1 Bayogenin 3-O-Cellobioside is a novel non-cultivar specific anti-blast metabolite
2 produced in rice in response to *Pyricularia oryzae* infection

3

4 Justice Norvienyeku^{1,2†}, Lili Lin^{1,2†}, Abdul Waheed^{1,2}, Xiaomin Chen^{1,2}, Jiandong
5 Bao^{1,2}, Ammarah Shabbir^{1,2}, Lianyu Lin^{1,2}, Zhenhui Zhong^{1,2}, Wajjiha Batool^{1,2}, ,
6 Sami Rukaiya Aliyu^{1,2}, Jie Zhou^{1,2}, Guodong Lu^{1,2}, Zonghua Wang^{1,3*}

7

8 ¹State Key Laboratory for Ecological Pest Control of Fujian and Taiwan Crops, College of
9 Life Science, Fujian Agriculture and Forestry University, Fuzhou, 350002, China

10 ²Fujian University Key Laboratory for Plant-Microbe Interaction, College of Plant Protection,
11 Fujian Agriculture and Forestry University, Fuzhou, 350002, China

12 ³Institute of Oceanography, Minjiang University, Fuzhou, 350108, China

13 **Abstract**

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

28

Introduction

Rice blast disease accounts for yield losses that could feed about 60 million rice consumers annually(1). *Pyricularia oryzae* (Syn: *Magnaporthe oryzae*), the fungus that causes rice blast disease(2), is ubiquitously present in all rice-producing regions across the globe. In addition to rice, blast fungus can infect wheat, barley, millet, sorghum, rye, and other cultivated as well as non-cultivated grass plants, and is therefore considered one of the most important plant pathogens (3).

Rice blast fungus initiates infection by producing asexual spores (conidia) which disperse and attach onto healthy host tissues (4). In favorable 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 (5). The appressoria further differentiates 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 (6, 7).

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, Piz-t, Pi-pita, Pi-gm , Pi9, Pi2, among others (8-10). However, rice blast evolves rapidly to overcome R-gene supported resistance (11), and inherent basal resistance does not offer full immunity against blast fungus. Rice cultivars from *japonica* and *indica* sub-species possess differential resistance against blast fungus, with *japonica* rice mainly pose inherent resistance whereas *indica* rice poses R-gene mediated resistance (9). Although rice-specific secondary metabolites (phytochemicals) such as oryzalexins, phytocassanes, momilactone, and sakuranetin can protect rice from bacterial and fungal pathogens, including blast fungus (12, 13), these compounds have not been

extensively exploited as potent defense molecules against blast pathogen, largely because most of these metabolites are cultivar-specific and were identified enhance in response to phytohormone treatment rather than pathogen treatment. Current advances in metabolomics technologies enable the identification of phytochemicals and defense signaling molecules that are induced in plant tissues during host-pathogen interactions. Here, we investigated how *P. oryzae*-mediated metabolome reprogramming affects the susceptibility or resistance of different rice cultivars to *P. oryzae*.

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) from both *indica* and *japonica* lineage (supplementary table 1) to *P. oryzae*. Briefly, we spray-inoculated three-week-old 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, we assessed the type and severity of lesions on leaf tissues according to a published rice blast lesion scoring index (14). 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).

We hypothesized that metabolites produced after inoculation with rice blast might inhibit infectious development of *P. oryzae*. To test this hypothesis, we extracted crude extracts from inoculated rice seedlings, as well as from non-inoculated controls. We used the crude leaf extracts to wash conidia from the *P. oryzae* Guy11 strain, prepared conidia suspensions, and inoculated a hydrophobic coverslip to induce appressorium formation *in vitro*. Crude extract from the untreated resistant cultivars inhibited conidia germination and appressorium formation, whereas crude extracts

from the untreated susceptible cultivars had no inhibition effect (Figure. 1C-D). However, in 5/6 cases, the crude leaf extracts from the inoculated seedlings inhibited germination and appressorium formation more than crude leaf extracts from the corresponding non-inoculated control seedlings (Figure. 1E-F). Thus, indicate the likely presence of anti-blast metabolites in crude extracts from both susceptible and resistant cultivars show increased production of upon inoculation *P. oryzae*.

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 (Figure.2A), harvested leaf tissues at 12-hours post inoculation, and extracted metabolites in methanol using QTOF-UPHPLC (see Methods). Also to ensure exclusion of fungi specific metabolites, we analyzed the metabolomes 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 (15). 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 diminish unwanted biological variables, background noise, and biologically insignificant metabolites (see Supplementary Fig.1a, Methods, and (15). We uncovered ~2121 metabolites in susceptible rice seedlings, and ~3450 metabolites in resistant rice seedlings (Supplementary Table 2a). Kyoto Encyclopedia of Genes and Genomes (KEGG) compound enrichment analysis

115 (16) revealed that 883 metabolites were produced exclusively in infected seedlings
116 (Figure.2 A &B) and (Supplementary Figure.1b). Of these 883 metabolites, 705 were
117 unknown. Of the 178 metabolites with a KEGG code, 18 were present in both
118 susceptible and resistant rice cultivars after inoculation, 114 were present exclusively
119 in the susceptible cultivars after inoculation, and 45 were solely present in the
120 resistant cultivars after inoculation (Figure.2B- D).

