and cold the plates were transferred to 22°C and kept in dark for 3 days  
348 to promote etiolation, which greatly simplifies the separation of shoots from

349 membranes. Afterwards the plates were incubated at long day conditons (16 h light/ 8  
350 h dark),  $120 \mu\text{E m}^{-2} \text{S}^{-1}$ , and at  $22^\circ\text{C}$  for further 7 days. The medium was then replaced  
351 with  $\frac{1}{2}$  MS without sucrose and the plants incubated for 24 hours before inoculation  
352 with the bacteria or mock and incubated further in the same conditions for 3 days,  
353 unless specified otherwise.

354 For the experiments with cut shoots and roots immediately before inoculation the  
355 shoots were cut with scissors and roots were freed from the membrane and placed  
356 directly into the nutrient solution.

357

### 358 **Bacterial strains and conditions for cocultivation experiments**

359 For co cultivation experiments 2 bacterial strains were used, *Pseudomonas* sp. CH267  
360 (Haney *et al.*, 2015), obtained from J. R. Dinneny, Stanford University and *B. glumae*  
361 PG1 (Gao *et al.*, 2015), obtained from K.-E. Jäger, Heinrich Heine Universität  
362 Düsseldorf, Germany. The bacteria were kept as glycerol stocks and plated freshly  
363 before experiment on LB plates supplemented with appropriate antibiotics.

364 For inoculation, overnight bacterial cultures were washed two times with sterile 10  
365 mM  $\text{MgCl}_2$  and final  $\text{OD}_{600}$  was measured. *Pseudomonas* sp. CH267 was diluted  
366 stepwise to  $\text{OD}_{600} = 0.0001$ , and *B. glumae* PG1 to  $\text{OD}_{600} = 0.0005$  in 10 mM  $\text{MgCl}_2$ .  
367 Eight  $\mu\text{l}$  of these suspensions were used for inoculation into each well. Eight  $\mu\text{l}$  of 10  
368 mM  $\text{MgCl}_2$  was used as mock treatment. Samples for DNA, RNA and camalexin  
369 (shoots, roots and exudates) were harvested after 3 days of inoculation, except the time  
370 course experiments.

371 Alternatively, plants were grown on square Petri dishes with  $\frac{1}{2}$  MS with sucrose for  
372 18 days and inoculated with 8  $\mu\text{l}$  of suspensions of *Pseudomonas* sp. CH267 ( $\text{OD}_{600} =$   
373 0.0001) or *B. glumae* PG1 ( $\text{OD}_{600} = 0.0005$ ) onto leaves or the bottom 2 mm of root  
374 tips. After 30 min drying the plates were returned to growth cabinet and grown for 3  
375 days at long days.

376

### 377 **Camalexin measurements**

378 Camalexin was extracted from 5-30 mg of plant material as described in (Koprivova  
379 *et al.*, 2019). For extraction of camalexin from exudates the media were centrifuged at  
380 maximum speed for 20 min at  $18^\circ\text{C}$  and purified using 1 ml solid phase extraction  
381 tubes (Discovery- DSC18) according to manufacturer's instructions. Samples were  
382 eluted with 90% (V/V) acetonitrile and 0.1% (V/V) formic acid, dried in a speed vac

383 and dissolved in 50  $\mu$ l of DMSO. 20  $\mu$ l was injected into HPLC and analysed as  
384 described above. For the quantification external standards were used ranging from 1  
385 pg to 1 ng per  $\mu$ l.

386

### 387 **Grafting experiments**

388 For grafting, the selected genotypes were first grown on vertical Petri dishes  
389 containing  $\frac{1}{2}$  MS with sucrose and 0.8% agarose for 5-6 days at short day conditions  
390 (10 h light / 14 h dark) and 22°C. Grafting was performed under binocular microscope  
391 in sterile conditions and the grafts were transferred onto fresh plates for further 18 days  
392 under the same short day light conditions. Graft unions were examined under the  
393 binocular to identify adventitious root formation. Healthy grafts were carefully  
394 transferred into 12 well plates with 1 ml of  $\frac{1}{2}$  MS medium placing the shoots onto  
395 sterile cut cups from 0.5 ml Eppendorf tubes in order to prevent them from direct  
396 contact with the liquid. The plants were inoculated with 8  $\mu$ l of *B. glumae* PG1  
397 suspension at OD<sub>600</sub> = 0.0005 into the solution or onto the leaves and further incubated  
398 for 3 days. Camalexin analysis in shoots, roots, and exudates of individual plants was  
399 performed as described above.

