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1 **Short title:**

2 Cell wall damage induced signalling in rice

3 **Article title:**

4 The dual function receptor kinase, OsWAKL21.2, is involved in elaboration of
5 lipaseA/esterase induced immune responses in rice

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14 One sentence Summary: (200 characters)

15 A novel rice receptor WAKL21 that sense cell wall damage caused by Xanthomonas secreted
16 cell wall degrading enzyme to induce immune responses.

17 **Author's contribution:**

18 R.V.S., K.K.M. and A.R. designed the experiments. A.R. and H.K.P. performed microarray.
19 A.R. and K.K.M. and S.J.H. performed cloning and transient expression studies. K.K.M.
20 performed publicly available transcriptome analysis, qRT-PCR analysis, Western blotting, and
21 biochemical characterization experiments and wrote the paper. A.R. and K.K.M. generated
22 transgenic Arabidopsis lines on which K.K.M. and S.J.H. performed experiments. K.K.M.,
23 H.K.P. and R.V.S. finalised the manuscript, which was approved by all authors.

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29 The author responsible for distribution of materials integral to the findings presented in this
30 article in accordance with the policy described in the Instructions for Authors
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32

33

34 Abstract:

35 Plant pathogens secrete cell wall degrading enzymes (CWDEs) to degrade various components
36 of the plant cell wall. Plants sense this cell wall damage as a mark of infection and induce
37 immune responses. Little is known about the plant functions that are involved in the elaboration
38 of cell wall damage-induced immune responses. Transcriptome analysis revealed that a rice
39 receptor kinase, *WALL-ASSOCIATED KINASE-LIKE 21* (*OsWAKL21.2*), is upregulated
40 following treatment with either *Xanthomonas oryzae* pv. *oryzae* (*Xoo*, a bacterial pathogen) or
41 LipA, a CWDE of *Xoo*. Downregulation of *OsWAKL21.2* attenuates LipA mediated immune
42 responses. Overexpression of *OsWAKL21.2* in rice mimics LipA treatment mediated induction
43 of immune responses and enhanced expression of defence related genes, indicating it could be
44 involved in the perception of LipA induced cell wall damage in rice. *Os*WAKL21.2 is a dual
45 function kinase having *in-vitro* kinase and guanylate cyclase activities. Ectopic expression of
46 *OsWAKL21.2* in Arabidopsis also activates plant immune responses. Interestingly,
47 *Os*WAKL21.2 needs kinase activity to activate rice immune responses while in Arabidopsis it
48 needs guanylate cyclase activity. Our study reveals a novel receptor kinase involved in
49 elaboration of DAMP induced rice immune responses that can activate similar immune
50 responses in two different species via two different mechanisms.

51 Introduction:

52 The plant cell wall acts as a formidable barrier for pathogens. Plant pathogens secrete a battery
53 of cell wall degrading enzymes (CWDEs) to degrade different components of the plant cell
54 wall (Hématy et al., 2009, Albersheim and Anderson-Prouty, 1975). CWDEs act as a double-
55 edged sword for pathogens as on one hand the activity of these enzymes leads to cell wall
56 degradation, on the other hand, it releases cell wall degradation products that can elicit plant
57 immune responses (Jha et al., 2005, Hahn et al., 1981). Such host derived molecules that can
58 elicit immune responses are called damage associated molecular patterns (DAMPs). Some
59 known cell wall degradation products that act as DAMPs include pectin degradation products

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60 oligogalacturonide (OG), hemicellulose degradation products such as xyloglucan oligomers,
61 and cellulose degradation products such as cellobiose and cellotriose (Gust et al., 2017, de
62 Azevedo Souza et al., 2017, Claverie et al., 2018). These DAMPs are sensed by membrane-
63 localised receptor-like kinases (RLKs) that activate the signaling cascade. Some known
64 receptors of the DAMPs are AtPEPR1/2 for plant elicitor peptides (Pep), AtDORN1 for eATP,
65 SYR1 for systemins and AtWAK1/2 for oligogalacturonide (OG) (Brutus et al., 2010, Gust et
66 al., 2017, Wang et al., 2018).

67 The wall-associated kinases (WAKs) constitute a unique class of receptor kinases which are
68 known to be closely associated with the plant cell wall (Verica and He, 2002). WAKs are
69 known to be involved in many physiological processes including cell elongation, pollen
70 development and abiotic and biotic stress tolerance (Kohorn, 2015). Members of the WAK
71 gene family have been known to interact with pectin and pectin degradation products (OGs).
72 AtWAK1 and AtWAK2 have been reported to interact with pectin and OGs *in vitro* (Kohorn
73 et al., 2006, Kohorn et al., 2009). Some proteins of the WAK gene family have also been known
74 to be involved in immune responses in many plant species such as Arabidopsis, rice, maize and
75 wheat (Zhang et al., 2017, Harkenrider et al., 2016, Zuo et al., 2015, Hurni et al., 2015,
76 Saintenac et al., 2018, Li et al., 2009, He et al., 1998, Hu et al., 2017). In most of the cases, a
77 receptor kinase or receptor-coreceptor complex recognises the ligand and triggers
78 phosphorylation events leading to activation of MAP kinase signaling and its downstream
79 targets (Meng and Zhang, 2013). However, some recent studies also indicate the presence of
80 an alternate signaling system in plants which is mediated by cyclic nucleotides such as cyclic
81 guanosine monophosphate (cGMP) and cyclic adenosine monophosphate (cAMP) (Gehring
82 and Turek, 2017). cGMP is generated by guanylate cyclases (GCs) and most of the reported
83 plant GCs are membrane localised receptor kinases that also contain a functional GC motif
84 inside the kinase domain (Gehring and Turek, 2017). Such kinases showing these dual activities
85 are called moonlighting kinases (Wong et al., 2015). In Arabidopsis, some receptor kinases
86 including a wall associated kinase like gene (*AtWAKL10*) are reported as such moonlighting
87 kinases (Meier et al., 2010).

88 Rice (*Oryza sativa*) serves as a staple food for more than half of the world population.
89 *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) causes the serious bacterial blight disease of rice.
90 CWDEs secreted by *Xoo* include cellulases, xylanases and lipases/esterases (LipA)
91 (Rajeshwari et al., 2005, Jha et al., 2007). LipA is an important CWDE of *Xoo* and deletion of
92 the LipA gene results in a significant reduction in the virulence of *Xoo* in rice (Jha et al., 2007).

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93 Treatment of rice tissue with purified LipA leads to the activation of plant immune responses
94 including callose deposition, programmed cell death and an enhanced tolerance towards *Xoo*
95 (Aparna et al., 2009). The mechanism of action of LipA on the cell wall is still not clear, but it
96 has been predicted that it acts by cleaving ester linkages in the rice cell wall (Aparna et al.,
97 2009). Heat inactivation or mutation of the active site residues of LipA abolishes the
98 biochemical activity as well as the ability to induce immune responses in rice, indicating that
99 the enzymatic activity of LipA is essential for the induction of immune response (Jha et al.,
100 2007, Aparna et al., 2009). However, the process through which rice senses the cell wall
101 damage caused by LipA and further activates immune responses is not clear. Several receptors
102 of DAMPs have been reported in the model plant Arabidopsis, but none of them in the case of
103 rice.

