Introduction

Rice blast disease accounts for yield losses that could feed about 60 million rice consumers annually (Uda et al., 2018). *Pyricularia oryzae* (Syn: *Magnaporthe oryzae*), the fungus that causes rice blast disease (Zhong et al., 2018), is ubiquitously present in rice-producing regions across the globe. In addition to rice, blast fungus can infect wheat, barley, millet, sorghum, rye and other cultivated and non-cultivated grass plants and is therefore considered one of the most important plant pathogens (Dean et al., 2005; Isam et al., 2016).

Rice blast fungus initiates infection by producing asexual spores (conidia) that settle and glue itself firmly to plant tissues (Marcel et al., 2010). Under favourable conditions, the spore propagule (inoculum) germinates and produces a short hyphae-like structure typically from the apical cell called germ tube, which later differentiates into a bulbous infectious structure known as appressoria (Ghatak, 2013; Wilson and Talbot, 2009). The appressoria further differentiate into rigid and robust penetration structure (penetration-peg) which is engaged by the blast pathogen to physically rupture the cuticle of susceptible host plants for successful invasion, colonization of host cells and the manifestation of blast symptoms (Fernandez and Orth, 2018; Howard and Valant, 1996).

Plants, on the other hand, possess two innate defence systems, namely pattern-triggered immunity (PTI) and effector-triggered immunity (ETI; Kanyuka and Rudd, 2019; Peng et al., 2018). While PTI represents the first line of defence, it is activated through the recognition of microbial-associated signatures by pattern-recognition receptors (PRRs) found on cell surfaces. PTI-mediated immune response usually involves rapid accumulation of defence hormones, including abscisic acid (ABA), salicylic acid (SA), jasmonic acid (JA) and other secondary metabolites bolster plant immunity against invading pathogens. In contrast, ETI is activated by the recognition of pathogen effector proteins by intracellularly localized nucleotide-binding, leucine-rich repeat proteins (NLRs) receptors. Current rice blast control strategies rely heavily on the use of rice cultivars with inherent basal resistance against the pathogen, as well as the breeding of resistant (R)-gene aided cultivars including CO39, Pi-b, Pi-4b, Pi-a, Pi-9, Pi-t, Pi-pita, Pi-gm, Pi-9b, Pi-2, among others (Deng et al., 2006; Huang et al., 2008; Promchuy et al., 2017; Yang et al., 2017). However, the rice blast pathogen undergoes evolutionary transformations that enable it to overcome R-gene supported resistance (Bao et al., 2017; de Jonge et al., 2013).

Beside R-genes, rice-specific metabolites, such as oryzalexins, phytocassanes, momilactones and sakuranetin, have been shown to inhibit bacterial and fungal pathogens, including blast fungus (Akatsuka et al., 1985; Otomo et al., 2004), while a more recent whole-metabolome profiling revealed differences between rice cultivars (Hu et al., 2014; Kusano et al., 2015). However, knowledge on the contributions of non-cultivar-specific mechanisms such as pathogen-induced resistant-related phytochemicals is scarce. Understanding how inherent versus pathogen-induced metabolomics differences influence the resistance or susceptibility different rice cultivars against the rice blast fungus would be crucial in advancing the breeding of durably resistant rice cultivars against the devastating rice blast. Here, we investigated how *P. oryzae*-mediated metabolome reprogramming affects the susceptibility or resistance of different rice cultivars to *P. oryzae*. 

Bayogenin 3-O-cellobioside confers non-cultivar-specific defence against the rice blast fungus *Pyricularia oryzae*

Justice Norvienyeku1,1,2, Lili Lin1,1, Abdul Waheed1, Xiaomin Chen1, Jiandong Bao1, Sami Rukaiya Aliyu1, Lianyu Lin1, Ammarah Shabbir1, Wajjiha Batool1, Zhenhui Zhong1, Jie Zhou1, Guodong Lu1 and Zonghua Wang1,2,*

1State Key Laboratory of Ecological Pest Control for Fujian and Taiwan Crops & Ministry of Education Key Laboratory of Biopesticides and Chemical Biology, College of Life Sciences, Fujian Agriculture and Forestry University, Fuzhou, China
2Institute of Oceanography, Minjiang University, Fuzhou, China

Summary

Rice cultivars from *japonica* and *indica* lineage possess differential resistance against blast fungus as a result of genetic divergence. Whether different rice cultivars also show distinct metabolomic changes in response to *P. oryzae*, and their role in host resistance, are poorly understood. Here, we examine the responses of six different rice cultivars from *japonica* and *indica* lineage challenged with *P. oryzae*. Both susceptible and resistant rice cultivars expressed several metabolites exclusively during *P. oryzae* infection, including the saponin Bayogenin 3-O-cellobioside. Bayogenin 3-O-cellobioside level in infected rice directly correlated with their resistant attributes. These findings reveal, for the first time to our knowledge that besides oat, other grass plants including rice produces protective saponins. Our study provides insight into the role of pathogen-mediated metabolomics reprogramming in host immunity. The correlation between Bayogenin 3-O-Cellobioside levels and blast resistance suggests that engineering saponin expression in cereal crops represents attractive and sustainable disease management.
Results

Raw leaf extracts from inoculated rice seedlings inhibit infectious development of *P. oryzae*

Firstly, we confirmed the susceptibility and resistance of six different rice cultivars (CO39, LTH, NPB Pi-B, Pi-4B and Pi-gm) (Figure 1a) from both indica and japonica lineage to *P. oryzae* (Table S1). Briefly, we spray-inoculated three-week-old rice seedlings with conidia suspensions prepared from the *P. oryzae* Guy11 strain, incubated them in a dark and humid chamber and transferred them to a growth chamber. At 7 days post-inoculation (hpi), we assessed the type and severity of lesions on leaf tissues per published standards index for scoring rice blast lesion (Ghazanfar *et al.*, 2009). We found that the CO39, NPB and LTH cultivars were highly susceptible, with a higher number of severe blast lesions (type 4 and 5 lesions), whereas Pi-gm, Pi-4b and Pi-b cultivars displayed moderate to complete immunity against blast fungus (Figure 1b).

To assess whether metabolites induced by rice blast infection are involved in the resistance against *P. oryzae*, we tested the anti-fungal activity of crude extracts from inoculated rice seedlings and. We used the crude leaf extracts to wash conidia from the *P. oryzae* Guy11 strain, prepared conidia suspensions and inoculated hydrophobic coverslips to induce appressorium formation *in vitro*. While the mock control (spore suspensions prepared with sterilized double deionized water), the internal control (spore suspensions prepared with 3% v/v methanol), and crude extracts from the untreated susceptible, and resistant rice cultivars have no inhibitory effect germination and appressorium formation (Figure 1c).