121 To determine the function of the 18 metabolites present in both susceptible and
122 resistant rice seedlings upon infection, we used their compound codes, chemical
123 formulae and common names to search publicly available metabolite libraries,
124 including KEGG compound, KEGG BRITE, PubChem Compound, The Human
125 Metabolome Database, The Small Molecule Pathway Database, and The Toxin and
126 Toxin Target Database (17). We found that 5/18 metabolites are known to be
127 functional phytochemicals: Podorhizol beta-D-glucoside, Bayogenin 3-O-cellobioside,
128 (+)-Syringaresinol O-beta-D-glucoside, 4-Methylsulfonylbutyl glucosinolate, and
129 Dihydromyricetin (Figure.2E-F) and (Supplementary Figure. 3). Indeed, Podorhizol
130 beta-D-glucoside and 4-Methylsulfonylbutyl glucosinolate are known to enhance
131 plant immunity against diverse pathogens (18, 19).

132 Intriguingly, unlike the other four metabolites, the levels of Bayogenin
133 3-O-cellobioside were relatively low in the inoculated, susceptible rice cultivars
134 compared to in the inoculated, resistant group (Figure. 2 G-K). Furthermore, we
135 observed that Bayogenin 3-O-cellobioside levels were about ~1000-fold higher in the
136 completely resistant cultivar Pi-gm than in the moderately resistant cultivars Pi-4b
137 and Pi-b (Figure.2K). Thus, Bayogenin 3-O-cellobioside levels increase specifically
138 upon inoculation of rice seedlings with *P. oryzae* and directly correlate with the extent
139 of resistance. Our results suggest that Bayogenin 3-O-cellobioside is a novel general
140 defense molecule produced in response to rice blast fungus infection, in both
141 susceptible and resistant rice cultivars.

142

143

144 Glycosylated Bayogenin inhibits infectious development of *P. oryzae* *in vitro*

145 Saponins are glycosylated triterpenoids, generated in many plants as a secondary
146 metabolite. Different saponins display potent insecticidal and fungicidal activities
147 against a broad range of plant parasites (20, 21). Unlike dicotyledonous plants,
148 monocots such as rice are considered triterpenoid-poor plants (22). Avenacin is a
149 saponin in oat (*Avena* spp) that provides defense against soil-borne fungal pathogens .
150 Bayogenin 3-O-cellobioside is a glycosylated saponin, consisting of a non-sugar
151 aglycone (Bayogenin) linked to a sugar glycone (Cellobioside). Because Bayogenin
152 3-O-cellobioside is not commercially available, we tested both non-glycosylated
153 Bayogenin and glycosylated Bayogenin (Bayogenin 3-O-β-D-glucopyranoside) in an
154 *in vitro* conidia germination and appressorium formation assays. We prepared
155 titrations of these compounds and used them to wash conidia produced by the *P*
156 *oryzae* Guy11 strain. Subsequently, we prepared conidia suspensions and inoculated a
157 hydrophobic coverslip. We observed a dose-dependent inhibition of conidia
158 germination and appressorium formation *in vitro* with increasing concentrations of
159 Bayogenin 3-O-β-D-glucopyranoside (5 nM/L-100 nM/L) (Figure.3 C and G). In
160 contrast, increasing the concentrations of the non-glycosylated Bayogenin did not
161 affect germination or appressorium formation, nor did the control treatments (Figure.3
162 D and G D).

163 To ascertain whether other saponins would similarly inhibit conidia germination
164 and appressorium formation, we used the *in vitro* assays to test two closely related
165 saponins, Hedegeranin, and Oleanolic acid. Despite their reported insecticidal effects
166 (23, 24), we found that washing conidia with 5-100 nM/L of Hedegeranin or
167 Oleanolic acid did not adversely affect conidia germination and appressorium
168 morphogenesis of *P. oryzae* *in vitro* (Figure.3 E, F, and G). Together, these findings
169 suggest that glycosylated Bayogenin, such as the Bayogenin 3-O-cellobioside, and
170 Bayogenin 3-O-β-D-glucopyranoside are potent phytochemicals that specifically
171 inhibit infectious development of *P. oryzae*.

172

173

174

175 **Differential expression of steroid biosynthesis enzymes during rice-*P. oryzae*** 176 **interaction**

177 Genes encoding enzymes involved in steroid biosynthesis, including β -amyirin
178 synthases, uridine diphosphate (UDP) glucuronyltransferases/ glycosyltransferases
179 (UDP/GTs), appear to mediate the biosynthesis of saponins in plants (22, 25, 26). We
180 identified a total of 6 rice β -amyirin synthases and 145 putative rice-specific UDP-GTs
181 from the publicly available glycosyltransferase database (27). Our bioinformatics
182 analysis revealed that the rice UDP-GTs family could be classified into 33
183 sub-families (groups) based on the alignment of a shared motif (Figure.4A). Further,
184 75 of this putative rice UDP-GTs are within genes clusters on multiple chromosomes
185 (Figure. 4B).

186 We hypothesized that increased expression of either β -amyirin synthases or UDP-GTs
187 might support the increased production of Bayogenin 3-O-cellobioside in rice
188 seedlings inoculated with *P. oryzae*. To examine how inoculation affects the
189 expression of these genes in different cultivars, we analyzed resistant (Pi-gm) and
190 susceptible (CO39, NPB) seedlings by RNA sequencing. We found that of the 102
191 UDP-GTs were expressed exclusively upon inoculation of Pi-gm, whereas 83 and 13
192 UDP-GTs were expressed exclusively upon inoculation of CO39 and NPB,
193 respectively (Fig.7C) and (see Supplementary Table 3). Also, two β -amyirin synthase
194 genes were significantly (~5-12 fold) up-regulated in Pi-gm and CO39 rice cultivars
195 in response to *P. oryzae* challenge (Figure.4D). Thus, various UDP-GT and β -amyirin
196 synthase genes are expressed only upon inoculation of rice with *P. oryzae*, in both
197 susceptible and resistant seedlings. Our findings also underscore the importance of
198 β -amyirin synthases and UDP-GTs in enforcing host immunity (28, 29). This
199 clustering, and the observation that a higher number of UDP-GT genes are expressed
200 upon inoculation of resistant cultivars compared to susceptible cultivars suggesting

201 that multiple UDP-GTs enhance rice immunity against blast fungus by producing
 202 diverse glycosides, likely including Bayogenin 3-O-cellobioside. Disruption of a
 203 β -amyirin synthase and UDP-GTs blocks avenacin biosynthesis in oat (30, 31).
 204 However, the extent to which β -amyirin synthases and UDP-GTs influence Bayogenin
 205 3-O-cellobioside biosynthesis, as well as the resistance or susceptibility of different
 206 rice cultivars to *P. oryzae*, remains to be determined.