400

### 401 **Expression analysis**

402 To determine transcript levels total RNA was isolated by standard phenol/chlorophorm  
403 extraction and LiCl precipitation. First strand cDNA synthesis was performed using  
404 QuantiTect Reverse transcription Kit (Quiagen) from 800 ng of total RNA.  
405 Quantitative real time RT-PCR (qPCR) was performed using gene-specific primers  
406 (Supplemental Table 1) and the fluorescent dye SYBR Green (Promega). All  
407 quantifications were normalized to the TIP41 (AT4G34270) gene. The RT-PCR  
408 reactions were performed in duplicate for each of the 4 independent samples.

409

### 410 **Determination of bacterial titre**

411 For the estimation of bacterial titre using qPCR the method from (Ross and Somssich,  
412 2016) was adapted. Genomic DNA was extracted using buffer containing 0.025 M  
413 EDTA, 0.2 M Tris pH 8.0, 0.25 M NaCl and 0.5% SDS. After 10 min incubation at  
414 65°C and subsequent centrifugation, supernatant was precipitated with equal volume  
415 of isopropanol, washed with 70% ethanol and resuspended in 100  $\mu$ l of sterile water.  
416 For the qPCR 13 ng of corresponding DNA samples were used with *Arabidopsis* (At

417 primer AT4G26410) and *B. glumae* PG1 specific primer (Burk1 for NR042931). The  
418 qPCR conditions were the same as for expression analysis. The qPCR reactions were  
419 performed in duplicate for each of the 4 independent samples. To relate the qPCR  
420 results to the bacterial titre, first serial dilutions of bacterial suspensions of different  
421 OD<sub>600</sub> have been plated on LB plates and the colonies were counted manually to link  
422 OD<sub>600</sub> and cfu. Subsequently, 10 µl of five 10-fold dilutions of bacterial suspensions  
423 with initial OD<sub>600</sub> = 1.8 were added to 30 mg of Arabidopsis leaves and the DNA  
424 extracted and analysed as above. Using calibration curves plotting  $\Delta Ct$  ( $Ct_{Bg} - Ct_{At}$ )  
425 and the cfu against the  $\log_{10}OD_{600}$  the bacterial titre can be estimated from the  $\Delta Ct$   
426 values.

427

428

#### 429 **ACKNOWLEDGEMENTS**

430 Research in SK's lab is funded by the Deutsche Forschungsgemeinschaft (DFG) under  
431 Germany's Excellence Strategy – EXC 2048/1 – project 390686111 and within the  
432 SPP 2125 DECRyPT.

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435

436 **FIGURE LEGENDS**

437 **Figure 1. Camalexin accumulation upon inoculation with PGP or pathogen**  
438 **bacteria.**

439 Arabidopsis plants were grown on a nylon net in hydroculture for 10 days and  
440 inoculated in the solution with *Pseudomonas sp.* CH267, *B. glumae* PG1 (BG), or  
441 MgCl<sub>2</sub> as mock. Camalexin was measured in leaves (A), roots (B), and exudates (C)  
442 sampled daily over 6 days. Data are presented as means ± S.D. from 4 biological  
443 replicates, each corresponding to at least 30 seedlings. Asterisks mark significant  
444 differences between the values of CH267 and PG1 treated plants (P<0.05, T-test).

445 **Figure 2. Characterisation of mutants in CYP71A genes involved in camalexin**  
446 **synthesis.**

447 The seedlings were grown on a nylon net in hydroculture for 10 days, inoculated in  
448 the solution with *B. glumae* PG1 or MgCl<sub>2</sub> as mock and incubated for 3 days. A  
449 Camalexin was measured in leaves, roots, and exudates of *B. glumae* PG1 treated  
450 plants. Data are presented as means ± S.D. from 4 biological replicates, each  
451 corresponding to at least 30 seedlings. Different letters mark significant differences in  
452 total camalexin (shoots + roots + exudates) between the genotypes (P<0.05, ANOVA).  
453 B Transcript levels of the genes of camalexin synthesis were compared by RT-qPCR  
454 in roots of mock (M) and *B. glumae* PG1 (BG) treated plants. Data are shown as  
455 heatmap of relative expression.