![Figure 1](image-url)

**Figure 1** Crude leaf extracts from pre-inoculated resistant rice cultivars significantly inhibits germination and appressorium formation in *P. oryzae*. (a). Showed blast symptoms and the susceptibility index of homogenously susceptible rice cultivars sprayed inoculation with conidia suspensions of *P. oryzae*. (b) Showed the development blast symptoms and resistance attributes displayed by moderate to completely resistant rice cultivars sprayed inoculation with conidia suspensions of *P. oryzae*. Note hypersensitive response (HR)-0 represents complete resistance, and lesion type 1–2 represents moderate resistance, while lesion type 3–5 signifies complete susceptibility. (c, d) displayed the morphology of appressorium formed by conidia treated with sterilized double deionized water as mock control and 3% v/v methanol as internal control, respectively. (e). Exhibit germination and appressorium formation characteristics of rice blast fungus spores treated with total crude extracts obtained from non-inoculated susceptible rice cultivars. (f). Exhibit germination and appressorium formation characteristics of rice blast fungus spores treated with total crude extracts obtained from pre-inoculated susceptible rice cultivars. (g). The micrograph displays the inhibitory effects of total crude extracts obtained from non-inoculated resistant rice cultivars on the germination of rice blast fungus spores. (h). The micrograph displays the inhibitory effects of total crude extracts obtained from pre-inoculated resistant rice cultivars on the germination of rice blast fungus spores. (i, j). The stacked column graph is a statistical presentation of *P. oryzae* spores treated with total crude extracts from resistant rice cultivars. Results for infection assay (a, b) were obtained from three biological experiments with five technical replicates *n* = 600 with a conidia concentration of 3.0 × 10^{-5}. Microscopy examination and statistical analysis, c, d, e, f, g & h (*n* = 750). Scale bars, 10 μm.
formation, we observed that in 5/6 cases, crude leaf extracts from the pre-inoculated resistant rice seedlings inhibited germination and appressorium formation (Figure 1c–j). Furthermore, to mimic the appressorium formation process in nature, leaf and sheath tissues of susceptible rice of cultivars (CO39 and NPB) and resistant cultivars (Pi-4b, and Pi-gm) were inoculated with spore suspensions and incubated under humid conditions in a dark chamber with a sustained temperature of 25 °C. Results obtained from microscopy examinations showed that only a few of the germinated spores could progress to form appressorium on leaf tissues of resistant rice cultivars while majority of spores inoculated leaf tissues of the susceptible germinated and differentiated into appressorium (Figure S5a). Additional leaf sheath histopathological assessment assays showed an apparent restriction of invasive hyphae to single cells of the resistant rice cultivars (Figure S5b). These results indicate the inhibitory effect of crude extracts from resistant cultivars pre-inoculated with the rice blast fungus may be due the cumulative effects anti-blast metabolites present in the crude leaf extract.

Resistant rice cultivars accumulate higher levels of Bayogenin 3-O-cellobioside upon inoculation with P. oryzae

To monitor the changes in rice seedlings due to P. oryzae infection, we performed metabolomic analysis of inoculated and non-inoculated rice cultivars. We spray-inoculated two-week-old susceptible (CO39, NPB and LTH) and resistant (Pi-gm, Pi-4B and Pi-B) rice seedlings with conidia suspensions along with non-inoculated controls (Fig S1), harvested leaf tissues at 12-hpi and extracted metabolites in methanol using QTOF-UPHPLC (see Methods). Also to ensure exclusion of fungi-specific metabolites, we analysed the metabolome of P. oryzae at different developmental stages including vegetative growth (mycelium stage), conidia (aberrant conidia/ resting stage) and conidia germination and appressorium formation stage (infectious development stage) (see Methods and (Norvienyeku et al., 2017)). Principal component analysis (PCA) of the data revealed the reproducible identification of metabolites in at least five out of the seven independent repeats (Figure 2a).

To uncover disease-relevant metabolites from the whole metabolome profiles, we developed a robust filtering system to identify of metabolites in at least five out of the seven independent repeats (Figure 2a). In addition, to identify of metabolites with a KEGG code, 18 were present in both susceptible and resistant rice cultivars after inoculation, 18 were present exclusively in the susceptible cultivars after inoculation, and 45 were present exclusively in resistant cultivars after inoculation (Figure 2b–d).

To determine the function of the 18 metabolites present in both susceptible and resistant rice seedlings upon infection, we used their compound codes, chemical formulae and common names to search publicly available metabolite libraries, including KEGG compound, KEGG BRITE, PubChem Compound, The Human Metabolome Database, The Small Molecule Pathway Database and The Toxin and Toxin Target Database (Kim et al., 2018). We found that 5/18 metabolites are known to be functional phytochemicals: podorhizol beta-D-glucoside, Bayogenin 3-O-cellobioside, (-+)-Syringaresinol O-beta-D-glucoside, 4-methylsulfonylbutyl glucosinolate and dihydromyricetin (Figure 2e,f and Figure S2). Indeed, podorhizol beta-D-glucoside and 4-methylsulfonylbutyl glucosinolate are known to enhance plant immunity against diverse pathogens (Abuyusuf et al., 2018; Kumaraswamy et al., 2011).

Intriguingly, unlike the other four metabolites, the levels of Bayogenin 3-O-cellobioside were relatively low in the inoculated, susceptible rice cultivars compared to in the inoculated, resistant group (Figure 2 g–k). Furthermore, we observed that Bayogenin 3-O-cellobioside levels were about ~ 1000-fold higher in the completely resistant cultivar Pi-gm than in the moderately resistant cultivars Pi-4b and Pi-b (Figure 2k). Thus, Bayogenin 3-O-cellobioside levels increase specifically upon inoculation of rice seedlings with P. oryzae and directly correlate with the extent of resistance. Our results suggest that Bayogenin 3-O-cellobioside is a novel general defence molecule produced in response to rice blast fungus infection, in both susceptible and resistant rice cultivars (Figure S3a–d).

Glycosylated Bayogenin inhibits the pathogenic development of P. oryzae in vitro

Saponins are glycosylated triterpenoids, generated in many plants as a secondary metabolite. Different saponins display potent insecticidal and fungicidal activities against a broad range of plant parasites (Abbruscato et al., 2014; Doughari, 2015). Unlike dicotyledonous plants, monocots such as rice are considered triterpenoid-poor plants (Osbourn, 2003). Avenacin is a saponin in oat (Avena spp) that provides defence against soil-borne fungal pathogens (Anderson et al., 2010). Bayogenin 3-O-cellobioside is a glycosylated saponin, consisting of a non-sugar aglycone (Bayogenin) linked to a sugar glycone (Cellobioside). Because Bayogenin 3-O-cellobioside is not commercially available, we tested both non-glycosylated Bayogenin and glycosylated Bayogenin (Bayogenin 3-O-beta-D-glucopyranoside) in an in vitro conidia germination and appressorium formation assays. We prepared titrations of these compounds and used them to wash conidia produced by the P. oryzae Guy11 strain. Subsequently, we prepared conidia suspensions and inoculated a hydrophobic cover slip. Relative to the mock control and the internal control (Figure 3a–c), we observed a dose-dependent inhibition of conidia germination and appressorium formation in vitro with increasing concentrations of Bayogenin 3-O-beta-D-glucopyranoside (5 nM/L–10 nM/L) (Figure 3c and g). In contrast, increasing the concentrations of the non-glycosylated Bayogenin did not affect germination or appressorium formation nor did the control treatments (Figure 3d and g d). We also observed a significant inhibition in the vegetative growth of P orate cultured on complete media (nutrient sufficient culture media) supplemented with Bayogenin 3-O-beta-D-glucopyranoside (5 nM/L–10 nM/L); however, the supplementation of culture medium with crude extracts from pre-inoculated and non-inoculated rice seedling
cultures has no significant effect on the vegetative development of the rice blast fungus (Fig S5a-d).