104 In this study, transcriptome analysis was initially performed to identify gene expression
105 changes that occur during LipA induced immune responses in rice. An enhanced transcript
106 level of a wall-associated kinase like gene, *OsWAKL21.2* was observed after treatment of rice
107 leaves with either purified LipA or the pathogen, *Xoo,* but not after treatment with a LipA
108 mutant of *Xoo*. Sequence alignment and biochemical studies indicate that OsWAKL21.2 is a
109 dual function receptor kinase that has an *in vitro* kinase as well as a GC activity. OsWAKL21.2
110 is a key component of signaling involved in LipA induced immunity as its downregulation
111 leads to attenuation of LipA induced immune response. Overexpression of *OsWAKL21.2* in
112 rice and ectopic expression in Arabidopsis induces plant defence response and confers
113 enhanced tolerance to subsequent bacterial infection. However, we have observed that the
114 mode of action of the receptor is dissimilar in rice and Arabidopsis. Our results suggest that
115 OsWAKL21.2 requires its kinase activity to induce immune response in rice, whereas, in
116 Arabidopsis, it requires GC activity.

117
Results:

118
Expression of *OsWAKL21.2* was enhanced after treatment of rice leaves with LipA

119 In order to identify rice functions that are potentially involved in early stages of LipA induced
120 immune responses, we performed transcriptome analysis of rice leaves at 30 minutes and 2hr
121 after infiltration with LipA. After 30 minutes, no gene was significantly altered while 78 genes
122 (74 unique set of genes) were differentially expressed (68up, 10 down) (>1.5 fold) after 2hr of
123 LipA treatment (Supplemental Fig. S1A, Supplemental Table 1). Pathway analysis using
124 MapMan indicates that more than one-third (31/78) of these genes might have roles in

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125 signaling, defence responses or in transcription/translation (Supplemental Fig. S1B). When
126 compared with a previous microarray (Ranjan et al., 2015) performed after 12hr of LipA
127 treatment, we observed 38 of these 78 genes are differentially expressed (37up, 1 down) at both
128 time points (Fig. 1A) (Supplemental Table 2). We compared with a publicly available
129 microarray dataset that was performed 24hr after treatment of rice leaves with various
130 *Xanthomonas oryzae* strains (GEO Acc. No. GSE36272), we observed some of these 38 genes
131 were commonly upregulated following *Xanthomonas oryzae* treatment (Supplemental Table
132 3). The upregulation of six of these commonly upregulated genes was validated by qRT-PCR
133 after treatment of rice leaves with either *Xoo* or LipA (Supplemental Fig. S1C). Three of the
134 37 genes that were most commonly upregulated after *Xanthomonas* treatments include a
135 putative wall-associated receptor kinase like gene (*OsWAKL21*, LOC_Os12g40419), a putative
136 ubiquitin ligase (*OsPUB38*, LOC_Os04g35680) and a putative fructose-bisphosphate aldolase
137 (LOC_Os08g02700) (Supplemental Table 3). Since our focus was on the perception of cell
138 wall damage in rice plants, we decided to explore further the function of wall-associated
139 receptor kinase OsWAKL21.

140 *OsWAKL21* has three splice variants [*OsWAKL21.1* (LOC_Os12g40419.1), *OsWAKL21.2*
141 (LOC_Os12g40419.2) and *OsWAKL21.3* (LOC_Os12g40419.3)] (Fig. 1B). qRT-PCR
142 analyses indicate that the second splice variant (*OsWAKL21.2*) is mainly upregulated in rice
143 leaves after either LipA or *Xoo* treatment (Fig. 1C). Interestingly, treatment of rice leaves with
144 LipA mutant of *Xoo* did not enhance expression of *OsWAKL21.2* while introduction of a LipA
145 complementing clone into the LipA mutant restores the ability to enhance expression of
146 *OsWAKL21.2* (Fig. 1D). Microarray data and qRT-PCR also revealed that out of the three
147 splice variants, the expression level of *OsWAKL21.2* is higher in leaves as compared to the
148 other two splice variants (Data not shown).

149 **Overexpression of *OsWAKL21.2* in rice mimics LipA induced immune responses**

150 Treatment of rice tissue with LipA induces immune responses such as callose deposition,
151 enhanced expression of defence related genes, activation of JA pathway and enhanced
152 tolerance against subsequent *Xoo* infection (Jha et al., 2007, Ranjan et al., 2015).
153 Agrobacterium mediated transient overexpression of *OsWAKL21.2* in young rice leaves
154 significantly induces callose deposition which is comparable to callose deposition induced by
155 LipA treatment (Fig. 2A,B). Transient overexpression of *OsWAKL21.2* in rice leaves also
156 enhances tolerance against subsequent *Xoo* infection leading to reduced lesion length caused

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157 by *Xoo* which is also observed following treatment with LipA (Fig. 2C, Supplemental Fig.
158 S2A). The overexpression of *OsWAKL21.2* was confirmed by qRT-PCR and Western blot
159 analysis (Supplemental Fig. S1B,C).

160 Plant immune responses are known to be modulated via the expression of defence-related
161 genes. Therefore, we tested the expression of some key defence-related genes of rice after the
162 transient overexpression of *OsWAKL21.2* in mid-veinal regions of rice leaves. *OsWAKL21.2*
163 overexpression in rice enhances expression of three pathogenesis-related genes (*OsPR1a*,
164 *OsPR10*/*OsPBZ14* and *OsPR10a/OsPBZ1)*, a somatic embryogenesis receptor kinase
165 (*OsSERK2*) and a phenylalanine ammonia lyase (*OsPAL3*) (Fig. 2D). We also tested expression
166 of 10 genes that are upregulated following LipA/*Xoo* treatment (Supplemental Table 3) in
167 microarray and observed seven of these ten genes are also significantly upregulated following
168 overexpression of *OsWAKL21.2* in rice (Supplemental Fig. S2D). These results indicate that
169 Agrobacterium-mediated transient overexpression of *OsWAKL21.2* in rice leaves mimics LipA
170 treatment in terms of induction of callose deposition, enhanced tolerance against subsequent
171 *Xoo* infection and enhances expression of defence-related genes and a number of the LipA
172 responsive genes.