207

208

209 DISCUSSION

210

211 We report that the rice metabolite Bayogenin 3-O-cellobioside (saponin) is a novel,
212 general defense molecule that accumulates exclusively in response to *P. oryzae*
213 infection, in susceptible and resistant rice cultivars from both japonica and indica
214 lineages. Further, the levels of Bayogenin 3-O-cellobioside after inoculation with *P.*
215 *oryzae* directly correlate with the resistance of the rice cultivars. Therefore,
216 susceptible and resistant rice cultivars display metabolomic differences not only
217 before infection but also after infection, which contribute to differential resistance to
218 rice blast pathogen.

219

220 Saponins are glycosylated triterpenoids, generated in many plants as a secondary
221 metabolite. Different saponins display potent insecticidal and fungicidal activities
222 against a broad range of plant parasites (20, 21, 32). Unlike dicotyledonous plants,
223 monocots such as rice are considered triterpenoid-poor plants (22). Avenacin is a
224 saponin in oat (*Avena* spp.) that provides defense against soil-borne fungal pathogens
225 (33). To our knowledge, Bayogenin 3-O-cellobioside represents the first example of a
226 saponin produced in rice.

227

228 β -amyrin synthases and UDP-GTs are key enzymes involved in the biosynthesis of
229 saponins in plants (34-36). We found that two out of four putative β -amyrin synthases
230 genes and a total of 106 putative UDP-GTs were specifically expressed during rice
231 blast fungus infection of different cultivars. These data suggest that rice, and likely
232 other monocots, are genetically capable of generating saponins and other glycosylated
233 steroids under defined conditions.

234

235 Our findings also underscore the importance of β -amyrin synthases and UDP-GTs in
236 enforcing host immunity (28, 29, 37). The clustering of rice UDP-GTs at single loci
237 on a limited number of chromosomes suggests that clusters are likely controlled by a
238 common regulator (38, 39). This clustering, and the observation that a higher number

239 of UDP-GT genes are expressed upon inoculation of resistant cultivars compared to
240 susceptible cultivars, suggests that multiple UDP-GTs enhance rice immunity against
241 blast fungus by producing diverse glycosides, likely including Bayogenin
242 3-O-cellobioside. Disruption of a β -amyrin synthase and UDP-GTs blocks avenacin
243 biosynthesis in oat (30, 31). The extent to which β -amyrin synthases and UDP-GTs
244 influence Bayogenin 3-O-cellobioside biosynthesis, as well as the resistance or
245 susceptibility of different rice cultivars to *P. oryzae*, remains to be determined.

246

247 Bayogenin 3-O-cellobioside is a glycoside consisting of a non-sugar aglycone
248 (Bayogenin) linked to a sugar glycone (cellobioside) (40). Glycosylation is required to
249 transform saponins to their bioactive state (41, 42). We found that spores from rice
250 blast fungus treated with glycosylated Bayogenin (Bayogenin
251 3-O- β -D-glucopyranoside) failed to germinate whereas spores treated with
252 non-glycosylated Bayogenin germinated and progressed to form functional
253 appressorium. The aglycone component of insecticidal saponins is not sufficient to
254 prevent *Phyllotreta nemorum* from feeding on the tissues of susceptible P-type of
255 *Barbarea vulgaris* (43). Similarly, glycosylation plays a crucial role in promoting the
256 fungicidal activities of Bayogenin.

257

258 Glycosides contribute to plant resistance against a broad range of parasitic insects and
259 herbivores (44). However, the glycosides Hederagenin, and Oleanolic acid, did not
260 inhibit spore germination or development of rice blast fungus. Bayogenin
261 3-O- β -D-glucopyranoside, on the other hand, significantly inhibited the germination
262 of *P. oryzae* spores, but did not adversely affect vegetative development of rice blast
263 fungus (data not shown), though there is limited knowledge on the evolution of
264 saponin biosynthesis in different plant families (45). Differences in saponin
265 bioactivity could be due to the composition of the core structure, functional groups,
266 and the affinity with which these amphiphilic compounds integrate and disrupt the
267 integrity of the targeted biological membrane systems (32, 45). Glycosylated

268 Bayogenin (specifically Bayogenin 3-O-cellobioside) appears to be a novel and potent
269 anti-fungal metabolite generated in both susceptible and resistant rice cultivars,
270 providing a chemical defense against rice blast fungus. Soyasapogenol glycosides
271 (*Lupinus angustifolius* L) and avenacin A (*Avena strigose*) display specific antifungal
272 activity against *Candida albicans* and *Gaeumannomyces graminis* var. *tritici*,
273 respectively, without a corresponding insecticidal effect(41, 46).