To ascertain whether other saponins would similarly inhibit conidia germination and appressorium formation, we used the in vitro assays to test two closely related saponins, Hedegeranin and oleanolic acid. Despite their reported insecticidal effects (Cai et al., 2017; Christensen et al., 2018; Kortbeek et al., 2018), we found that washing conidia with 5–100 nM/L of Hedegeranin or

Figure 3 Glycosylated Bayogenin exclusively inhibits the germination and appressorium formation in conidium produced by the rice blast fungus. (a). Represents the negative control group that portrays the morphological characteristics of appressorium produced by P. oryzae conidia suspensions prepared with double deionized water (ddH2O). (b). Displayed the impact of 3% and 5% per cent methanol on conidia germination and appressorium formation (positive control group). (c). The micrograph represents the inhibitory effects of different concentrations of glycosylated Bayogenin (Bayogenin 3-O-β-D-glucopyranoside) on conidia germination and appressorium development. (d) Showed the influence of non-glycosylated Bayogenin on germ tube speciation and appressorium development in P. oryzae. (e) Showed the impact of Hedegeranin on conidia germination, and appressorium formation in P. oryzae conidium. (f). Showed germination and appressorium formation characteristics of P. oryzae conidia treated with oleanolic acid. (g). The stacked column bar graph is a statistical display of the effect of different saponins on conidia germination and appressorium morphogenesis. Statistical computation was performed using average values obtained from three biological experiments with three replicate each time for all treatment (n = 750). Scale bars, 10 μm.
oleanolic acid did not adversely affect conidia germination and appressorium morphogenesis of *P. oryzae* in vitro (Figure 3e, f, and g). Together, these findings suggest that glycosylated Bayogenin, such as the Bayogenin 3-O-cellobioside, and Bayogenin 3-O-β-D-glucopyranoside are potent phytochemicals that specifically the infectious development of *P. oryzae*.

**Differential expression of steroid biosynthesis enzymes during rice–*P. oryzae* interaction**

Genes encoding enzymes involved in steroid biosynthesis, including β-amyrin synthases, uridine diphosphate (UDP) glucuronoyltransferases and UDP-glycosyltransferases (UDP-GTs), appear to mediate the biosynthesis of saponins in plants (Augustin et al., 2012; Haralampidis et al., 2002; Itkin et al., 2013; Liu et al., 2018; Osbourn, 2003; Sawai and Saito, 2011). We identified a total of 6 rice β-amyrin synthases and 145 putative rice-specific UDP-GTs from the publicly available glycosyltransferase database (Chandran et al., 2016). Our bioinformatics analysis revealed that the rice UDP-GTs family can be classified into 33 subfamilies (groups) based on the alignment of a shared motif (Figure 4a). Furthermore, 75 of these putative rice UDP-GTs are within genes clusters on multiple chromosomes (Figure 4b).

We hypothesized that increased expression of β-amyrin synthases and/or UDP-GTs might support the increased production of Bayogenin 3-O-cellobioside in rice seedlings inoculated with *P. oryzae*. To examine how inoculation affects the expression of these genes in different cultivars, we analysed resistant (Pi-gm) and susceptible (CO39, NPB) seedlings by RNA sequencing. We found that 102 UDP-GTs were expressed exclusively upon inoculation of Pi-gm, whereas 83 and 13 UDP-GTs were expressed exclusively upon inoculation of CO39 and NPB, respectively (Figure 4c). Also, two β-amyrin synthase genes were significantly (5- to 12-fold) up-regulated in Pi-gm and CO39 rice cultivars in response to *P. oryzae* challenge (Figure 4d).

Thus, various UDP-GT and β-amyrin synthase genes are expressed onl upon inoculation of rice with *P. oryzae*, in both susceptible and resistant seedlings. We supposed that differential expression of steroid biosynthesis enzymes may contribute to the induction of Bayogenin 3-O-cellobioside upon inoculation of rice with *P. oryzae*.

**Two independent UDP-glucosyltransferase rice mutants showed compromised resistance against *P. oryzae***

From transcriptomic analyses, we asserted that UDP-GTs-mediated biogenesis of glycosides during *P. oryzae* infection partly promotes rice cultivars’ resistance against the blast fungus. To validate this position, we obtained three UDP-GTs genes (Os01g0176200, Os02g0329933 and Os05g0550100), one putative β-amyrin synthase (Os06g0483200) mutant and the parental control Zhonghua 11 (ZH11) lines (Table S3) obtained from Rice Mutant Database (RMD) resource centre (Zhang et al., 2006). Two of the UDP-GTs (Os01g0176200 and Os02g0329933) were comparatively and exclusively up-regulated in the resistant rice cultivars Pi-gm during *P. oryzae* infection. Results obtained from the individual rice mutant lines inoculated with spore suspensions of the *P. oryzae* (FJ81278) isolate and incubated for 7 days showed that while the resistance exhibited by Os05g0550100 and Os06g0483200 null mutant rice lines against the blast fungus was comparable to the wild-type control group ZH11 and Pi-gm, T-DNA-mediated disruption of Os01g0176200 and Os02g0329933 partially attenuated the resistance of the ZH11 against the blast fungus; hence, typical blast lesions developed on leaf tissues of the ko_Os01g0176200 and ko_Os02g0329933 rice lines. However, the level of blast susceptibility displayed by the ko_Os01g0176200 and ko_Os02g0329933 mutant lines was lower compared to the susceptibility recorded for the almost homogenously blast susceptible CO39 rice cultivar (Figure 5a).

Additional histopathological assays instituted in this study to ascertain the impact of the individual UDP-GTs and the β-amyrin synthase genes disruptions on the development of the blast fungus in planta through the inoculating leaf sheaths excised from the respective rice lines with FJ81278 asexual spores in suspension and incubated under dark and humid conditions. Subsequent microscopic examination rice sheath epidermal tissues at 24, 48 and 72 hpi showed that ko_Os05g0550100, ko_Os06g0483200, ZH11 and Pi-gm posed sustained resistance and restricted the pathogenic development of the FJ81278 isolate by restricting either the penetration or the colonization efficiency of the invading fungus in vivo. Conversely, the blast fungus invaded and successfully colonized sheath tissues of the Os01g0176200 and ko_Os02g0329933 rice lines at 24-hpi and extended to secondary and tertiary cells at 48 and 72 hpi, respectively (Figure 5b). The mild susceptibility displayed by the Os01g0176200 and ko_Os02g0329933 T-DNA mutagenic lines informed our reasoning that more than one UDP-GTs likely contributes to the resistance of rice against the blast fungus directly or indirectly by mediating the glycosylation of steroidal compounds including Bayogenin 3-O-cellobioside.