456 **Figure 3. Tissue specificity of camalexin synthesis**

457 Arabidopsis seedlings were grown for 14 days on an agar plate, inoculated with  
458 *Pseudomonas sp.* CH267 (A) or *B. glumae* PG1 (BG) (B) either on the leaves or on  
459 the root tips, and incubated for 3 days. Camalexin accumulation in leaves and roots  
460 was determined by HPLC. Data are presented as means ± S.D. from 3 biological  
461 replicates, each corresponding to 3 individual roots or shoots. C DNA was isolated  
462 from the roots and shoots and subjected to qPCR with primers against *B. glumae* PG1  
463 and Arabidopsis TIP41 gene as control. Using previously established calibration  
464 between Ct values, OD and cfu, the qPCR data were expressed as cfu, presented as  
465 means ± S.D. from 4 biological replicates, each corresponding to 3 individual roots or  
466 shoots. Different letters mark values significantly different at P<0.05 (T-test).

467 **Figure 4. Camalexin in cut roots and shoots**

468 Arabidopsis seedlings were grown on a nylon net in hydroculture for 10 days, the  
469 shoots were cut with scissors, the roots removed from the net and both placed

470 separately to the solution. The shoots, roots, and intact plants were inoculated with *B.*  
471 *glumae* PG1 and further incubated for 3 days. Camalexin accumulation in shoots,  
472 roots, and exudates was determined by HPLC. Data are presented as box plots from at  
473 least 8 biological replicates, each corresponding to about 30 individual roots or shoots.  
474 The box extends from the 25<sup>th</sup> to 75<sup>th</sup> percentiles, the line is plotted at the median, the  
475 whiskers extend from minimum to maximum values.

476 **Figure 5. Analysis of camalexin in grafted plants.**

477 Homografts of *Arabidopsis* WT, *cyp79b2 cyp79b3 (b2/b3)*, and *pad3* and the  
478 heterografts of the WT with the mutants were grown for 18 after the grafting,  
479 transferred onto cut caps of Eppendorf tubes and placed with only the roots submerged  
480 into the hydroculture solution. The plants were then inoculated with *B. glumae* PG1  
481 into the solution (**A**) or onto the leaves (**B**) and further incubated for 3 days. Camalexin  
482 accumulation in shoots, roots, and exudates was determined by HPLC. Data are  
483 presented as box plots from at least 8 individual grafts. The box extends from the 25<sup>th</sup>  
484 to 75<sup>th</sup> percentiles, the line is plotted at the median, the whiskers extend from minimum  
485 to maximum values.