274

275 **Conclusion**

276

277 Inherent metabolite differences between distinct rice cultivars have been associated
278 with their distinct morphological and physiological characteristics (47-49). However,
279 little is known about pathogen-induced metabolite differences between various rice
280 cultivars and the potential impact on resistance or susceptibility traits. Beyond
281 Bayogenin 3-O-Cellobioside, we found that other previously reported defense-related
282 metabolites, such as abscisic acid glucoside ester (50),
283 aurantio-obtusin- β -D-glucoside(51), carlinoside (52, 53) and sakuranin (derivative of
284 sakuranetin) (54-57), were specifically produced in resistant rice cultivars challenged
285 with rice blast fungus. Thus, resistant rice cultivars possess a metabolomic advantage
286 over susceptible rice cultivars both before and during infection.

287 Overall, we report for the first time that diverse cultivars of rice produce a novel
288 saponin (Bayogenin 3-O-Cellobioside) with anti-blast properties upon rice blast
289 infection. We propose that β -amyrin synthases and/or UDP-GTs support saponin
290 biosynthesis in rice (Fig.5). Our study provides insight into pathogen-mediated
291 metabolomic reprogramming in host plants, and its impact on the resistant or
292 susceptibility. The correlation between Bayogenin 3-O-Cellobioside levels and blast
293 resistance suggests that engineering saponin expression in cereal crops represents an
294 attractive and sustainable disease control strategy.

295

296

297

298 **Materials and Methods**

299 **1. Preparation of rice samples for metabolomics assay**

300 2-3-week-old rice seedlings were sprayed-inoculated with conidia suspensions
 301 ($1.5\text{-}2.0 \times 10^5$ conidia/mL containing 0.02% Tween20) and incubated in a humid
 302 chamber along with the control groups (sprayed with water containing 0.02%
 303 Tween20) for 12 hours at 27 °C. Leaf tissues were harvested from respective groups
 304 separately (inoculated and non-inoculated groups) 12 hours post-inoculation. The
 305 harvested leaf tissues were ground in liquid nitrogen to yield a fine and
 306 homogeneously blended powder. 0.1 g of the leaf powder was mixed with 1000 µl of
 307 50% methanol and incubated in shaking incubator for 12 hours at 4 °C. The contents
 308 were centrifuged at 13000 rpm for 15 minutes at 4 °C. The supernatant was pipetted
 309 into new eppendorf tubes and 15 µl of the extracts were diluted 10-fold with 70% (v/v)
 310 cold methanol and filtered with 0.2µm Millex Millipore membrane into sample bottles
 311 with glass insert. The diluted extracts were stored at 4 °C and were later used for
 312 non-targeted whole-metabolome analysis. Whole-metabolome profiling data was
 313 generated with 2777 C UPLC system (Waters, UK) type of Liquid chromatography
 314 and Xevo G2-XS QTOF (Waters, UK) mass spectrometry instruments (BGI.Tech
 315 metabolomics platform at ShengZheng). The HPLC assay was conducted with six
 316 technical replicates.

317

318 **2. Culturing and preparation of Magnaporthe oryzae mycelia for Metabolomic**

319 **Assay**

320 Wild-type *P. oryzae* Guy11 samples for metabolomics assays were cultured in
 321 complete liquid media (CM) (6 g yeast extract, 6 g casamino acid, 10 g sucrose in 1L
 322 distilled water) in a shaking incubator operating at 150 rpm at 28.5 °C for 5 days. The
 323 cultured strains were subsequently filtered and thoroughly rinsed with sterilized
 324 double deionized water (ddH₂O) and freeze-dried in 70% (v/v) methanol for 24 hours
 325 in (Labconco Free Zone 12L). The dried hyphae tissues were ground into powder
 326 using a pestle and mortar. 0.16 mg of the ground hyphae was mixed with 1.5mL of

327 50% (v/v) methanol, vortexed vigorously to yield a uniform mixture, and incubated in
328 a water bath at 65°C for 1 hour. After incubation, the mixture was centrifuged for 10
329 minutes at 12000 rpm. The supernatant was aliquoted into a new 2.0 mL sterilized
330 eppendorf tube, 15µL of the supernatant was diluted 10-fold with 70% (v/v) methanol
331 and filtered with 0.2µm Millex Millipore membrane into sample bottles with glass
332 insert and stored at 4°C for metabolic analysis.

333

334 **3. Harvesting and preparation of conidia for metabolomic Assay**

335 To generate conidia for metabolomics analysis, a mycelial plug of Wild-type *P. oryzae*
336 Guy11 strains were grown on rice bran medium, at 27°C with constant exposure to
337 light. After 10 days the conidia were harvested, washed with sterile distilled water,
338 and was observed under the microscope. The washed conidia were then filtered and
339 centrifuged for 10 minutes at 12000 rpm. The conidia were ground in liquid-nitrogen
340 to yield fine powdered. 0.10 mg of the conidia powder was mixed with 1.5mL of 50%
341 (v/v) methanol, vortexed vigorously to yield a uniform mixture and incubated in a
342 water bath at 65°C for 1 hour. After incubation, the mixture was centrifuged for 10
343 minutes at 12000 rpm. The supernatant was aliquoted into a new 2.0 mL sterilized
344 Eppendorf tube, 15µL of the supernatant was diluted 10-fold with 70% (v/v) methanol
345 and filtered with 0.2µm Millex Millipore membrane into sample bottles with glass
346 insert and stored at 4°C for metabolic analysis.