**DISCUSSION**

We report that the rice metabolite Bayogenin 3-O-cellobioside (saponin) is a novel, general defence molecule that accumulates exclusively in response to *P. oryzae* infection, in susceptible and resistant rice cultivars from both japonica and indica linages. The levels of Bayogenin 3-O-cellobioside after inoculation with *P. oryzae* directly correlated with the resistance attributes of the individual rice cultivars. Therefore, susceptible and resistant rice cultivars display metabolomic differences not only before infection but also after infection and also likely accounts for their differential resistance to rice blast pathology.

Saponins are glycosylated triterpenoids, generated in many plants as a secondary metabolite. Different saponins display potent insecticidal and fungicidal activities against a broad range of plant parasites (Abbruscato et al., 2014; Augustin et al., 2011; Doughari, 2015). Unlike dicotyledonous plants, monocots such as rice are considered triterpenoid-poor plants (Osbourn, 2003). Avenacin is a saponin in oat (*Avena* spp.) that provides defence against soil-borne fungal pathogens (Anderson et al., 2010). To our knowledge, Bayogenin 3-O-cellobioside represents the first example of a saponin produced in rice.

β-amyrin synthases and UDP-GTs are critical enzymes that catalyze saponin biosynthesis in plants (Sui et al., 2011; Suzuki et al., 2002; Tava et al., 2011). We found that two out of the four putative β-amyrin synthases genes and a total of 106 putative UDP-GTs were explicitly expressed during rice blast fungus infection of different cultivars. These data suggest that rice, and likely other monocots, are genetically capable of generating saponins and other glycosylated steroids under defined conditions.

Our findings also underscore the importance of β-amyrin synthases and UDP-GTs in enforcing host immunity (Boachon et al., 2014; Qi et al., 2006, Wang et al., 2016). The clustering of rice UDP-GTs at single loci on a limited number of
chromosomes suggests that common regulators likely control clusters (Chen et al., 2017; Fischer et al., 2016). This clustering, and the observation that a higher number of UDP-GT genes expressed upon inoculation of resistant cultivars compared to susceptible cultivars, suggests that multiple UDP-GTs enhance rice immunity against blast fungus by producing diverse glycosides, likely including Bayogenin 3-O-cellobioside. Disruption of a \( \beta \)-amyrin synthase and UDP-GTs blocks avenacin biosynthesis in oat (Geisler et al., 2013; Tamura et al., 2017). The extent to which \( \beta \)-amyrin synthases and UDP-GTs, especially Os05g0550100 and Os06g0483200, influence Bayogenin 3-O-cellobioside biosynthesis, as well as the resistance or susceptibility of different rice cultivars to \( P. \) oryzae, remains to be determined.

Bayogenin 3-O-cellobioside is a glycoside consisting of a non-sugar aglycone (Bayogenin) linked to a sugar glycone.

**Figure 4** Clustering and expression profile of putative saponin biosynthesis gene in rice during \( P. \) oryzae infection. (a) The Neighbour-joining tree showed the clustering 145 rice diphosphate glucuronyltransferases (UDP/GTs) into 33 different subfamilies (groups) based on conserved alignable motifs. Each group is defined by a colour shade and consists of 1-15 genes. (b) Displayed the chromosomal distribution and locations of UDP/GTs gene clusters in rice. The blue each vertical bar with upper and lower or (long and short) arms represents rice chromosome. The position of the blue circle (connecting the upper and lower arms) on each chromosome indicate represents the centromeric region. The numbering (Chr1-Chr12) on top of each vertical blue bar corresponds to chromosome number. Set of genes aligned to red solid lines projecting from the chromosome represents a single cluster; therefore, the number of red solid lines on each chromosome reflects the number of UDP/GTs gene clusters identified on the respective chromosome. (c) Showed the comparative expression pattern of 108 differentially expressed UDP/GTs susceptible (CO39 and NPB) and resistant (P-gm) rice cultivars challenged with the rice blast fungus. (d) The heatmap represent folds expression pattern of rice-specific \( \beta \)-amyrin synthase genes in blast resistant and susceptible rice cultivars challenged with \( P. \) oryzae. Note the expression these 106 were exclusively in response to infection and were not detected in the non-inoculated control groups. Each coloured dot in or outside box plot represents the unique expression level (log2 FPKM) of UDP/GTs gene in the treated rice cultivars with a \( P \leq 0.05 \). The asterisks at the whiskers indicate the lower and upper outliers.
Glycosylation is required to transform saponins to their bioactive state (Mugford and Osbourn, 2012b; Townsend et al., 2006). We found that spores from rice blast fungus treated with glycosylated Bayogenin (Bayogenin 3-O-β-D-glucopyranoside) failed to germinate, whereas spores treated with non-glycosylated Bayogenin (cellobioside) (Hostettmann and Marston, 2005). Glycosylation is required to transform saponins to their bioactive state (Mugford and Osbourn, 2012b; Townsend et al., 2006). We found that

Figure 5 T-DNA-mediated mutagenesis of UDP-GTs partially compromised the resistance of rice cultivar Zhonghua 11 against the rice blast fungus. (a) The infection assay showed the resistance or susceptibility level of three individual UDP-GTs genes mutant rice lines (Os05g0550100, Os02g0329933 and Os01g0176200) and a β-amyrin synthase gene (Os06g0483200) against P. oryzae (FJ81278) isolate compared to resistant control rice lines (ZH11 and Pi-gm) and susceptible control rice line (CO39). spores were inoculated on leaf tissues of susceptible (CO39 and NPB) and resistant (Pi-gm and Pi-4b) rice cultivars.

The histopathological micrograph showed the impact of individual disruption of UDP-GTs and β-amyrin synthase on the endogenous pathogenic development of P. oryzae. Leaves of two-week-old ZH11 seedlings sprayed sterilized double deionized water containing 0.02% v/v Tween20 was used as the mock control. For sheath inoculation, sheaths from respective rice lines were inoculated with conidia in suspension, the water used in preparing the conidia suspensions was shared and used to treat sheaths obtained from the individual rice lines as the mock control. Note: FJ81278 was used for the inoculation assay because the rice mutant lines used in this investigation were generated from cultivar Zhonghua 11 rice cultivar known to contain an R-gene that responses to Avr-Pi-zt.
germinated and progressed to form functional appressorium. The aglycone component of insecticidal saponins is not sufficient to prevent *Phyllotreta nemorum* from feeding on the tissues of susceptible P-type of *Barbarea vulgaris* (Nielsen et al., 2010). Similarly, glycosylation plays a crucial role in promoting the fungicidal activities of Bayogenin.