173 **Transient downregulation of *OsWAKL21.2* attenuates LipA induced immune responses**
174 **in rice**

175 We next checked the effect of transient knockdown of *OsWAKL21.2* by Virus-induced gene
176 silencing (VIGS) on LipA induced immune responses. It was observed that the downregulation
177 was not retained by all leaves for a long time which was also observed previously using this
178 vector system (Kant and Dasgupta, 2017). So, an alternative approach was used for assessment
179 of callose deposition after LipA treatment (Supplemental Fig. S3). We categorized the leaf
180 samples qualitatively into three classes based on the amount of callose deposition as low,
181 medium and high callose deposits (Low<30 deposits/leaf, Medium~30-80 deposits/leaf or
182 High>80 deposits/leaf) (Fig. 3A). Following LipA treatment, about 30-40% of the leaf samples
183 showed high callose deposition, 10-15% showed low callose deposition while the rest of them
184 (about 50%) showed a medium level of callose deposition (Fig. 3B). A similar ratio was
185 observed if the seedlings were previously treated with VIGS-EV (Fig. 3C). The number of
186 leaves showing low callose deposition significantly increased to more than 50% in WAKL-
187 RNAi lines (WRI 1-300, WRI 450-600 and WRI 1-600 correspond to the fragment of
188 *OsWAKL21.2* that was used for downregulation) while there was a reduction in the leaves that

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189 showed high or medium callose deposition (Fig. 3C). In RNAi lines, the leaves that show low
190 callose deposition following LipA treatment also show significantly lower transcript/protein
191 level of OsWAKL21.2 which was not observed in the leaves that show high callose deposition
192 (Fig. 3D, Supplemental Fig. S4A).

193 Prior treatment of the mid-vein of rice leaves with LipA or the overexpression of *OsWAKL21.2*
194 enhances tolerance of the plant against subsequent *Xoo* infection. We decided to check if the
195 downregulation of *OsWAKL21.2* in rice enhances susceptibility towards *Xoo* or affects LipA
196 induced tolerance towards *Xoo*. VIGS mediated transient downregulation of *OsWAKL21.2* in
197 rice mid vein attenuates LipA induced enhanced tolerance against subsequent *Xoo* infection
198 (Fig. 3E, Supplemental Fig. S4B). qRT-PCR and Western blotting studies using anti-
199 OsWAKL21 antibodies indicated the downregulation of *OsWAKL21.2* in the mid vein
200 following VIGS mediated *OsWAKL21.2* downregulation (Fig. 3F, Supplemental Fig. S4C).
201 There was slight but usually non-significant reduction on transcript level of other splice
202 variants and no significant difference was observed in transcript level of other predicted off-
203 target genes (Supplemental Fig.S5). This suggests that optimal expression of *OsWAKL21.2* in
204 rice leaves is required for LipA induced tolerance against *Xoo*.

205 **Ectopic expression of *OsWAKL21.2* in transgenic Arabidopsis lines induces plant immune**
206 **responses**

207 In order to determine whether expression of *OsWAKL21.2* would activate immune responses
208 in other plants, we generated stable Arabidopsis transgenic lines expressing *OsWAKL21.2*
209 under a 17-β-estradiol (Est) inducible promoter. Expression of *OsWAKL21.2* in transgenic lines
210 was examined after treatment with the inducer through qRT-PCR and Western blotting
211 (Supplemental Fig. S6A,B). We observed that ectopic expression of *OsWAKL21.2* in
212 Arabidopsis also results in an enhanced callose deposition (Fig. 4A,B). Ectopic expression of
213 *OsWAKL21.2* in Arabidopsis also enhances tolerance against subsequent *Pseudomonas*
214 *syringae* pv. *tomato* DC3000 (*Pst* DC3000) infection leading to reduction in *in planta* growth
215 of *Pst* DC3000 (Fig. 4C). In Arabidopsis, the Salicylic acid (SA) and Jasmonic acid (JA)
216 pathways are widely known to be involved in immune responses. We examined the expression
217 of key genes linked to these pathways in Arabidopsis transgenic lines. The ectopic expression
218 of *OsWAKL21.2* in Arabidopsis resulted in a significant increase in the transcript levels of key
219 SA pathway-related genes (*AtPR2, AtPR5,* and *AtWRKY33*) and *AtGSL5*, a major callose
220 synthase of Arabidopsis (Fig. 4D) (Jacobs et al., 2003, Janda and Ruelland, 2015). The

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221 transcript level of the key JA responsive gene *AtPDF1.2* was found to be decreased, indicating
222 that the JA pathway might be downregulated in Arabidopsis following *OsWAKL21.2*
223 expression (Fig. 4D). Overall, this data implies that in Arabidopsis, *OsWAKL21.2* enhances
224 callose deposition, enhanced expression of SA pathway related genes, and in addition,
225 enhances tolerance against subsequent *Pst* DC3000 infection.

226 **OsWAKL21.2 is a membrane localizing moonlighting receptor kinase having *in vitro***
227 **kinase and guanylate cyclase activities**

228 Sequence analysis of OsWAKL21.2 indicated that it is a receptor-like serine/threonine kinase
229 that accommodates an N-terminal extracellular galacturonan binding domain (GBD), an
230 epidermal growth factor (EGF) like repeat and an intracellular C-terminal kinase domain,
231 resembling other known wall-associated kinases (Fig. 5A). The analyses of OsWAKL21.2 also
232 revealed the presence of a putative guanylate cyclase (GC) motif inside the kinase domain
233 (Supplemental Fig. S7A,C). The tool 'GCpred' also predicted the presence of a plant GC motif
234 (from residue 569-585) inside the kinase domain of OsWAKL21.2 (Supplemental Fig. S7B)
235 (Xu et al., 2018). EGFP tagged recombinant OsWAKL21.2:EGFP localize to the cell
236 membrane in onion epidermal cell indicating it is a membrane bound receptor (Fig. 5B).

237 The biochemical characterization was performed by cloning the intracellular kinase domain of
238 OsWAKL21.2 ($OsWAKL21_{376-725}$) with an N-terminal 6x His tag and expressing it in *E. coli*
239 cells. The purified cytoplasmic domain of OsWAKL21.2 showed an autophosphorylation
240 activity when incubated with γ-^{32}P-ATP indicating that it is an active kinase (Fig. 5C). For the
241 guanylate cyclase activity, the same purified protein was incubated with GTP and cGMP was
242 detected by qualitative and quantitative assays. cGMP was detected only when GTP was
243 incubated with purified $OsWAKL21_{376-725}$ (Fig. 5D, Supplemental figure S7D). The rate of
244 cGMP synthesis was 2.1±0.75 pM/µg protein/hr (Fig. 5D) which is comparable to other known
245 plant GCs such as AtPEPR1, AtPSKR1 and AtGC1. The biochemical analyses strongly
246 suggests that OsWAKL21.2 is a dual-function enzyme having kinase and guanylate cyclase
247 activity.