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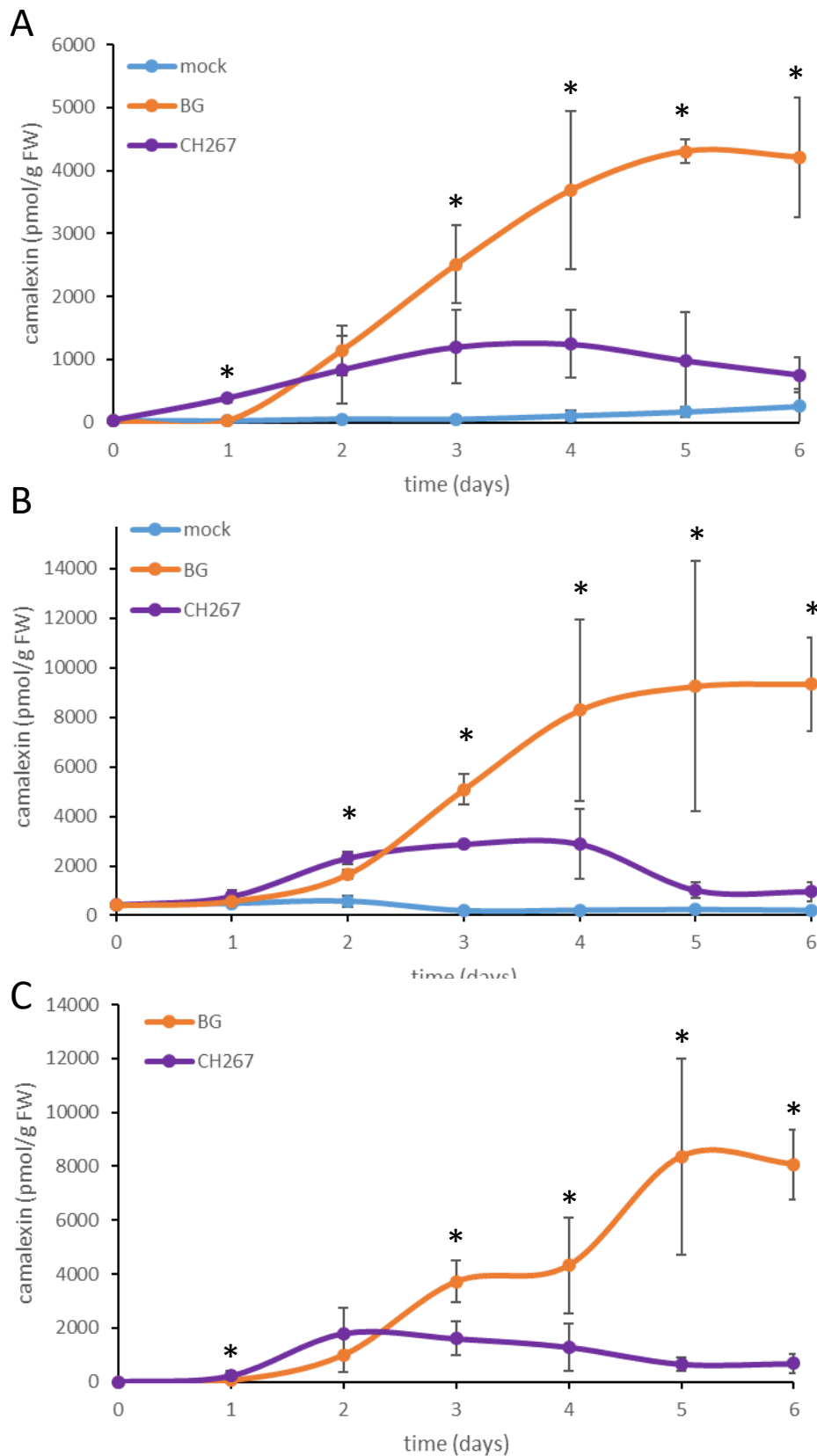
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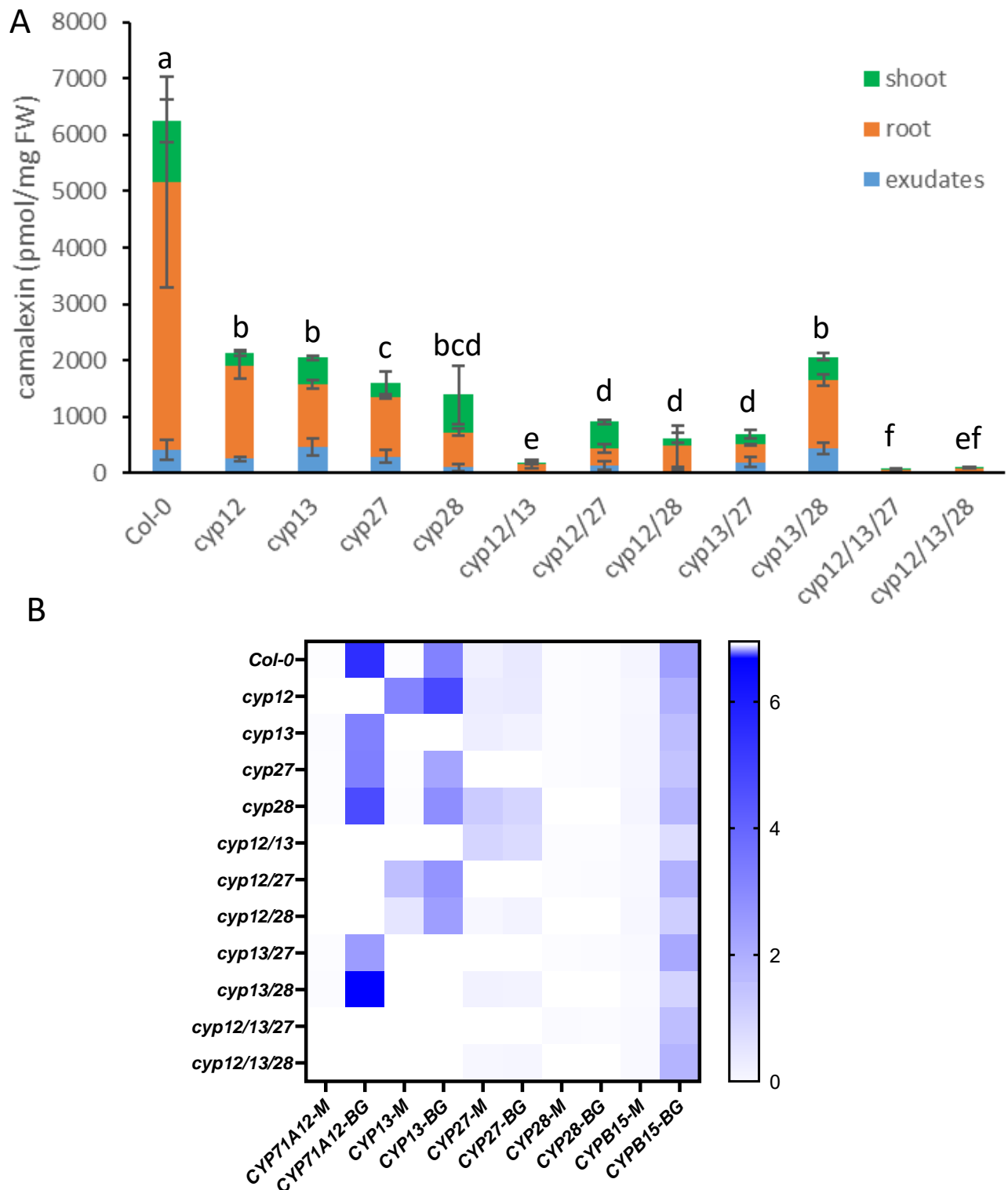