**Figure 6** Action model Bayogenin 3-O-cellobioside biosynthesis in rice compared to other types of saponins. In reference to our results, we stipulated that rice (susceptible and resistant) cultivars exposed to persistent irritations from *P. oryzae* undergo metabolomics reprogramming which results in the generation of Bayogenin 3-O-cellobioside (glycosylated Bayogenin) and other types of saponins through the enzymatic actions of Beta-amyrin synthases and UDP/GTs. Glycosylated exerts specific inhibitory effect on the germination of *P. oryzae* spores.
Glycosides contribute to plant resistance against a broad range of parasitic insects and herbivores (Mugford and Osbourn, 2012a). However, treatment with alternative glycosides, including Hederagenin and oleanolic acid, did not inhibit spore germination and subsequent pathogenic development of the rice blast fungus. Bayogenin 3-O-β-D-glucopyranoside, on the other hand, significantly inhibited the germination of *P. oryzae* spores and the vegetative development of rice blast fungus. However, there is limited knowledge on the evolution of saponin biosynthesis in different plant families (Augustin et al., 2012). Differences in saponin bioactivity have been attributed to the composition of the targeted biological membrane systems (Augustin et al., 2012). Differences in the core structure, functional groups and the affinity with which the respective groups separately (inoculated and non-inoculated groups) 12 h post-inoculation. The harvested leaf tissues were ground in liquid nitrogen to yield a fine and homogeneously blended powder. 0.1 g of the leaf powder was mixed with 1000 µL of 50% methanol and incubated in shaking incubator for 12 h at 4 °C. The contents were centrifuged at 13226 × g for 15 min at 4 °C. The supernatant was pipetted into new Eppendorf tubes, and 15 µL of the extracts was diluted 10-fold with 70% (v/v) cold methanol and filtered with 0.2 µm Milex Millipore membrane into sample bottles with glass insert. The diluted extracts were stored at 4 °C and were later used for non-targeted whole-metabolome analysis. Whole-metabolome profiling data were generated with 2777 C UPLC system (Waters, UK) type of liquid chromatography and Xevo G2-XS QTOF (Waters, UK) mass spectrometry instruments (BGI.Tech metabolomics platform at ShengZhen). The HPLC assay was conducted with six technical replicates.

Culturing and preparation of Magnaporthe oryzae mycelia for Metabolomic assay

Wild-type *P. oryzae* Guy11 samples for metabolomics assays were cultured in complete liquid media (CM) (6 g yeast extract, 6 g casamino acid, 10 g sucrose in 1L distilled water) in a shaking incubator operating at 150 rpm at 28.5 for 5 days. The cultured strains were subsequently filtered and thoroughly rinsed with sterilized double deionized water (dH2O) and freeze-dried in 70% (v/v) methanol for 24 h in (Labconco Free Zone 12L). The dried hyphae tissues were ground into powder using a pestle and mortar. 0.16 mg of the ground hyphae was mixed with 1.5 mL of 50% (v/v) methanol, vortexed vigorously to yield a uniform mixture and incubated in a water bath at 65 °C for 1 h. After incubation, the mixture was centrifuged for 10 min at 11269 × g. The supernatant was aliquoted into a new 2.0-mL sterilized Eppendorf tube, and 15 µL of the supernatant was diluted 10-fold with 70% (v/v) methanol and filtered with 0.2 µm Milex Millipore membrane into sample bottles with glass insert and stored at 4 °C for metabolic analysis.

Harvesting and preparation of conidia for metabolomic assay

To generate conidia for metabolomics analysis, a mycelial plug of wild-type *P. oryzae* Guy11 strains were grown on rice bran medium, at 27 °C with constant exposure to light. After 10 days, the conidia were harvested, washed with sterile distilled water and were observed under the microscope. The washed conidia were then filtered and centrifuged for 10 min at 11269 × g. The conidia were ground in liquid nitrogen to yield fine powdered. 0.10 mg of the conidia powder was mixed with 1.5 mL of 50% (v/v) methanol, vortexed vigorously to yield a uniform mixture and incubated in a water bath at 65 °C for 1 h. After incubation, the mixture was centrifuged for 10 min at 11269 × g. The supernatant was aliquoted into a new 2.0-mL sterilized Eppendorf tube, and 15 µL of the supernatant was diluted 10-fold with 70% (v/v) methanol and filtered with 0.2 µm Milex Millipore membrane into sample bottles with glass insert and stored at 4 °C for metabolic analysis.

**Conclusion**

Inherent metabolite differences between distinct rice cultivars have been associated with their distinct morphological and physiological characteristics (Hu et al., 2014; Kusano et al., 2015; Schauer et al., 2006). However, little is known about pathogen-induced metabolite differences between various rice cultivars and the potential impact on resistance or susceptibility traits. Beyond Bayogenin 3-O-Cellobioside, we found that other previously reported defence-related metabolites, such as abscisic acid glucoside ester (Piotrowska and Bajguz, 2011), aurantio-obtusin-D-glucoside (Kumar et al., 2015), carlosin (Das et al., 2016; Ling and Weilin, 2016) and sakuranin (derivative of sakuranetin) (Gupta et al., 1972; Hasegawa et al., 2014; Kodama et al., 1992; Narasimhachari and Seshadri, 1952), were specifically produced in resistant rice cultivars challenged with rice blast fungus. Thus, resistant rice cultivars possess a metabolomic advantage over susceptible rice cultivars both before and during infection.

Overall, we report for the first time that diverse cultivars of rice produce a novel saponin (Bayogenin 3-O-Cellobioside) with anti-blast properties upon rice blast infection. We propose that β-amyrin synthases and UDP-GTs support saponin biosynthesis in rice (Figure 6). Our study provides insight into pathogen-mediated metabolomic reprogramming in host plants and their impact on the resistance or susceptibility. The correlation between Bayogenin 3-O-Cellobioside levels and blast resistance suggests that engineering saponin expression in cereal crops represents an attractive and sustainable disease control strategy.

**Materials and methods**

**Preparation of rice samples for metabolomics assay**

Two- to 3-week-old rice seedlings were sprayed-inoculated with conidia suspensions (1.5–2.0 × 10⁸ conidia/mL containing 0.02% Tween20) and incubated in a humid chamber along with the control groups (sprayed with water containing 0.02% Tween20) for 12 h at 27 °C. Leaf tissues were harvested from respective groups separately (inoculated and non-inoculated groups) 12 h post-inoculation. The harvested leaf tissues were ground in liquid nitrogen to yield a fine and homogeneously blended powder. 0.1 g of the leaf powder was mixed with 1000 µL of 50% methanol and incubated in shaking incubator for 12 h at 4 °C. The contents were centrifuged at 13226 × g for 15 min at 4 °C. The supernatant was pipetted into new Eppendorf tubes, and 15 µL of the extracts was diluted 10-fold with 70% (v/v) cold methanol and filtered with 0.2 µm Milex Millipore membrane into sample bottles with glass insert. The diluted extracts were stored at 4 °C and were later used for non-targeted whole-metabolome analysis. Whole-metabolome profiling data were generated with 2777 C UPLC system (Waters, UK) type of liquid chromatography and Xevo G2-XS QTOF (Waters, UK) mass spectrometry instruments (BGI.Tech metabolomics platform at ShengZhen). The HPLC assay was conducted with six technical replicates.