248 **Kinase activity of OsWAKL21.2 is essential for induction of immune responses in rice**
249 **but not in Arabidopsis**

250 Considering that OsWAKL21.2 is a receptor kinase, we hypothesized that kinase activity of
251 the protein would be required for the induction of immune responses. Based on homology with
252 other plant receptor kinases, we mutated four active site residues (K407, D507, T542, T547)

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253 to alanine and generated a kinase-deficient mutant (OsWAKL21.2-kinase deficient or
254 *OsWAKL21.2*-kd). Purified kinase domain of OsWAKL21.2-kd had almost lost kinase activity
255 but it retains GC activity (Supplemental Fig. S8A,B,C). Furthermore, we observed that
256 Agrobacterium-mediated transient overexpression of the full-length OsWAKL21.2-kd in rice
257 leaves neither enhanced callose deposition nor affected tolerance towards *Xoo* (Fig. 6A,B, and
258 Supplemental Fig. S9A,B). *OsWAKL21.2*-kd also did not enhance the expression of key
259 defence-related genes (Fig. 6C), thus indicating that the kinase activity of OsWAKL21.2 is
260 required for induction of immune responses in rice.

261 In order to further investigate the role of the kinase activity of OsWAKL21.2 in the induction
262 of plant immune responses, we generated transgenic Arabidopsis lines expressing
263 *OsWAKL21.2*-kd. Interestingly, we observed that the ectopic expression of *OsWAKL21.2*-kd
264 in Arabidopsis caused an increase in callose deposition (Fig. 6D, Supplemental Fig. S9C,D).
265 Similar results were observed in four different transgenic lines. In Arabidopsis, the expression
266 of *OsWAKL21.2*-kd increased tolerance towards *Pst* DC3000 and also changed the expression
267 of defence-related genes in a similar pattern as *OsWAKL21.2* (Fig. 6E,F). As mentioned above,
268 this mutant did not induce immune responses in rice, indicating that the kinase activity of
269 OsWAKL21.2 is vital for the induction of immune responses in rice but not in Arabidopsis.

270 **GC activity of *OsWAKL21.2* is required for induction of immune responses in**
271 **Arabidopsis but not in rice**

272 Owing to the fact that the kinase-deficient mutant of *OsWAKL21.2* induced immune responses
273 in Arabidopsis, we decided to investigate whether the GC activity of OsWAKL21.2 might have
274 role in induction of immune responses in Arabidopsis. In order to test this hypothesis, we
275 initially induced the expression of *OsWAKL21.2* in Arabidopsis in the presence of a GC
276 inhibitor LY83583 and observed that the GC inhibitor attenuates *OsWAKL21.2* and
277 *OsWAKL21.2*-kd induced callose deposition in Arabidopsis (Supplemental Fig. S10). In order
278 to confirm this, we generated a mutant of OsWAKL21.2 that lacked the GC activity
279 (*OsWAKL21.2*-GC Deficient or *OsWAKL21.2*-gcd) but retained the kinase activity
280 (Supplemental Fig. S8A,B,C) (Ma et al., 2012). Ectopic expression of *OsWAKL21.2*-gcd did
281 not induce either callose deposition or enhance tolerance towards *Pst* DC3000 (Fig. 7A,B, and
282 Supplemental Fig. S9C,D). Furthermore, *OsWAKL21.2*-gcd failed to significantly alter the
283 expression of most of the defence-related genes that are differentially regulated by
284 *OsWAKL21.2* in Arabidopsis (Fig. 7C). Ectopic expression of *OsWAKL21.2* in Arabidopsis

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285 leaves also enhances in planta cGMP level which was not observed when *OsWAKL21.2-gcd*
286 was expressed in transgenic Arabidopsis plants (Supplemental Fig. S11A,B,C). However,
287 transient overexpression of *OsWAKL21.2*-gcd induces immune responses in rice that were
288 similar to the ones induced by the wild-type OsWAKL21.2 (Fig. 7D,E,F, Supplemental Fig.
289 S9A,B). These observations clearly indicated that the GC activity of OsWAKL21.2 is essential
290 for induction of Arabidopsis immune responses but not for induction of immune responses in
291 rice.

292 ***OsWAKL21.2* possibly induces the JA pathway in rice while it activates SA pathway in**
293 **Arabidopsis**

294 The results in this study indicated that kinase activity of *OsWAKL21.2* is required to induce
295 rice immune responses and that the GC activity is required for induction of Arabidopsis
296 immune responses. Our previous report indicated that the JA pathway is activated in rice leaves
297 after treatment with LipA (Ranjan et al., 2015). We selected a subset of ten genes that were
298 earlier predicted to be associated with the JA pathway in rice and were found to be upregulated
299 after 12hr of LipA infiltration (Ranjan et al., 2015). We tested the expression of these 10 genes
300 and observed that 8 out of 10 genes showed significant upregulation after *OsWAKL21.2*
301 overexpression (Fig. 8A). This indicates that overexpression of *OsWAKL21.2* in rice enhances
302 expression of JA pathway related genes.

303 The results above (Fig. 4D) suggested that expression of SA related genes was enhanced after
304 ectopic expression of *OsWAKL21.2* in Arabidopsis. We further tested the expression of some
305 more SA pathway related Arabidopsis genes (*AtSID2*, *AtCBP60g, AtSARD1, AtSH3, AtNPR3*
306 and *AtWRKY38*) after ectopic expression of *OsWAKL21.2* and observed significantly enhanced
307 expression of these genes (Fig. 8B). In order to validate the role of the SA pathway in
308 *OsWAKL21.2* induced immune responses in Arabidopsis, we made the crosses between
309 *OsWAKL21.2* transgenic lines with *NahG* transgenic lines that do not accumulate SA (Delaney
310 et al., 1994). Transgenic offspring lines that express both *OsWAKL21.2* and *NahG* did not show
311 enhanced callose deposition while sister lines that expressed only *OsWAKL21.2* showed
312 enhanced callose deposition after treatment with estradiol (Fig. 8C, Supplemental Fig. S11C).
313 This observation indicated that *OsWAKL21.2* induces immune responses in Arabidopsis via
314 activation of the SA pathway.

315

316

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317 **Discussion:**

318 CWDEs are important virulence factors secreted by microbial plant pathogens. *Xoo* secretes
319 numerous CWDEs to degrade the rice cell wall and treatment of rice with *Xoo* secreted purified
320 CWDEs such as Cellulase A (ClsA), Cellobiosidase (CbsA) and Lipase/esterase (LipA) leads
321 to activation of plant immune responses (Jha et al., 2007). Earlier we have shown that the
322 biochemical activity of LipA is required for the induction of rice immune responses (Aparna
323 et al., 2009). This indicates that the rice plant is capable of recognizing cell wall degradation
324 products as DAMPs and further induce immune responses. The molecular players involved in
325 the perception of cell wall damage caused by CWDEs in rice is yet to be deciphered. To discern
326 the functions involved in LipA induced immune responses, we performed transcriptome
327 analyses at various time points following LipA treatment. Comparison with online available
328 microarray indicates a handful of genes that are commonly upregulated following LipA or Xoo
329 treatment. One such gene was the second splice variant of a rice Wall-associated kinase-like
330 gene 21 (*OsWAKL21.2*). The wall-associated kinase (WAK) is the only gene family known to
331 recognize plant cell wall-derived DAMPs (Kohorn, 2015). Our study suggests that the
332 expression of *OsWAKL21.2* is enhanced after treatment of rice leaves with either LipA or *Xoo*
333 but not after treatment with a LipA mutant of *Xoo*. This indicates that the increase in
334 *OsWAKL21.2* expression after *Xoo* treatment is specifically because of the presence of LipA
335 in *Xoo*. We also observed that it is a membrane localized receptor kinase having in vitro kinase
336 and guanylate cyclase activity.