347

348 **4. Generation of Appressorium for Metabolomic assays**

349 For appressorium formation metabolome profiling, appressoria were generated by
350 dropping an aliquot of 1.0 mL per of conidia suspension (1×10^5) on fisher scientific
351 hydrophobic slide surface and incubated in a humid chamber at 26°C without light.
352 Appressorium formation was observed after 12 hours using an optical microscope.
353 Solution drops containing the developed appressorium were pipetted into sterilized
354 EP-tubes and centrifuged for 5 minutes at 5000 rpm. The liquid was pipetted-out and
355 the pellet was transferred, frozen and ground in liquid nitrogen to yield a fine powder

356 using pestle and mortar. 0.10 mg of the powder generated was mixed with 1.5mL of
357 50% (v/v) methanol, vortexed vigorously to yield a uniform mixture and incubated in
358 a water bath at 65 °C for 1 hour. After incubation, the mixture was centrifuged for 10
359 minutes at 12000 rpm. The supernatant was aliquoted into new 2.0 mL sterilized
360 Eppendorf tubes, 15µL of the supernatant was diluted 10-fold with 70% (v/v)
361 methanol and filtered with 0.2µm Millex Millipore membrane into sample bottles with
362 glass insert and stored at 4 °C for metabolic analysis.

363 **5. Pathogenicity Assay**

364
365 For plant infection assays, conidia were collected from strains cultured on rice-bran
366 medium for 7-10 days. Conidial suspensions were adjusted to $1.5-2.0 \times 10^5$
367 conidia/mL in 0.02% Tween solution and sprayed onto 3-4-week-old susceptible
368 (CO39, LTH & NPB) and resistant (*Pi-b*, *Pi-4b*, & *Pi-gm*) rice seedlings. Inoculated
369 plants were incubated in a dark, humid chamber at 25 °C for 24 hours, and then moved
370 to another humid chamber with a 12 hour photoperiod. The plants were examined for
371 disease symptoms at 7-days post-inoculation.

372

373 **6. Evaluating the influence of rice leaf extracts on conidia germination and** 374 **appressorium formation**

375 Conidia were collected from 7-day-old rice-bran medium. Conidial suspensions were
376 adjusted to $1.5-2.0 \times 10^5$ conidia/mL in 0.02% Tween solution and sprayed onto
377 3-4-week-old susceptible (CO39, LTH & NPB) and resistant (*Pi-b*, *Pi-4b*, & *Pi-gm*)
378 rice seedlings. Inoculated plants were incubated in a dark, humid chamber at 25°C for
379 24 hours, then moved to another humid chamber with 12 hour photoperiod. The
380 inoculated rice leaves were then grounded in liquid nitrogen into a fine powder. About
381 1g of crushed leaves were dissolved in 4 ml of 80% methanol and incubated at 4 °C
382 on a shaking incubator overnight. After overnight shaking, the mixture was
383 centrifuged for 10 minutes at 13000 g to obtain the supernatant. The supernatant was

384 then filtered with non-sterilized millex syringe driven membrane. The substrate syrup
385 was used to directly wash conidia from the culture plates. 20 uL of the conidia
386 suspensions was placed on a fisher scientific hydrophobic microscope cover glass and
387 incubated in a dark humid chamber at 26°C before proceeding to appressorium
388 formation.

389

390 **7. RNA extraction and generation of Illumina RNA sequencing library**

391 Total RNA was extracted from the inoculated rice seedlings (C_Co39, C_NPB, and
392 C_gm) along with their non-inoculated control group T_Co39, T_NPB, and T_gm.
393 The extraction of total RNA from inoculated and non-inoculated control samples was
394 carried-out with RNeasy pure Plant Kit (Qiagen, Beijing) by following processes
395 recommended by the manufacturer. RNA degradation and contamination were
396 measured by running the extracted RNAs on 1% agarose gels. RNA integrity was
397 assessed using the RNA Nano 6000 Assay Kit of the Bioanalyzer 2100 system
398 (Agilent Technologies, CA, USA). The RNA concentration was measured with an
399 RNA Assay Kit in Qubit 2.0 Fluorometer (Life Technologies, CA, USA). The RNA
400 concentration was measured with an RNA Assay Kit in Qubit 2.0 Fluorometer (Life
401 Technologies, CA, USA). The cDNA library was sequenced on the Illumina
402 sequencing platform (Illumina HiSeq 2000) with 150 bp pair-end reads length and 300
403 bp insert size by Gene Denovo Co. (Guangzhou, China). Novogene in-house Perl
404 script was used to select clean reads by removing adaptor sequences, low-quality
405 sequences (reads with more than 50% of bases quality lower than 20) and reads with
406 more than 5% N bases. The reference genome of Nipponbare genome *Oryza sativa*
407 Japonica and gene model annotation files were downloaded from the genome website
408 directly (58). Index of the reference genome was built using Hisat2 v2.0.4, and
409 paired-end clean reads were aligned to the reference genome using Hisat2 v2.0.4. We
410 selected Hisat2 as the mapping tool for that Hisat2 can generate a database of splice
411 junctions based on the gene model annotation file and thus a better mapping result
412 than other non-splice mapping tools.

413 **Additional information**

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

417 **Acknowledgements**

418 This work was supported by National Natural Science Foundation of China
419 (No.U1805232, No.31770156) and National Key Research and Development Program
420 of China (2016YFD0300707)