Wild-type *P. oryzae* Guy11 samples for metabolomics assays were cultured in complete liquid media (CM) (6 g yeast extract, 6 g casamino acid, 10 g sucrose in 1L distilled water) in a shaking incubator operating at 150 rpm at 28.5 for 5 days. The cultured strains were subsequently filtered and thoroughly rinsed with sterilized double deionized water (dH2O) and freeze-dried in 70% (v/v) methanol for 24 h in (Labconco Free Zone 12L). The dried hyphae tissues were ground into powder using a pestle and mortar. 0.16 mg of the ground hyphae was mixed with 1.5 mL of 50% (v/v) methanol, vortexed vigorously to yield a uniform mixture and incubated in a water bath at 65 °C for 1 h. After incubation, the mixture was centrifuged for 10 min at 11269 × g. The supernatant was aliquoted into a new 2.0-mL sterilized Eppendorf tube, and 15 µL of the supernatant was diluted 10-fold with 70% (v/v) methanol and filtered with 0.2 µm Milex Millipore membrane into sample bottles with glass insert and stored at 4 °C for metabolic analysis.

**Harvesting and preparation of conidia for metabolomic assay**

To generate conidia for metabolomics analysis, a mycelial plug of wild-type *P. oryzae* Guy11 strains were grown on rice bran medium, at 27 °C with constant exposure to light. After 10 days, the conidia were harvested, washed with sterile distilled water and were observed under the microscope. The washed conidia were then filtered and centrifuged for 10 min at 11269 × g. The conidia were ground in liquid nitrogen to yield fine powdered. 0.10 mg of the conidia powder was mixed with 1.5 mL of 50% (v/v) methanol, vortexed vigorously to yield a uniform mixture and incubated in a water bath at 65 °C for 1 h. After incubation, the mixture was centrifuged for 10 min at 11269 × g. The supernatant was aliquoted into a new 2.0-mL sterilized Eppendorf tube, and 15 µL of the supernatant was diluted 10-fold with 70% (v/v) methanol and filtered with 0.2 µm Milex Millipore membrane into sample bottles with glass insert and stored at 4 °C for metabolic analysis.

**Generation of appressorium for metabolomic assays**

For appressorium formation metabolome profiling, appressoria were generated by dropping an aliquot of 1.0 mL per of conidia suspension (1 × 10⁵) on fisher scientific hydrophobic slide surface and incubated in a humid chamber at 26 °C without
light. Appressorium formation was observed after 12 h using an optical microscope. Solution drops containing the developed appressorium were pipetted into sterilized EP tubes and centrifuged for 5 min at 1957 × g. The liquid was pipetted out, and the pellet was transferred, frozen and ground in liquid nitrogen to yield a fine powder using pestle and mortar. 0.10 mg of the powder generated was mixed with 1.5 mL of 50% (v/v) methanol, vortexed vigorously to yield a uniform mixture and incubated in a water bath at 65°C for 1 h. After incubation, the mixture was centrifuged for 10 min at 11269 × g. The supernatant was aliquoted into new 2.0-mL sterilized Eppendorf tubes, and 15 μL of the supernatant was diluted 10-fold with 70% (v/v) methanol and filtered with 0.2 μm Millex Millipore membrane into sample bottles with glass insert and stored at 4°C for metabolic analysis.

**Pathogenicity assay**

For plant infection assays, conidia were collected from strains cultured on rice bran medium for 7–10 days. Conidial suspensions were adjusted to 1.5–2.0 × 10^5 conidia/mL in 0.02% Tween solution and sprayed onto 3- to 4-week-old susceptible (CO39, LTH and NPB) and resistant (Pi-b, Pi-4b and Pi-gm) rice seedlings. Inoculated plants were incubated in a dark, humid chamber at 25°C for 24 h and then moved to another humid chamber with a 12-h photoperiod. The plants were examined for disease symptoms at 7 days post-inoculation.

**Evaluating the influence of rice leaf extracts on conidia germination and appressorium formation**

Conidia were collected from 7-day-old rice bran medium. Conidial suspensions were adjusted to 1.5–2.0 × 10^5 conidia/mL in 0.02% Tween solution and sprayed onto 3- to 4-week-old susceptible (CO39, LTH and NPB) and resistant (Pi-b, Pi-4b and Pi-gm) rice seedlings. Inoculated plants were incubated in a dark, humid chamber at 25°C for 24 h and then moved to another humid chamber with a 12-h photoperiod. The inoculated rice leaves were then grounded in liquid nitrogen to a fine powder. About 1 g of crushed leaves was dissolved in 4 mL of 80% methanol and incubated at 4°C on a shaking incubator overnight. After overnight shaking, the mixture was centrifuged for 10 min at 13,000 g to obtain the supernatant. The supernatant was then filtered with non-sterilized millex syringe-driven membrane. The substrate syrup was used to directly wash conidia from the culture plates. Twenty μL of the conidial suspensions was placed on a fisher scientific hydrophobic microscope cover glass and incubated in a dark and humid chamber at 26°C before proceeding to appressorium formation.

**RNA extraction and generation of Illumina RNA sequencing library**

Total RNA was extracted from the inoculated rice seedlings (C5_Co39, C_NB and C_gm) along with their non-inoculated control group T_Co39, T_NB and T_gm. The extraction of total RNA from inoculated and non-inoculated control samples was carried out with RNAprep pure Plant Kit (Tiangen, Beijing) by following processes recommended by the manufacturer. RNA degradation and contamination were measured by running the extracted RNAs on 1% agarose gels. RNA integrity was assessed using the RNA Nano 6000 Assay Kit of the Bioanalyzer 2100 system (Agilent Technologies, CA, USA). The RNA concentration was measured with an RNA Assay Kit in Qubit 2.0 Flurometer (Life Technologies, CA, USA). The RNA concentration was measured with an RNA Assay Kit in Qubit 2.0 Flurometer (Life Technologies, CA, USA). The cDNA library was sequenced on the Illumina sequencing platform (IlluminaHiSeq 2000) with 150 bp pair-end reads length and 300 bp insert size by Gene Denovo Co. (Guangzhou, China). Novogene in-house Perl script was used to select clean reads by removing adaptor sequences, low-quality sequences (reads with more than 50% of bases quality lower than 20) and reads with more than 5% N bases. The reference genome of Nipponbare genome Oryza sativa Japonica and gene model annotation files was downloaded from the genome website directly (Sakai et al., 2013). Index of the reference genome was built using Hisat2 v2.0.4, and paired-end clean reads were aligned to the reference genome using Hisat2 v2.0.4. We selected Hisat2 as the mapping tool for that Hisat2 can generate a database of splice junctions based on the gene model annotation file and thus a better mapping result than other non-splice mapping tools.

**Additional information**

Accession codes: Details of the RNA-Seq data generated in this study have been deposited in the NCBI Sequence Read Archive database and can be accessed with the GEO accession code: GSE126961.

**Acknowledgements**

This work was supported by National Key Research and Development Program of China (2016YFD0300707) and National Natural Science Foundation of China (No.U1805232, No.31770156). Prof. Zuhua He of Institute of Plant Physiology and Ecology, Shanghai Institute for Biological Sciences, CAS provided Pigm rice seeds used in this study.