337 Downregulation of some WAK gene family members in rice such as *OsWAK14, OsWAK91,*
338 *OsWAK92* or *Xa4-WAK* have been reported to enhance the susceptibility of rice plants towards
339 subsequent infection (Delteil et al., 2016, Hu et al., 2017). We downregulated the expression
340 of *OsWAKL21.2* in rice leaves using VIGS. Although downregulation of *OsWAKL21.2* did not
341 alter susceptibility against *Xoo*, it attenuated LipA induced tolerance to *Xoo* and callose
342 deposition in rice indicating that it is a key component of signaling activated after LipA
343 treatment. Our previous observation suggests that it is the activity and not the structure of LipA
344 that is required for induction of immune responses in rice indicating the role of DAMPs in
345 LipA induced immunity (Jha et al., 2007, Aparna et al., 2009). Some WAKs are already
346 reported as receptors of DAMPs (OGs) in Arabidopsis (Kohorn et al., 2009, Brutus et al.,
347 2010). *OsWAKL21.2* is a receptor that is induced after the LipA treatment at early and late time
348 points and is essential for the recognition of cell wall damage induced by LipA indicating it
349 might be an upstream component in signalling activated following LipA treatment.

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350 Treatment of rice leaves with LipA leads to callose deposition, activation of JA pathway,
351 enhanced expression of some defence related genes and enhanced tolerance against subsequent
352 *Xoo* infection (Ranjan et al., 2015, Jha et al., 2007). Callose deposition is a hallmark of the
353 immune response that is observed after treatment of the plant tissue with CWDEs (including
354 LipA) or DAMPs (Jha et al., 2007, Galletti et al., 2008). We also observed that the
355 overexpression of *OsWAKL21.2* in rice and ectopic expression in Arabidopsis leaves leads to
356 the fortification of the cell wall in the form of callose deposition. Activation of the immune
357 response leads to an increased tolerance towards subsequent infection in plants. We also
358 observed that OsWAKL21.2 induced immune responses lead to enhanced tolerance against
359 subsequent bacterial infection in rice and Arabidopsis. Overexpression of several other WAKs
360 such as *OsWAK1* (Li et al., 2009), *OsWAK25* (Harkenrider et al., 2016), *OsWAK14*, *OsWAK91*
361 or *OsWAK92* (Delteil et al., 2016), *AtWAK2* (Kohorn et al., 2009), *AtWAK1* (Brutus et al.,
362 2010), and Ta-*WAKL4* (Saintenac et al., 2018) has been reported to enhance tolerance towards
363 subsequent infections in different plant species. Immune responses are usually correlated with
364 enhanced expression of defence-related genes. The overexpression of *OsWAKL21.2* in the mid-
365 vein of rice leaves enhanced the expression of five defence-related and LipA responsive genes.
366 The key defence-related genes upregulated by *OsWAKL21.2* overexpression include *OsPR1a*
367 *(Park et al., 2008)*, *OsPR10a (Bai et al., 2011)*, *OsPR10 (Harkenrider et al., 2016)*, *OsSERK2*
368 (Chen et al., 2014) and *OsPAL3* (Chen et al., 2018) which are well categorized as defence-
369 related genes implicated in tolerance against *Xoo.* Interestingly, four of these five key defence
370 genes (except *OsPR1a*) that are upregulated by *OsWAKL21.2* overexpression are also
371 upregulated after 12hr of LipA treatment in a microarray that was earlier done in the lab
372 (Ranjan et al., 2015). Overexpression of *OsWAKL21.2* also enhances the expression of most of
373 the tested LipA responsive genes (7/10) and most of the tested JA pathway related LipA
374 responsive genes (8/10) indicating that the overexpression of *OsWAKL21.2* partially mimics
375 LipA treatment condition. These results establish that the overexpression of *OsWAKL21.2* in
376 rice mimics the LipA treatment condition in rice plants leading to callose deposition, enhanced
377 expression of JA pathway related genes, enhanced tolerance against *Xoo* and the increased
378 expression of defence-related genes. This indicates that *OsWAKL21.2* could be a major
379 upstream component in the signaling process that is activated after cell wall damage caused by
380 LipA.

381 Ectopic expression of *OsWAKL21.2* leads to enhanced expression of the SA responsive genes
382 such as *AtPR2*, *AtPR5* and *AtWRKY33* and downregulation of the JA responsive gene,

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383 *AtPDF1.2* indicating that *OsWAKL21.2* likely activates the SA pathway in Arabidopsis. We
384 observed enhanced expression of several other SA biosynthesis-, regulation- and response-
385 related genes in Arabidopsis (*AtSID2, AtSARD1, AtCBP60G, AtNPR3, AtWRKY33, AtWRKY38*
386 and *AtSH3*) (Janda and Ruelland, 2015). Activation of SA pathway in Arabidopsis enhances
387 expression of biotic stress-responsive callose synthase *AtGSL5* (Dong et al., 2008) which was
388 also upregulated following ectopic expression of *OsWAKL21.2*. We also found that the
389 transgenic plants expressing *OsWAKL21.2* and *NahG* did not show callose deposition,
390 demonstrating that SA accumulation is required for *OsWAKL21.2* induced immune response
391 in Arabidopsis. These outcomes also explain the enhanced tolerance towards *Pst* DC3000, as
392 an activation of the SA pathway in Arabidopsis leads to increased tolerance towards *Pst*
393 DC3000 (Xin and He, 2013). The results indicate that *OsWAKL21.2* when expressed
394 ectopically in Arabidopsis acts as a defence gene and activates SA pathway-mediated immune
395 responses. Some members of the WAK gene family in Arabidopsis such as *AtWAK1, AtWAK2,*
396 *AtWAK3, AtWAK5* and *AtWAKL10* are known as SA responsive genes treatment with SA leads
397 to the enhanced expression of these genes indicating correlation of SA pathway and WAKs in
398 Arabidopsis (He et al., 1998, He et al., 1999, Meier et al., 2010).