421

422 Reference

- 423 1. Uda M, *et al.* (2018) Antimicrobial Activity of Plant Extracts from Aloe Vera, Citrus
424 Hystris, Sabah Snake Grass and Zingiber Officinale against Pyricularia Oryzae that
425 causes Rice Blast Disease in Paddy Plants. *IOP Conference Series: Materials Science
426 and Engineering*, (IOP Publishing), p 012009.
- 427 2. Zhong Z, *et al.* (2018) Population genomic analysis of the rice blast fungus reveals
428 specific events associated with expansion of three main clades. *The ISME journal*:1.
- 429 3. Dean RA, *et al.* (2005) The genome sequence of the rice blast fungus Magnaporthe
430 grisea. *Nature* 434(7036):980.
- 431 4. Marcel S, Sawers R, Oakeley E, Angliker H, & Paszkowski U (2010) Tissue-adapted
432 invasion strategies of the rice blast fungus Magnaporthe oryzae. *The Plant Cell*
433 22(9):3177-3187.
- 434 5. Wilson RA & Talbot NJ (2009) Under pressure: investigating the biology of plant
435 infection by Magnaporthe oryzae. *Nature Reviews Microbiology* 7(3):185.
- 436 6. Howard RJ & Valent B (1996) Breaking and entering: host penetration by the fungal
437 rice blast pathogen Magnaporthe grisea. *Annual Reviews in Microbiology*
438 50(1):491-512.
- 439 7. Fernandez J & Orth K (2018) Rise of a Cereal Killer: The Biology of Magnaporthe
440 oryzae Biotrophic Growth. *Trends in microbiology*.
- 441 8. Promchuay A, Nilthong S, & Jantasuriyarat C (2017) Investigation of Pid3 Rice Blast
442 Resistant Gene in Northern Upland Rice Varieties (Oryza sativa L.), Thailand Using
443 Molecular Markers. *Journal of Advanced Agricultural Technologies Vol* 4(3).
- 444 9. Huang C-L, Hwang S-Y, Chiang Y-C, & Lin T-P (2008) Molecular evolution of the Pi-ta
445 gene resistant to rice blast in wild rice (Oryza rufipogon). *Genetics* 179(3):1527-1538.
- 446 10. Deng Y, Zhu X, Shen Y, & He Z (2006) Genetic characterization and fine mapping of
447 the blast resistance locus Pigm (t) tightly linked to Pi2 and Pi9 in a broad-spectrum
448 resistant Chinese variety. *Theoretical and applied genetics* 113(4):705-713.
- 449 11. Bao J, *et al.* (2017) PacBio sequencing reveals transposable elements as a key
450 contributor to genomic plasticity and virulence variation in Magnaporthe oryzae.
451 *Molecular plant* 10(11):1465-1468.
- 452 12. Akatsuka T, Kodama O, Sekido H, Kono Y, & Takeuchi S (1985) Novel Phytoalexins
453 (Oryzaalexins A, B and C) Isolated from Rice Blast Leaves Infected with Pyricularia
454 oryzae. Part I: Isolation, Characterization and Biological Activities of Oryzaalexins Part
455 II: Structural Studies of Oryzaalexins. *Agricultural and biological chemistry*
456 49(6):1689-1701.
- 457 13. Otomo K, *et al.* (2004) Diterpene cyclases responsible for the biosynthesis of
458 phytoalexins, momilactones A, B, and oryzaalexins A–F in rice. *Bioscience,
459 biotechnology, and biochemistry* 68(9):2001-2006.
- 460 14. Ghazanfar MU, Habib A, & Sahi S (2009) Screening of rice germplasm against
461 Pyricularia oryzae the cause of rice blast disease. *Pak. J. Phytopathol* 21(1):41-44.
- 462 15. Norvienyeku J, *et al.* (2017) Methylmalonate-semialdehyde dehydrogenase
463 mediated metabolite homeostasis essentially regulate conidiation, polarized

- 464 germination and pathogenesis in *Magnaporthe oryzae*. *Environmental microbiology*
- 465 19(10):4256-4277.
- 466 16. Hashimoto K, *et al.* (2009) Comprehensive analysis of glycosyltransferases in
- 467 eukaryotic genomes for structural and functional characterization of glycans.
- 468 *Carbohydrate research* 344(7):881-887.
- 469 17. Kim S, *et al.* (2018) PubChem 2019 update: improved access to chemical data.
- 470 *Nucleic acids research* 47(D1):D1102-D1109.
- 471 18. Kumaraswamy KG, Kushalappa AC, Choo TM, Dion Y, & Rioux S (2011) Mass
- 472 spectrometry based metabolomics to identify potential biomarkers for resistance in
- 473 barley against fusarium head blight (*Fusarium graminearum*). *Journal of chemical*
- 474 *ecology* 37(8):846-856.
- 475 19. Abuyusuf M, *et al.* (2018) Glucosinolate Profiling and Expression Analysis of
- 476 Glucosinolate Biosynthesis Genes Differentiate White Mold Resistant and
- 477 Susceptible Cabbage Lines. *International journal of molecular sciences* 19(12):4037.
- 478 20. Doughari J (2015) An overview of plant immunity. *J. Plant Pathol. Microbiol*
- 479 6(11):10.4172.
- 480 21. Abbruscato P, *et al.* (2014) Triterpenoid glycosides from *medicago sativa* as
- 481 antifungal agents against *Pyricularia oryzae*. *Journal of agricultural and food*
- 482 *chemistry* 62(46):11030-11036.
- 483 22. Osbourn AE (2003) Saponins in cereals. *Phytochemistry* 62(1):1-4.
- 484 23. Cai F, *et al.* (2017) *Medicago truncatula* Oleanolic-Derived Saponins Are Correlated
- 485 with Caterpillar Deterrence. *Journal of chemical ecology* 43(7):712-724.
- 486 24. Kortbeek RW, van der Gragt M, & Bleeker PM (2018) Endogenous plant metabolites
- 487 against insects. *European Journal of Plant Pathology*:1-24.
- 488 25. Itkin M, *et al.* (2013) Biosynthesis of antinutritional alkaloids in solanaceous crops is
- 489 mediated by clustered genes. *Science*:1240230.
- 490 26. Liu C, Ha CM, & Dixon RA (2018) Functional Genomics in the Study of Metabolic
- 491 Pathways in *Medicago truncatula*: An Overview. *Functional Genomics in Medicago*
- 492 *truncatula*, (Springer), pp 315-337.
- 493 27. Chandran AKN, *et al.* (2016) Updated Rice Kinase Database RKD 2.0: enabling
- 494 transcriptome and functional analysis of rice kinase genes. *Rice* 9(1):40.
- 495 28. Wang Y, *et al.* (2016) Transcriptome profiling of Huanglongbing (HLB) tolerant and
- 496 susceptible citrus plants reveals the role of basal resistance in HLB tolerance.
- 497 *Frontiers in plant science* 7:933.
- 498 29. Boachon B, *et al.* (2014) Role of two UDP-Glycosyltransferases from the L group of
- 499 *arabidopsis* in resistance against *pseudomonas syringae*. *European journal of plant*
- 500 *pathology* 139(4):707-720.
- 501 30. Geisler K, *et al.* (2013) Biochemical analysis of a multifunctional cytochrome P450
- 502 (CYP51) enzyme required for synthesis of antimicrobial triterpenes in plants.
- 503 *Proceedings of the National Academy of Sciences* 110(35):E3360-E3367.
- 504 31. Tamura K, *et al.* (2017) Cytochrome P450 monooxygenase CYP716A141 is a unique
- 505 β -amyrin C-16 β oxidase involved in triterpenoid saponin biosynthesis in *Platycodon*
- 506 *grandiflorus*. *Plant and Cell Physiology* 58(5):874-884.