**Conflict of interest**

All authors have consented to the submission of this manuscript to *Plant Biotechnology Journal* for possible consideration for publication and declared no conflict of interest.

**Author contributions**

J.N., Z.W and Lili Lin perceived and design the research. J.N. and Z.W. acquired funding for the research. J.N., Lianyu Lin, A.W., X.C., S.R.A., A.S. and W.B. prepared and process the experimental samples and carried out the experiments. J.N., J.B., Lianyu Lin, Lili Lin and Z.Z analyse metabolomics and RN-Seq data. J.N., Z.W., I.Z. and G.L. prepared the manuscript.

**References**

Abbruscato, P., Tosi, S., Crispino, L., Blazzi, E., Menin, B., Picco, A.M., Pecetti, L. et al. (2014) Triterpenoid glycosides from medicago sativa as anti-fungal agents against *Pyricularia oryzae*. *Journal of Agricultural and Food Chemistry* 62, 11030–11036.

Abujyusuf, M., Robin, A., Lee, J.-H., Jung, H.-I., Kim, H.-T., Park, J.-I. and Nou, I.-S. (2018) Glucosinolate profiling and expression analysis of glucosinolate biosynthesis genes differentiate white mold resistant and susceptible cabbage lines. *Int. J. Mol. Sci.* 19, 4037.

Akatsuka, T., Kodama, O., Sekido, H., Kono, Y. and Takeuchi, S. (1985) Novel phytoalexins (Oryzalexins A, B and C) isolated from rice blast leaves infected with *Pyricularia oryzae*. *part i: isolation, characterization and biological
activities of oryzalexins part ii: structural studies of oryzalexins. Agric. Biol. Chem. 49, 1689–1701.

Anderson, J.P., Gleason, C.A., Foley, R.C., Thrall, P.H., Burdon, J.B. and Singh, K.B. (2010) Plants versus pathogens: an evolutionary arms race. Funct. Plant Biol. 37, 499–512.

Augustin, J.M., Bak, S.D., Shindota, T., Sanmiya, K., Nielsen, J.K., Khakimov, B., Olsen, C.E., et al. (2012) UDP-glycosyltransferases from the UGT73C subfamily in Barabara vulgaris catalyse Sapogenin 3-O-glucosylation in Saponin-mediated Insect resistance. Plant Physiology 160(4), 1881–1895.

Augustin, J.M., Kuzina, V., Andersen, S.B. and Bak, S. (2011) Molecular activities, biosynthesis and evolution of triterpenoid saponins. Phytochemistry 72, 435–457.

Bao, J., Chen, M., Zhong, Z., Tang, W., Lin, L., Zhang, X., Jiang, H. et al. (2017) PacBio sequencing reveals transposable elements as a key contributor to genomic plasticity and virulence variation in Magnaportha oryzae. Molecular plant 10, 1465–1468.

Boachon, B., Gamir, J., Pastor, V., Erb, M., Flors, V. and Mauch-Mani, B. (2014) Role of two UDP-Glycosyltransferases from the L group of Arabidopsis in resistance against pseudomonas syringae. Eur. J. Plant Pathol. 140, 707–720.

Cai, F., Watson, B.S., Meeke, D., Huwman, D.W., Wherrett, D.I., Ben, C., Gentzbleil, L. et al. (2017) Medicago truncatula oleic-acid-derived saponins are correlated with caterpillar deterrence. J. Chem. Ecol. 43, 712–724.

Chandran, A.K.N., Yoo, Y.-H., Cao, P., Sharma, R., Sharma, M., Dardick, C., Ronald, P.C. and et al. (2016) Updated rice kinase database RKD 2.0: enabling transcriptome and functional analysis of rice kinase genes. Rice 9, 40.

Chen, J., Zeng, X., Yang, Y.L., Xing, Y.M., Zhang, Q., Li, J.M., Ma, K. et al. (2017) Genomic and transcriptomic analyses reveal differential regulation of diverse terpenoid and polyketides secondary metabolites in Hericium erinaceus. Sci. Rep. 7, 10151.

Christensen, S., Enge, S., Jensen, K.R., Muller, C., Kaer, L.P., Agerbirk, N., Heilmann, M., et al. (2018) Different herbivore responses to two co-occuring chemotypes of the wild crucifer Barbarea vulgaris. Arthropod-Plant Interactions 1–12.

Das, S., Mukherjee, S., Kundu, R., Bhattacharya, P., Dua, B. and Bhattacharya, S.S. (2016) Variations in soil alter availability of carlinoide: an anti-hepatitic compound from Cajanus cajan (Linn.) leaves.Curr. Sci. 00113891, 110.

Dean, R.A., Talbot, N.J., Ebbole, D.J., Farman, M.L., Mitchell, T.K., Orbach, M.J., Thor, M. et al. (2005) The genome sequence of the rice blast fungus Magnaportha oryzae. Nature 434, 980.

Deng, Y., Zhi, X., Shen, Y. and He, Z. (2006) Genetic characterization and fine mapping of the blast resistance locus Pigm (t) tightly linked to P2 and P9 in a broad-spectrum resistant Chinese variety. Theoretical and Applied Genetics 113, 705–713.

Doughari, J.(2015) An overview of plant immunity. J. Plant Pathol. Microbiol 6 (10), 4172.

Fernandez, J. and Orth, K. (2018) Rise of a cereal killer: the biology of Magnaportha oryzae biotrophic growth. Trends in Microbiology 26(7), 582–597.

Fischer, J., Schroeckh, V. and Brakhage, A.A. (2016) Awakening of fungal secondary metabolite gene clusters. (Schmoll, M. & Dattenb., eds), pp. 405–424. New York, NY: Springer.

Geiser, K., Hughes, R.K., Sainsbury, F., Lomonossoff, G.P., Rezek, M., Fairhurst, S., Olsen, C.E. et al. (2013) Biochemical analysis of a multifunctional cytochrome P450 (CYP51) enzyme required for synthesis of antimicrobial terpenes in plants. Proc. Natl Acad. Sci. 110, E3360–E3367.

Ghatak, A. (2013) Relationships between rice neck blast and leaf blast epidemics. Pantnagar-263145 (Uttarakhand): GB Plant University of Agriculture and Technology.

Ghazanfar, M.U., Habib, A. and Sahi, S. (2009) Screening of rice germplasm against Pyricularia oryzae the cause of rice blast disease. Pak. J. Phytopathol. 21, 41–44.