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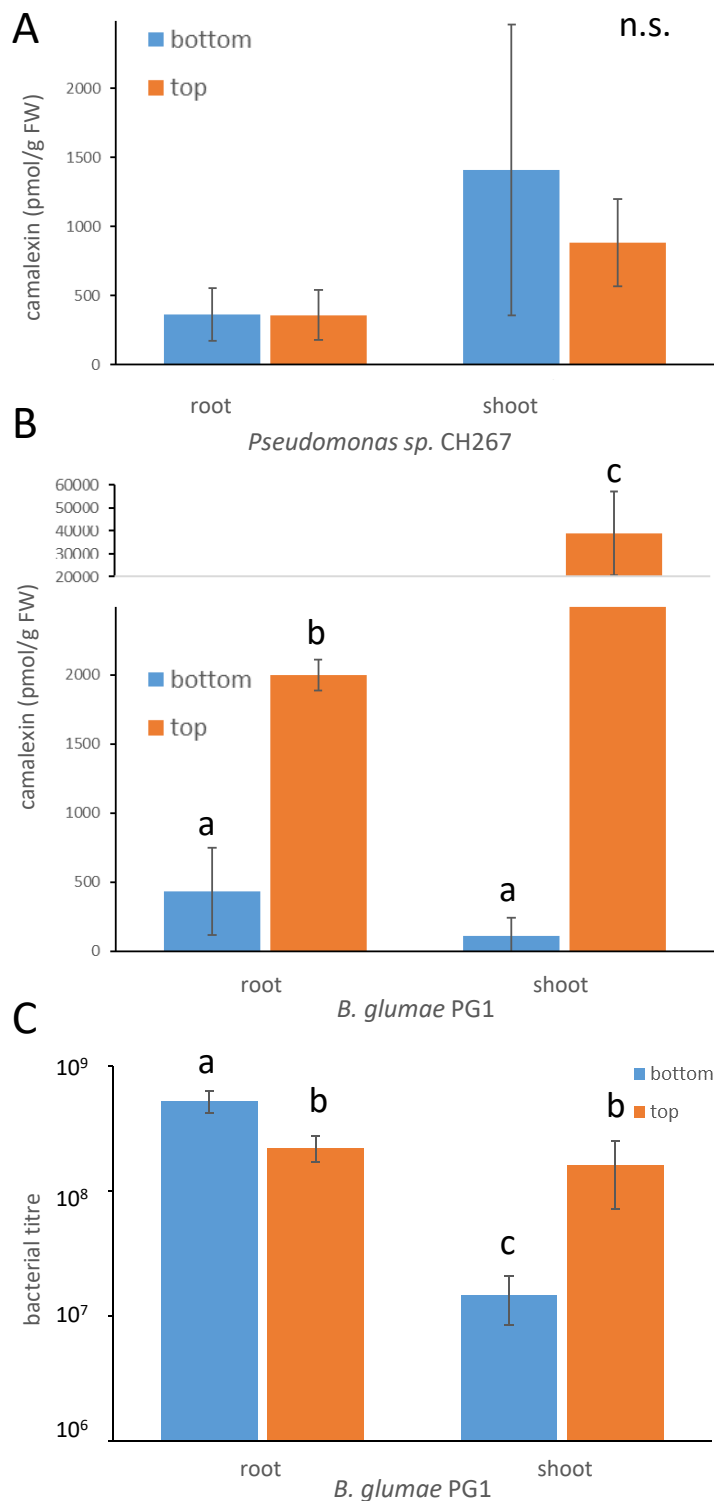
**Figure 1. Camalexin accumulation upon inoculation with PGP or pathogen bacteria.**