- 507 32. Augustin JM, Kuzina V, Andersen SB, & Bak S (2011) Molecular activities,
508 biosynthesis and evolution of triterpenoid saponins. *Phytochemistry* 72(6):435-457.
- 509 33. Anderson JP, *et al.* (2010) Plants versus pathogens: an evolutionary arms race.
510 *Functional plant biology* 37(6):499-512.
- 511 34. Suzuki H, Achnine L, Xu R, Matsuda SP, & Dixon RA (2002) A genomics approach to
512 the early stages of triterpene saponin biosynthesis in *Medicago truncatula*. *The Plant*
513 *Journal* 32(6):1033-1048.
- 514 35. Sui C, *et al.* (2011) Transcriptome analysis of *Bupleurum chinense* focusing on genes
515 involved in the biosynthesis of saikosaponins. *BMC genomics* 12(1):539.
- 516 36. Tava A, Scotti C, & Avato P (2011) Biosynthesis of saponins in the genus *Medicago*.
517 *Phytochemistry reviews* 10(4):459-469.
- 518 37. Qi X, *et al.* (2006) A different function for a member of an ancient and highly
519 conserved cytochrome P450 family: from essential sterols to plant defense.
520 *Proceedings of the National Academy of Sciences* 103(49):18848-18853.
- 521 38. Fischer J, Schroeckh V, & Brakhage AA (2016) Awakening of fungal secondary
522 metabolite gene clusters. *Gene Expression Systems in Fungi: Advancements and*
523 *Applications*, (Springer), pp 253-273.
- 524 39. Chen J, *et al.* (2017) Genomic and transcriptomic analyses reveal differential
525 regulation of diverse terpenoid and polyketides secondary metabolites in *Herichium*
526 *erinaceus*. *Scientific reports* 7(1):10151.
- 527 40. Hostettmann K & Marston A (2005) *Saponins* (Cambridge University Press).
- 528 41. Townsend B, Jenner H, & Osbourn A (2006) Saponin glycosylation in cereals.
529 *Phytochemistry Reviews* 5(1):109-114.
- 530 42. Mugford ST & Osbourn A (2012) Saponin Synthesis and Function 28. *Isoprenoid*
531 *Synthesis in Plants and Microorganisms: New Concepts and Experimental*
532 *Approaches*:405.
- 533 43. Nielsen JK, Nagao T, Okabe H, & Shinoda T (2010) Resistance in the plant, *Barbarea*
534 *vulgaris*, and counter-adaptations in flea beetles mediated by saponins. *Journal of*
535 *chemical ecology* 36(3):277-285.
- 536 44. Mugford ST & Osbourn A (2012) Saponin synthesis and function. *Isoprenoid*
537 *Synthesis in Plants and Microorganisms*, (Springer), pp 405-424.
- 538 45. Augustin JM, *et al.* (2012) UDP-glycosyltransferases from the UGT73C Subfamily in
539 *Barbarea vulgaris* catalyse Sapogenin 3-O-glucosylation in Saponin-mediated Insect
540 resistance. *Plant physiology*: pp. 112.202747.
- 541 46. Woldemichael GM & Wink M (2002) Triterpene glycosides of *Lupinus angustifolius*.
542 *Phytochemistry* 60(4):323-327.
- 543 47. Hu C, *et al.* (2014) Metabolic variation between japonica and indica rice cultivars as
544 revealed by non-targeted metabolomics. *Scientific reports* 4:5067.
- 545 48. Kusano M, *et al.* (2015) Using metabolomic approaches to explore chemical diversity
546 in rice. *Molecular plant* 8(1):58-67.
- 547 49. Schauer N, *et al.* (2006) Comprehensive metabolic profiling and phenotyping of
548 interspecific introgression lines for tomato improvement. *Nature biotechnology*
549 24(4):447.