399 Ligand binding onto receptor kinases triggers phosphorylation that is further conveyed
400 downstream via phosphorylation by/of kinases and their targets (Macho and Zipfel, 2014). Few
401 receptor kinases such as AtBRI1, AtPSKR1, AtPEPR1, *AtWAKL10* and HpPEPR1 are also
402 known to possess dual enzymatic activity i.e. they poses GC activity along with kinase activity
403 (Ma et al., 2012, Gehring and Turek, 2017, Swiezawska et al., 2017, Swiezawska et al., 2015,
404 Meier et al., 2010) . OsWAKL21.2 also possess such dual activity which is comparable with
405 other plant GCs. Treatment with a GC inhibitor and mutations in active site residues of the GC
406 motif showed that the GC activity of OsWAKL21.2 is required to induce immune responses in
407 Arabidopsis but not in rice. GCs convert GTP to cGMP which acts as a secondary signaling
408 molecule (Gehring and Turek, 2017). Overexpression of plant GCs *AtBRI1*, *AtPSKR1* and
409 *AtPEPR1* in Arabidopsis leads to a partial increase in cytoplasmic cGMP concentrations
410 (Gehring and Turek, 2017). Similarly, we have also observed that the ectopic expression of
411 *OsWAKL21.2* in Arabidopsis leads to a significant increase in the concentration of *in planta*
412 cGMP. Some of the moonlighting kinases such as AtPEPR1, AtBRI1 and AtPSKR1 are already
413 known for their direct or modulatory role in Arabidopsis immune responses (Lozano-Durán
414 and Zipfel, 2015, Igarashi et al., 2012). AtPEPR1 is receptor of DAMP (Pep's) and it's GC
415 activity is required for activation of immune responses (Ma et al., 2012). *AtWAKL10* has also

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416 been predicted as a defence-related gene that is similar to *OsWAKL21.2*. These observations
417 testify the possible involvement of GCs in Arabidopsis immune response. We have found that
418 in rice, OsWAKL21.2 requires the kinase activity for its function, whereas, in Arabidopsis, it
419 requires the GC activity. This does not rule out possible role of GC activity of OsWAKL21 in
420 rice as it might be involved in some other functions not studied here or is required for other
421 splice variants of this gene.

422 CWDEs secreted by *Xoo* cause degradation of the rice cell wall that leads to the release of cell
423 wall derived DAMPs. These DAMPs, in turn, induce rice immune responses, but the
424 mechanisms by which these DAMPs are perceived and recognized are obscure. Employing a
425 variety of analyses, we have found that the rice receptor kinase *OsWAKL21.2* is required for
426 the activation of plant immune responses post-LipA treatment. This suggests that *OsWAKL21.2*
427 could be either a receptor or a co-receptor for cell wall damage and possibly the first DAMP
428 receptor identified in rice. Overexpression of *OsWAKL21.2* in plants induces immune
429 responses and enhances tolerance towards hemibiotrophic pathogens. We observed that this
430 receptor kinase is a moonlighting kinase having *in vitro* guanylate cyclase activity along with
431 kinase activity making it one of the few moonlighting kinases known in plants and the first one
432 in rice. An interesting observation about *OsWAKL21.2* is that for the induction of immune
433 responses in rice, the kinase activity is required, but in Arabidopsis, the guanylate cyclase
434 activity is needed. Fig. 8D represents a mechanistic model of the role of OsWAKL21.2 in the
435 induction of immune responses in rice and Arabidopsis. Future studies would be aimed at
436 identifying interacting partners of OsWAKL21.2 that are involved in elaboration of LipA
437 induced immune responses. Furthermore, the possibility of using this gene to provide enhanced
438 tolerance to bacterial pathogens in a variety of crops including monocots and dicots can be
439 explored.

440 Materials and Methods:

441 Plant materials and growth conditions

442 Rice (*Oryza sativa* ssp. *indica*) variety TN1 (Taichung native 1) which is susceptible to
443 *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) was used for plant experiments. All the rice experiments
444 were performed in either the growth chamber (12hr Day//Night) or greenhouse at 28°C.
445 *Arabidopsis thaliana* ecotype Columbia (Col-0) and *NahG* lines were used for Arabidopsis
446 experiments. Transgenic lines were generated using the floral dip method (Clough and Bent,
447 1998). Transgenic plants were selected by adding hygromycin and/or kanamycin (*NahG* lines)

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448 to the final concentration of 20µg/ml or 50µg/ml respectively. Plants were maintained in
449 growth chamber at 22°C day and 18°C night temperature at about 70% humidity and with 12hr
450 day/night cycle. Leaves of 4-5-week-old plants that are in rosette state were used for
451 experiments.

452 **Bacterial cultures**

453 *Xoo* wild type strain BXO43 (lab isolate) was used as a rice pathogen. The LipA mutant
454 (BXO2001) of *Xoo* (BXO43) and its complemented strain (BXO2008) was also used in this
455 study (Rajeshwari et al., 2005). *Pseudomonas syringae* pv. *tomato* DC3000 (*Pst* DC3000) was
456 used as an Arabidopsis pathogen. Transient transformation in rice and floral dip of Arabidopsis
457 was performed using *Agrobacterium tumefaciens* strain LBA4404. *E. coli* BL21-AI was used
458 for recombinant protein expression for biochemical assays.

459 **LipA purification from *Xoo***

460 *Xoo* BXO2008, a LipA overproducing strain derived from BXO2001 was used for LipA
461 overproduction and purification and LipA was purified by the protocol described previously
462 (Aparna et al., 2007). The purity and activity of the enzyme was tested by running on a SDS-
463 PAGE gel and by activity on tributyrin containing plates.

464 **Microarray analysis**

465 The leaf treatment and microarray analysis was performed as described previously (Ranjan et
466 al., 2015). RNA was isolated from 25-30 leaves after 30min or 2hr of treatment with LipA
467 (0.5mg/ml) or buffer. Processed data and '.cel' files were also submitted to gene expression
468 omnibus (GEO-NCBI, Acc. No. GSE53940). RMA and PLIER16 algorithms were used for
469 analysis and probes showing significant differential expression (FC $\geq$ 1.5-fold and $p<0.05$) in
470 both analyses were considered as differentially expressed genes.

471 **Vector construction and site-directed mutagenesis**

472 Gateway™ cloning technology was used for cloning. *OsWAKL21.2* was amplified using rice
473 cDNA and cloned into pENTR-D-TOPO (Invitrogen™). The gene was subcloned using LR
474 clonase reaction (Invitrogen™) into pMDC7 plasmid (Curtis and Grossniklaus, 2003) for plant
475 expression studies and in pH7FWG2 plasmid (Karimi et al., 2002) for localization experiments.
476 In pMDC7, the target gene sequence is cloned downstream to XVE promoter, which is 17-β-
477 estradiol inducible. 20µM of 17-β-estradiol (Sigma Aldrich) was used in all overexpression
478 studies as an inducer while 0.1% DMSO was used as a control (uninduced condition). Kinase
479 domain OsWAKL21$_{376\text{-}725}$ was cloned into bacterial expression vector pDEST17 (Invitrogen)
480 and transformed into *E. coli* BL21-AI for recombinant protein expression. The constructs in
481 pENTR-D-TOPO were used for site-directed mutagenesis (Zheng et al., 2004). The mutant

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482 versions were then transferred into desired destination vectors using LR clonase reaction. All
483 the clones and mutations were confirmed using Sanger sequencing. All the plant expression
484 constructs were introduced into *Agrobacterium tumefaciens* strain LBA4404.
485 LBA4404:XVE$_{pro}$:*OsWAKL21.2*, LBA4404:XVE$_{pro}$:*OsWAKL21.2*-kd and
486 LBA4404:XVE$_{pro}$:*OsWAKL21.2*-gcd were used for transient transformation in rice and for
487 generation of Arabidopsis transgenic lines.