Arabidopsis plants were grown on a nylon net in hydroculture for 10 days and inoculated in the solution with *Pseudomonas sp.* CH267, *B. glumae* PG1 (BG), or  $MgCl_2$  as mock. Camalexin was measured in leaves (A), roots (B), and exudates (C) sampled daily over 6 days. Data are presented as means  $\pm$  S.D. from 4 biological replicates, each corresponding to at least 30 seedlings. Asterisks mark significant differences between the values of CH267 and PG1 treated plants ( $P < 0.05$ , T-test).



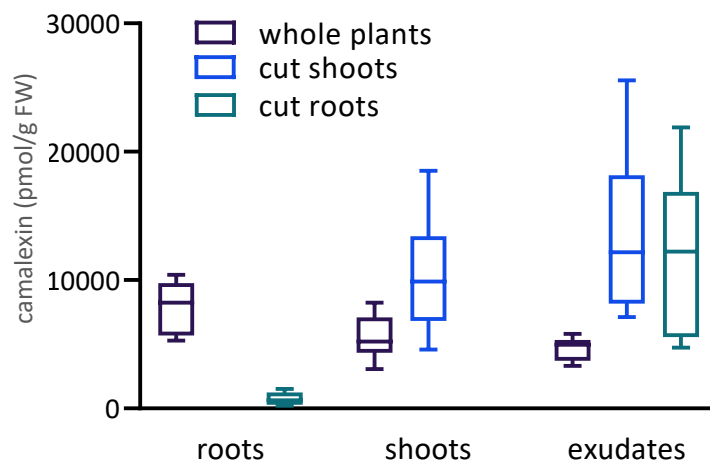
**Figure 2. Characterisation of mutants in *CYP71A* genes involved in camalexin synthesis.**

The seedlings were grown on a nylon net in hydroculture for 10 days, inoculated in the solution with *B. glumae* PG1 or  $MgCl_2$  as mock and incubated for 3 days. **A** Camalexin was measured in leaves, roots, and exudates of *B. glumae* PG1 treated plants. Data are presented as means  $\pm$  S.D. from 4 biological replicates, each corresponding to at least 30 seedlings. Different letters mark significant differences in total camalexin (shoots + roots + exudates) between the genotypes ( $P < 0.05$ , ANOVA). **B** Transcript levels of the genes of camalexin synthesis were compared by RT-qPCR in roots of mock (M) and *B. glumae* PG1 (BG) treated plants. Data are shown as heatmap of relative expression.