- 550 50. Piotrowska A & Bajguz A (2011) Conjugates of abscisic acid, brassinosteroids,
551 ethylene, gibberellins, and jasmonates. *Phytochemistry* 72(17):2097-2112.
- 552 51. Kumar Y, *et al.* (2015) Metabolic profiling of chickpea-Fusarium interaction identifies
553 differential modulation of disease resistance pathways. *Phytochemistry*
554 116:120-129.
- 555 52. Ling Y & Weilin Z (2016) Genetic and biochemical mechanisms of rice resistance to
556 planthopper. *Plant cell reports* 35(8):1559-1572.
- 557 53. Das S, *et al.* (2016) Variations in soil alter availability of carlinoside: an anti-hepatitic
558 compound from *Cajanus cajan* (Linn.) leaves. *Current Science (00113891)* 110(11).
- 559 54. Kodama O, Miyakawa J, Akatsuka T, & Kiyosawa S (1992) Sakuranetin, a flavanone
560 phytoalexin from ultraviolet-irradiated rice leaves. *Phytochemistry*
561 31(11):3807-3809.
- 562 55. Hasegawa M, *et al.* (2014) Analysis on blast fungus-responsive characters of a
563 flavonoid phytoalexin sakuranetin; accumulation in infected rice leaves, antifungal
564 activity and detoxification by fungus. *Molecules* 19(8):11404-11418.
- 565 56. Gupta S, Ravindranath B, & Seshadri T (1972) Polyphenols of *Juglans nigra*.
566 *Phytochemistry* 11(8):2634-2636.
- 567 57. Narasimhachari N & Seshadri T (1952) Components of the bark of *Prunus pudum*.
568 *Proceedings of the Indian Academy of Sciences-Section A*, (Springer), p 202.
- 569 58. Sakai H, *et al.* (2013) Rice Annotation Project Database (RAP-DB): an integrative and
570 interactive database for rice genomics. *Plant and Cell Physiology* 54(2):e6-e6.

571

572

573

Bayogenin 3-O-Cellobioside is a novel non-cultivar specific anti-blast metabolite produced in rice in response to *Pyricularia oryzae* infection

List of Figures and Figure Legends

Figure 1: Crude leaf extracts from pre-inoculated resistant rice cultivars significantly inhibits germination and appressorium formation in *P. oryzae*. (A). Shown blast symptoms and the susceptibility index of homogenously susceptible rice cultivars sprayed inoculation with conidia suspensions of *P. oryzae*. (B) Shown 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 represent complete resistance, lesion type 1-2 represent moderate resistance while lesion type 3-5 signifies complete susceptibility.(C) Exhibit germination and appressorium formation characteristics of rice blast fungus spores treated with total crude extracts obtained from pre-inoculated and non-inoculated susceptible rice cultivars. (D). The micrograph display the inhibitory effects of total crude extracts obtained from pre-inoculated and non-inoculated resistant rice cultivars on the germination of rice blast fungus spores. (E). The stacked column graph is a statistical presentation of *P. oryzae* spores treated with total crude extracts from susceptible rice cultivars. (F). 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) was 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 (n=750), Scale bars, 10 μ m.

Figure 2: Susceptible and resistant rice cultivars undergo common and differential metabolome reprogramming in response to *P. oryzae* infection. (A). PCA showed the number of common and differential metabolites recorded exclusively in susceptible and resistant rice cultivars challenged with rice blast fungus under positive (Pos.) ionization mode. (B). Shown the number of common and different metabolites recorded in susceptible and resistant rice cultivars inoculated with the rice blast

fungus under both negative (Neg⁻) and Pos⁺ ionization mode. (C). The graphical matrix showed the list, clusters and abundance intensity of metabolite generated exclusively in susceptible rice cultivars in response to rice blast fungus infection under (Neg⁻) ionization mode. (D). The heat-map showed names and cluster of unique metabolites identified exclusively in resistant rice cultivars inoculated with *P. oryzae* under (Pos⁺) ionization mode along with their comparative mass abundance intensities across the respective cultivars. (E) Represent the chromatograph of metabolites identified exclusively in both susceptible and resistant rice cultivars inoculated with the rice blast fungus under positive (Pos⁺) ionization mode. (F) Displayed the chromatograph of common metabolites identified in susceptible and resistant rice cultivars challenged with the rice blast fungus under negative (Neg⁻) ionization mode. (G-K). Displayed mass abundances recorded for non-cultivars specific metabolites identified in both susceptible and resistant rice cultivars under under positive (Pos⁺) and negative (Neg⁻) ionization mode. Structures for the respective metabolites are presented in supplementary figure 3. Metabolomics data was obtained from one biological replicate with six technical replicates (n= 240, seedling population per replicate =40). Metabolites with a mass abundance $\geq$ 1000, mass error $\leq \pm 3$ T-test P-value (q-value) ≤ 0.05 , relative standard deviation (RSD) $\leq 30\%$ est P-value (q-value) ≤ 0.05 , relative standard deviation (RSD) $\leq 30\%$ and a minimum score of 25% were filtered and used for comparative metabolomics analysis (A, B, C, & D). Quantitative inter-metabolomics variation between groups was analyzed with ANOVA (G, H, I, J, & K).

41
42

43

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% percent 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.

Figure 4: Clustering and expression profile of putative saponin biosynthesis gene in rice during *P. oryzae* infection. (A). the Neighbor-joining tree showed the clustering 145 rice diphosphate glucuronyltransferases (UDP/GTs) into 33 different sub-families (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 represent rice chromosome. The position of the blue circle (connecting the upper and lower arms) on each chromosome indicate represent 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 represent 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 (Pi-gm) rice cultivars challenged with the rice blast fungus. Note the expression these 106 was exclusively in response to infection and were not detected in the non-inoculated control groups. Each coloured dot in or outside box plot represent the unique expression level ($\log_2$ 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.

72

73

74 **Figure 5:** Schematic representation of different types of saponins their exclusive insecticidal and fungicidal effects. *P.*
75 *oryzae* mediated metabolome-reprogramming result in the generation of Bayogenin 3-O-Cellobioside in resistant and susceptible
76 rice cultivars from both *japonica* and *indica* lineages combined enzymatic activities multiple diphosphate glucuronyltransferases
77 and β-amyrin synthases. Like avenacin, Bayogenin 3-O-Cellobioside represent a novel rice saponin with anti-blast effect.

78

79