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

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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.*,

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

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

RESULTS

Both PGP and pathogenic bacteria trigger camalexin synthesis and exudation

Previous work showed that camalexin can be exuded from plant roots incubated with PGP bacteria or the bacterial-derived peptide elicitor flagellin, which is the prerequisite of camalexin function in shaping microbiome function (Koprivova *et al.*, 2019; Millet *et al.*, 2010). PGP bacteria and flagellin trigger also camalexin accumulation in roots, as does infection with root fungal pathogen *Verticillium longisporum* (Iven *et al.*, 2012). To test, whether camalexin synthesis and exudation is triggered also by bacterial pathogens we incubated *Arabidopsis* growing in hydroculture with *Burkholderia glumae* PG1 (Gao *et al.*, 2015) or a PGP bacterium *Pseudomonas sp.* CH267 (Haney *et al.*, 2015; Koprivova *et al.*, 2019). To obtain a better picture of a control of camalexin synthesis we analysed its accumulation in roots and shoots and in the exudates. Both bacteria triggered camalexin synthesis in all three compartments, whereas minimal camalexin levels were detected in roots and shoots of 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

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

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

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

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

The first question addressed using *B. glumae* PG1 was the contribution of the “old” (CYP71A12 and A13) and “new” (A27 and A28) CYP71A isoforms to camalexin synthesis and exudation. All mutants clearly showed reduced total accumulation of camalexin, when the concentrations in shoots, roots, and exudates were summed (Figure 2A). This was particularly true for the concentration in the roots, which contributed most to the total camalexin; not surprisingly, as the pathogen was inoculated by the roots. However, with exception of *cyp71a28* all other mutants showed also reduced accumulation in the shoots. This result is in contrast to previous experiments with the “old” *cyp71a12* and *cyp71a13*, as in the former, upon abiotic elicitation with UV light or AgNO₃ camalexin in leaves was not affected and upon treatment with spores of fungal pathogen *Plectosphaerella cucumerina* BMM even increased (Muller *et al.*, 2015; Pastorczyk *et al.*, 2020). On the other hand it agrees with the measurements of camalexin in roots of soil grown plants, where all four single mutants showed lower concentrations (Koprivova *et al.*, 2019). The data also clearly demonstrate the very high contribution of CYP71A12 and CYP71A13 to camalexin synthesis in all compartments, despite previous conclusion that CYP71A12 is responsible for root synthesis and exudation upon elicitation with flagellin (Millet *et al.*, 2010). However, the data also show that even in the absence of these two enzymes, some camalexin is produced and this production is dependent on CYP71A27. Interestingly, the loss of the individual genes affects also transcript levels of the other

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

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

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

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

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

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

MATERIALS AND METHODS

Plant material and growth conditions

Arabidopsis thaliana L. accession Col-0 was used as wild type alongside mutants in camalexin synthesis *cyp71a12* (GABI_127H03), *cyp71a13* (SALK_105136), *pad3* (SALK_026585), *cyp71a27* (SALK_053817) and *cyp71a28* (SALK_064792). The double mutants *cyp71a12 cyp71a13* and *cyp79b2 cyp79b3* were obtained from H. Frerigmann and T. Gigolashvili, University of Cologne, respectively.

For camalexin and expression analyses plants were surface sterilized with chlorine gas. Seeds were suspended in 0.1 % agarose, distributed onto square 1 cm x 1 cm sterile nylon membranes (about 30 seeds per sample) and placed in 12 well plates on top of 1 ml of ½ Murashige Skoog (MS) medium with 0.5 % sucrose. After stratification for 2 days in dark and cold the plates were transferred to 22°C and kept in dark for 3 days 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°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 MgCl_2 and final 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 MgCl_2 .
367 Eight μl of these suspensions were used for inoculation into each well. Eight μl of 10
368 mM 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 μl 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°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 µl of DMSO. 20 µl 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 µl.

386

387 **Grafting experiments**

388 For grafting, the selected genotypes were first grown on vertical Petri dishes
389 containing ½ 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 ½ 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 µl of *B. glumae* PG1
397 suspension at OD₆₀₀ = 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 µl 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 OD₆₀₀ have been plated on LB plates and the colonies were counted manually to link
 422 OD₆₀₀ and cfu. Subsequently, 10 µl of five 10-fold dilutions of bacterial suspensions
 423 with initial OD₆₀₀ = 1.8 were added to 30 mg of Arabidopsis leaves and the DNA
 424 extracted and analysed as above. Using calibration curves plotting ΔCt (Ct_{Bg} - Ct_{At})
 425 and the cfu against the log₁₀OD₆₀₀ the bacterial titre can be estimated from the ΔCt
 426 values.

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 431 Germany's Excellence Strategy – EXC 2048/1 – project 390686111 and within the
 432 SPP 2125 DECrypT.

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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 MgCl₂ as mock. Camalexin was measured in leaves (A), roots (B), and exudates (C)
442 sampled daily over 6 days. Data are presented as means $\pm$ 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 MgCl₂ 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 $\pm$ 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
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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
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464 between Ct values, OD and cfu, the qPCR data were expressed as cfu, presented as
465 means $\pm$ 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,
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 473 least 8 biological replicates, each corresponding to about 30 individual roots or shoots.
 474 The box extends from the 25th to 75th 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
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 484 to 75th percentiles, the line is plotted at the median, the whiskers extend from minimum
 485 to maximum values.

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

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