488 **Callose deposition assay in rice and Arabidopsis**

489 For callose deposition assay in rice, 12-14 days old leaves were used for Agrobacterium-
490 mediated transient transformation (Pillai et al., 2018, Jha et al., 2010). The suspension was
491 infiltrated in third rice leaf using a needleless 1ml syringe with inducer [20μM 17-β-estradiol;
492 (Est), Sigma-Aldrich] or control (0.1% DMSO). Leaves collected for callose deposition were
493 stained with aniline blue according to Millet et al. (2010) (Millet et al., 2010). Callose
494 deposition was visualized under blue light (excitation wavelength 365nm) in ECLIPSE Ni-E,
495 epifluorescence microscope (Nikon, Japan) with 10X magnification. Eight images (~1mm^2
496 each) were captured from each leaf from the zone of infiltration and proximal region. The
497 number of callose deposits in all eight images for a leaf was added to get callose deposition per
498 leaf (per 8mm^2). Average was calculated for 10-12 leaves for each treatment.

499 For callose deposition in Arabidopsis transgenic plants, similar size of rosette stage leaves were
500 infiltrated either with 100μl of 0.1%DMSO or 20μM estradiol using the needleless 1.0 ml
501 syringe. After 12hr, leaves were collected and stained for callose deposition and observed under
502 the microscope as mentioned above for rice. Nearly 40-50 images per leaf were captured and
503 the number of callose deposits in each image was added to get number of callose deposits in
504 one leaf. For each sample average was calculated for 3 such leaves obtained from three separate
505 plants.

506 **Virulence assay in rice and Arabidopsis**

507 About 60 days old TN1 rice plants were used for infection of *Xoo*. For transient overexpression
508 in rice mid-vein, 200μl actively growing Agrobacterium (LBA4404) resuspended in 10mM
509 MES + 10mM $MgCl_2$ + 200μM acetosyringone (final OD 0.8) [with or without inducer (20μM
510 17-β-estradiol] was injected using a 1.0 ml syringe. After 24hr, about 1cm above
511 Agrobacterium injection site, the mid-veins of leaves were pin-pricked with needle touched to
512 fresh *Xoo* colony. Lesion length caused by *Xoo* was measured after 10 days of *Xoo* infection.

513 *Pseudomonas syringae* pv. *tomato* (*Pst* DC3000) was used for infection in Arabidopsis leaves.
514 Similar size leaves from five different rosette stage plants were infiltrated with either 0.1%
515 DMSO or 20μM estradiol. After 12hr, leaves were infected with actively growing culture of

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516 *Pst* DC3000 (Diluted to OD 0.02) by infiltration using a needleless 1.0 ml syringe. Colony
517 forming unit (CFU) at 0dpi (days post infection) and 2dpi was calculated.

518 **Downregulation of *OsWAKL21.2* using virus-induced gene silencing (VIGS)**

519 Virus-induced gene silencing was used for Agrobacterium-mediated transient downregulation
520 of *OsWAKL21.2* in rice. Three RNAi constructs of different length from unique 5'-end of
521 *OsWAKL21.2* were cloned in pRTBV-MVIGS (Purkayastha et al., 2010). Downregulation was
522 performed with a modified protocol mentioned previously (Purkayastha et al., 2010, Kant and
523 Dasgupta, 2017). For callose deposition studies, just germinated rice seedlings (1 day old) were
524 dipped in activated Agrobacterium culture (in 10mM MES+10mM $MgCl_2$+200µM
525 acetosyringone) for 24hr (Supplemental figure S3). 10 days after Agrobacterium treatment, the
526 third leaf of each plant was infiltrated with LipA using a needleless syringe (0.5mg/ml) (at least
527 40 leaves for each Agrobacterial strain). After 16hr, a small piece (~1.5cm) of each leaf around
528 the zone of infiltration was collected for callose deposition while the rest of the leaf piece was
529 stored for transcript/protein quantification. Each leaf was collected separately for callose and
530 transcript/protein quantification and labelled. Callose deposition was observed qualitatively as
531 mentioned above for callose deposition assay. Rest of the part of 4-5 leaves that showed either
532 low or high callose deposition were pooled and RNA/protein was isolated from those pooled
533 leaves for qRT-PCR or Western blotting.

534 For virulence assay after downregulation of *OsWAKL21.2*, mid-veins of 60 days old rice plants
535 were injected with 200µl activated Agrobacterium along with either buffer or LipA (0.5mg/ml)
536 (n>40). After 24hr, mid-veins of 10 leaves were collected (3cm each) for *OsWAKL21.2*
537 transcript/protein quantification while remaining 20-30 leaves were infected with a freshly
538 growing colony of *Xoo* as mentioned earlier. Lesion length caused by *Xoo* was measured after
539 10 days of infection.

540 **Purification of recombinant protein and *in vitro* biochemical assays**

541 The recombinant kinase domain of OsWAKL21.2, OsWAKL21.$2_{376\text{-}725}$ with 6X-His tag was
542 cloned, expressed and purified from *E. coli* BL21-AI. 50µg of purified recombinant protein
543 was used for kinase or guanylate cyclase assay in a 50µl reaction. The purified protein was
544 incubated with 10µCi of [γ-^{32}P] ATP in kinase assay buffer (50mM Tris (pH 7.5), 10mM
545 $MgCl_2$, 2mM $MnCl_2$, 0.5mM $CaCl_2$, 1mM DTT and 20mM ATP) for 1hr at room temperature
546 (Li et al., 2009), run on 10% SDS-PAGE gel and gel was subsequently exposed to
547 phosphoimager screen which was later scanned in phosphoimager (Personal molecular imager,
548 Biorad) instrument.