Gupta, S., Ravindranath, B. and Seshadri, T. (1972) Polyphenols of Juggans nigr. Phytochemistry 11, 2634–2636.
Narasimhachari, N. and Seshadri, T. (1952) Components of the bark of Prunus puddum. Proceedings of the Indian Academy of Sciences – Section A 35, 202.
Nielsen, J.K., Nagao, T., Okabe, H. and Shinoda, T. (2010) Resistance in the plant, Barbarea vulgaris, and counter-adaptations in flea beetles mediated by saponins. J. Chem. Ecol. 36, 277–285.
Norvienyeku, J., Zhong, Z., Lin, L., Dang, X., Chen, M., Lin, X., Zhang, H. et al. (2017) Methylmalonate-semialdehyde dehydrogenase mediated metabolite homeostasis essentially regulate conidiation, polarized germination and pathogenesis in Magnaporthe oryzae. Environ. Microbiol. 19, 4256–4277.
Osbourn, A.E. (2003) Saponins in cereals. Phytochemistry, 62, 1–4.
Otomo, K., Karino, Y., Motegi, A., Kenmoku, H., Yamane, H., Mitsuhashi, W., Okawa, H. et al. (2004) Diterpene cyclases responsible for the biosynthesis of phytoalexins, monolactones A, B, and oryzalexins A-F in rice. Biosci. Biotechnol. Biochem. 68, 2001–2006.
Peng, Y., van Wersch, R. and Zhang, Y. (2018) Convergent and divergent signaling in PAMP-triggered immunity and effector-triggered immunity. Mol. Plant Microbe Interact. 31, 403–409.
Piotrowska, A. and Bajguz, A. (2011) Conjugates of abscisic acid, brassinosteroids, ethylene, gibberellins, and jasmonates. Phytochemistry 72, 2097–2112.
Prongchayu, A., Nilthong, S. and Jantasujiyarat, C. (2017) Investigation of Pin3 rice blast resistant gene in northern upland rice varieties (Oryza sativa L.), Thailand using molecular markers. Journal of Advanced Agricultural Technologies 4, 209–214.
Qi, X., Bakht, S., Qin, B., Leggett, M., Hemmings, A., Mellon, F., Eagles, J. et al. (2006) A different function for a member of an ancient and highly conserved cytochrome P450 family: from essential sterols to plant defense. Proc. Natl Acad. Sci. 103, 18848–18853.
Sakai, H., Lee, S.S., Tanaka, T., Numa, H., Kim, J., Kawahara, Y., Wakimoto, H. et al. (2013) Rice Annotation Project Database (RAP-DB): an integrative and interactive database for rice genomics. Plant Cell Physiol. 54, ed.
Sawai, S. and Saito, K. (2011) Triterpenoid biosynthesis and engineering in plants. Frontiers in Plant Science 2, 25.
Schauer, N., Semel, Y., Roessner, U., Gur, A., Balbo, I., Carrari, F., Pleban, T. et al. (2006) Comprehensive metabolic profiling and phenotyping of interspecific introgression lines for tomato improvement. Nat. Biotechnol. 24, 447.
Sui, C., Zhang, J., Wei, J., Chen, S., Li, Y., Xu, J., Jin, Y. et al. (2011) Transcriptome analysis of Bupleurum chinense focusing on genes involved in the biosynthesis of saikosaponins. BMC Genom. 12, 539.
Suzuki, H., Achnine, L., Xu, R., Matsuda, S.P. and Dixon, R.A. (2002) A genomics approach to the early stages of triterpene saponin biosynthesis in Medicago truncatula. Plant J. 32, 1033–1048.
Tamura, K., Teranishi, Y., Udeta, S., Suzuki, H., Kawano, N., Yoshimatsu, K., Saito, K. et al. (2017) Cytochrome P450 monoxygenase CYP716A141 is a unique β-amyrin C-16β oxidase involved in triterpenoid saponin biosynthesis in Platycodon grandiflorus. Plant Cell Physiol. 58, 874–884.
Tava, A., Scotti, C. and Avato, P. (2011) Biosynthesis of saponins in the genus Medicago. Phytochem. Rev. 10, 459–469.
Townsend, B., Jenner, H. and Osbourn, A. (2006) Saponin glycosylation in cereals. Phytochem. Rev. 5, 109–114.
Ueda, M., Shaari, N.H., Said, N.S., Ibrahim, N.H., Akhir, M.A., Hashim, M.K.R., Salimi, M. et al. (2018) Antimicrobial activity of plant extracts from aloe vera, citrus hystricx, sabah snake grass and zingeriber officinale against pyricularia oryzae that causes rice blast disease in paddy plants. IOP Conference Series: Materials Science and Engineering 318, 012009.
Wang, Y., Zhou, L., Yu, X., Stover, E., Luo, F. and Duan, Y. (2016) Transcriptome profiling of Huanglongbing (HLB) tolerant and susceptible citrus plants reveals the role of basal resistance in HLB tolerance. Frontiers in plant science 7, 933.
Wilson, R.A. and Talbot, N.J. (2009) Under pressure: investigating the biology of plant infection by Magnaporthe oryzae. Nat. Rev. Microbiol. 7, 185.
Woldemicheal, G.M. and Wink, M. (2002) Triterpene glycosides of Lupinus angustifolius. Phytochemistry 60, 323–327.
Yang, Y., Zhang, H., Xuan, N., Chen, G., Liu, X., Yao, F. and Ding, H. (2017) Identification of blast resistance genes in 358 rice germplasms (Oryza sativa L.) using functional molecular markers. Eur. J. Plant Pathol. 148, 567–576.
Zhang, J., Li, C., Wu, C., Xiong, L., Chen, G., Zhang, Q. and Wang, S. (2006) RMD: a rice mutant database for functional analysis of the rice genome. Nucleic Acids Res. 34, D745–D748.
Zhong, Z., Chen, M., Lin, L., Han, Y., Bao, J., Tang, W., Lin, L. et al. (2018) Population genomic analysis of the rice blast fungus reveals specific events associated with expansion of three main clades. ISME J. 12, 1867–1878.

Supporting information
Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1 Different rice cultivars undergo common and differential metabolome reprogramming in response to P. oryzae infection.

Figure S2 Structure formulae of 19 non-cultivar specific metabolites identified in resistant and susceptible rice cultivars exclusively during P. oryzae infection.

Figure S3 Exclusive generation of Bayogenin 3-O-Cellobioside in P. oryzae inoculated resistant and susceptible rice cultivars

Figure S4 Development of P. oryzae on leaf and sheath tissues of susceptible and resistant rice cultivars (a) displayed germination and appressorium in P. oryzae spores inoculated on leaf tissues of susceptible (CO39, and NPB), and resistant (Pi-gm, and Pi-4b) rice cultivars (b) showed the in planta infectious development of P. oryzae in sheath tissues of susceptible (CO39, and NPB), and resistant (Pi-gm, and Pi-4b) rice cultivars

Figure S5 Impact of Bayogenin 3-O-β-D-glucopyranoside, and crude leaf extracts from pre-inoculated and non-inoculated resistant rice cultivars on the vegetative development of P. oryzae

Table S1 Impact of Bayogenin 3-O-β-D-glucopyranoside, and crude leaf extracts from pre-inoculated and non-inoculated resistant rice cultivars on the vegetative development of P. oryzae

Table S2 (a) Total Metabolites from leaf extract of Control and treatment groups. (b) Total Metabolites recorded in P. oryzae.

Table S3 List of transgenic rice lines obtained from the Rice Mutant Database (RMD) and used in assessing the immune-enhancing role of UDP-GTs and β-amyrin synthases during host-pathogen interaction.