# ABSTRACT

Tryptophan-derived specialized metabolites are among central components of Brassicaceae immunity, yet the extent to which their defense function depends on their biochemical activity rather than on the spatial and temporal context of their accumulation remains unclear. To address this question, two indole phytoalexin pathways have been re-engineered in the susceptible to infection *Arabidopsis thaliana pen2 cyp71a12 cyp71a13* triple mutant background, which is deficient in camalexin production and lacks the pre-invasive indole glucosinolate-based defense. The introduced genes were placed under the control of the pathogen-inducible, epidermis-active *PENETRATION 2* (*PEN2*) and *CYP81F2* promoters, and the resulting lines were challenged with the necrotrophic fungus *Plectosphaerella cucumerina*.

The native camalexin branch was re-engineered to change its cellular localization. Expression of *CYP71A13*, encoding the first camalexin-specific enzyme, alone supported only modest camalexin accumulation. Combined epidermal expression of *CYP71A13* and *Phytoalexin Deficient 3*, encoding the last enzyme on camalexin branch, markedly improved pathway completion upon infection, indicating that the remaining required glutathione *S*-transferase and γ-glutamyl peptidase activities are induced locally and can be recruited without additional engineering. Of note, the pools of camalexin hydroxylation and glycosylation products in the transgenic lines were clearly enriched relative to wild-type plants, indicating that relocation of this phytoalexin to the epidermis exposes it to elevated detoxification activity as compared with its native mesophyll site. Despite this partial diversion, the engineered branch significantly restricted *P. cucumerina* proliferation relative to the background mutant, demonstrating that camalexin retains its defense function in a non-native cellular compartment.

The foreign brassinin pathway from *Brassica rapa* was introduced into the same mutant background. Strikingly, expression of the *B. rapa dithiocarbamate S-methyltransferase* gene alone was sufficient to yield brassinin in the PEN2 background activity, indicating that the core route from the indole glucosinolate pool to the dithiocarbamate intermediate is conserved between the two species and does not strictly depend on a *Brassica*-specific glucosinolate-hydrolyzing enzyme. Co-expression of the *B. rapa brassinin-associated β-thioglucoside glucohydrolase* gene (*BABG*) further enhanced brassinin accumulation, identifying it as a more efficient contributor to indol-

3-ylmethyl-dithiocarbamate formation than the native *A. thaliana* enzymes. In the engineered background, brassinin was further processed by native hydroxylation and glycosylation activities, whereas formation of cyclobrassinin and spirobrassinin required additional expression of *B. rapa CYP71CR1* and *CYP71CR2*, respectively. As in the case of camalexin, the brassinin-producing lines displayed a significant restriction of *P. cucumerina* proliferation.

Together, these results show that both a native and a foreign indole phytoalexin can be functionally reconstituted in pathogen-challenged epidermal cells of a susceptible *A. thaliana* background, where they significantly restrict fungal proliferation. This supports the interpretation that the immune function of tryptophan-derived metabolites is shaped mainly by their chemical identity and not so much dependent on cellular localization of their production. This also highlights BABG as a particularly effective glucosinolate-hydrolyzing enzyme for engineering foreign indolic phytoalexins in *A. thaliana*.