### Figure 3. Tissue specificity of camalexin synthesis

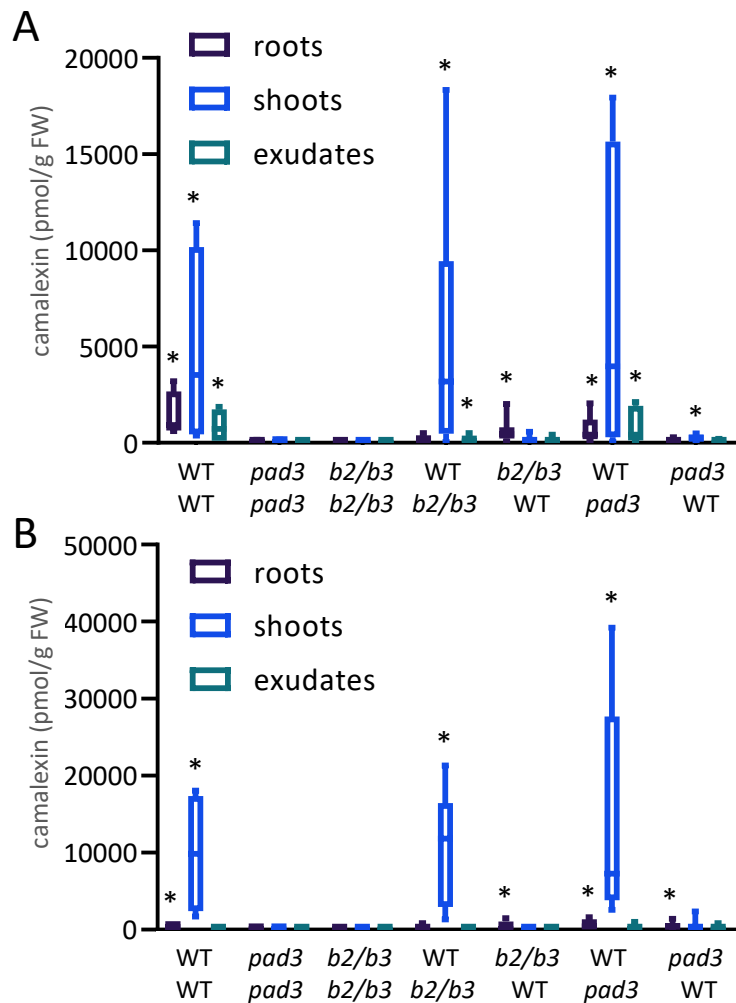
Arabidopsis seedlings were grown for 14 days on an agar plate, inoculated with *Pseudomonas sp. CH267* (A) or *B. glumae* PG1 (BG) (B) either on the leaves or on the root tips, and incubated for 3 days. Camalexin accumulation in leaves and roots was determined by HPLC. Data are presented as means  $\pm$  S.D. from 3 biological replicates, each corresponding to 3 individual roots or shoots. C DNA was isolated from the roots and shoots and subjected to qPCR with primers against *B. glumae* PG1 and Arabidopsis TIP41 gene as control. Using previously established calibration between Ct values, OD and cfu, the qPCR data were expressed as cfu, presented as means  $\pm$  S.D. from 4 biological replicates, each corresponding to 3 individual roots or shoots. Different letters mark values significantly different at  $P < 0.05$  (T-test).



**Figure 4. Camalexin in cut roots and shoots**

Arabidopsis seedlings were grown on a nylon net in hydroculture for 10 days, the shoots were cut with scissors, the roots removed from the net and both placed separately to the solution. The shoots, roots, and intact plants were inoculated with *B. glumae* PG1 and further incubated for 3 days. Camalexin accumulation in shoots, roots, and exudates was determined by HPLC. Data are presented as box plots from at least 8 biological replicates, each corresponding to about 30 individual roots or shoots. The box extends from the 25<sup>th</sup> to 75<sup>th</sup> percentiles, the line is plotted at the median, the whiskers extend from minimum to maximum values.





**Figure 5. Analysis of camalexin in grafted plants.**

Homografts of *Arabidopsis* WT, *cyp79b2 cyp79b3* (*b2/b3*), and *pad3* and the heterografts of the WT with the mutants were grown for 18 after the grafting, transferred onto cut caps of Eppendorf tubes and placed with only the roots submerged into the hydroculture solution. The plants were then inoculated with *B. glumae* PG1 into the solution (**A**) or onto the leaves (**B**) and further incubated for 3 days. Camalexin accumulation in shoots, roots, and exudates was determined by HPLC. Data are presented as box plots from at least 8 individual grafts. The box extends from the 25<sup>th</sup> to 75<sup>th</sup> percentiles, the line is plotted at the median, the whiskers extend from minimum to maximum values.