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549 GC assay was also performed from the same purified recombinant protein in GC assay buffer
550 [50mM Tris (pH 7.5), 2mM $MgCl_2$, 1mM $MnCl_2$, 0.5mM $CaCl_2$, 0.2mM NONOate (Sigma)]
551 modified from the protocol described previously (Meier et al., 2010). The reaction was
552 incubated at 37°C for either 1hr or 12hr. The 1hr reaction was used for quantitative analysis
553 while 12hr reactions were used for qualitative analysis. cGMP produced after 1hr was
554 quantified using cGMP enzyme immunoassay kit (Sigma-Aldrich, Cat. No- CG201) according
555 to manufacturer's protocol and the data was analyzed using online tool 'Elisaanalysis'
556 (https://elisaanalysis.com/app). For qualitative analysis, the resultant product was blotted on
557 nitrocellulose membrane (Amersham, Cat No. RPN203E) and dried in the laminar hood with
558 UV on for 1hr. The nucleotides were further crosslinked to the membrane by keeping in UV
559 transilluminator for 30min. The membrane was blocked, washed and further incubated with
560 anti cGMP antibody (1:1000, Sigma-Aldrich, Cat. No- G4899) and processed as mentioned in
561 Western blot section.

562 **RNA isolation and gene expression analysis**

563 For qRT-PCR, RNA was isolated by the protocol of Sánchez et al. (2008) with some
564 modifications (Oñate-Sánchez and Vicente-Carbajosa, 2008, Couto et al., 2015). For rice, 10-
565 12 leaf pieces (or mid-vein pieces) were crushed together for each treatment unless mentioned
566 otherwise. For Arabidopsis, three leaf pieces from separate plants were crushed together for
567 each treatment. cDNA was made from 5µg of total RNA [RNA to cDNA EcoDry™ Premix
568 (Oligo dT), (Clontech)] according to the manufacturer's protocol. qRT-PCR was performed
569 with diluted cDNA using Power SYBR™ Green PCR Master Mix (Thermo Fisher Scientific)
570 in ViiA 7 Real-Time PCR System (Applied Biosystems). Relative expression was calculated
571 in enzyme or 17-β-estradiol treated leaves with respect to mock/control (buffer or 0.1%
572 DMSO) treated leaves. The fold change was calculated using $2^{-\Delta\Delta Ct}$ method (Livak and
573 Schmittgen, 2001). *OsActin1* and *AtActin2* were used as internal control for rice and
574 Arabidopsis respectively. All the primers for qRT-PCR were designed using QuantPrime
575 (Arvidsson et al., 2008).

576 **Protein isolation and Western blotting**

577 For Western blot the protein was isolated from 10-12 leaf pieces of rice or three leaves of
578 Arabidopsis using the protocol described previously with minor modifications (Rohila et al.,
579 2006). 20µg of total protein was loaded in 10% SDS-PAGE gel for Western blot/Coomassie
580 brilliant blue staining. The protein was transferred to PVDF membrane (Millipore) and
581 processed for blotting. Anti $OsWAKL21_{376\text{-}725}$ antibodies were generated in the rabbit in our
582 institute animal house facility and used in dilution of 1:100. HRP tagged anti-Rabbit IgG

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583 secondary antibody (Abcam) (dilution 1:50000) was used and the blot was visualized in
584 chemidoc (Vilber Lourmat).

585 **Localization of OsWAKL21.2**

586 The localization of OsWAKL21.2 was observed by transient transformation of onion peel cell
587 as described previously (Sun et al., 2007). Os*WAKL21.2* was cloned into Gateway compatible
588 vector pH7FWG2 (Karimi et al., 2002) and transformed in onion peel using Agrobacterium-
589 mediated transient transformation. The GFP signal was visualized under GFP filter in
590 ECLIPSE Ni-E, epifluorescence microscope (Nikon, Japan).

591 **cGMP quantification**

592 cGMP was quantified in leaves of rosette stage transgenic Arabidopsis plants by the method
593 used by, Dubovskaya et al. (2011), Nan et al. (2014) and Chen et al. (2018) with minor
594 modifications (Chen et al., 2018, Dubovskaya et al., 2011, Nan et al., 2014). Six similar sized
595 leaves (total approximate 200mg) were collected from different plants for untreated control
596 (UT). 3-3 similar size leaves from three different plants were infiltrated either with 0.1%
597 DMSO or 20μM estradiol. Two leaves from each plant (total 6 leaves, ~200mg) were collected
598 for cGMP quantification while the third leaf was used for testing of expression of
599 *OsWAKL21.2*. After 3hr of infiltration, leaves were collected and crushed in a fine powder
600 using liquid nitrogen. The powder was resuspended in 2ml ice cold 6% (v/v) trichloroacetic
601 acid (TCA) and was collected in the 5ml tube. After brief vortexing (10s), tubes were
602 centrifuged twice at 1000g for 15min at 4°C and supernatant was collected each time in the
603 5ml tube. The aqueous supernatant was washed 7-8 times with water-saturated diethyl ether.
604 The solvent was evaporated in cold vacuum centrifuge at 4°C (SCANVAC, CoolSafe). cGMP
605 was quantified in the extract using cGMP enzyme immunoassay kit (Sigma-Aldrich, Cat. No-
606 CG201) according to the manufacturer's protocol. Data were analyzed using the online tool
607 Elisaanalysis (https://elisaanalysis.com/app).

608 **Analyses of publicly available transcriptome data**

609 Rice microarray data performed after *Xanthomonas oryzae* treatment was obtained from GEO,
610 NCBI (Acc. No. GSE36272). '.cel' files were downloaded, analyzed and processed using
611 expression console (Affymetrix) using RMA based normalization. '.chp' files obtained after
612 analysis were used in TAC software (Transcriptome analysis console v3.0, Affymetrix) for
613 relative expression analysis. Genes that show FC ≥ 1.5-fold with $p<0.05$ were considered as
614 differentially expressed.

615 **Statistical analysis**

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616 All experiments were independently performed at least thrice. All data represented here
617 indicate mean ± SE (standard error). The results of lesion length, callose deposition and
618 bacterial growth in CFU were analysed by one-way ANOVA ($p<0.05$) followed by the Tukey-
619 Kramer test. The results of qRT-PCR were analyzed by Student's *t*-test and the genes that show
620 significanty altered expression ($p<0.05$) between control and treated were considered as
621 differentially expressed.

622 **Accession numbers**

623 The PLIER16 and RMA processed microarray data files generated and used in this experiment
624 are submitted to gene expression omnibus (GEO) (https://www.ncbi.nlm.nih.gov/geo/) under
625 the accession number GSE53940. Other publicly available microarray data used in our analysis
626 was harvested from GEO under the accession numbers GSE49242 and GSE36272. Accession
627 numbers of genes referred in this study are provided in supplemental table 5.

628

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633 Chatterjee (DBT-CDFD) for providing *NahG* transgenic lines and *Pseudomonas syringae*
634 DC3000 strain.

635

636

637

638 Supplemental data:

639 **Supplemental Table 1:** List of probe sets that show differential expression after 2hr of LipA
640 treatment.

641 **Supplemental Table 2:** List of differentially expressed genes after 2hr and 12hr of LipA
642 treatment.

643 **Supplemental Table 3:** Frequency of differentially expressed genes after LipA treatment in
644 the microarray data performed after 24hr of *Xanthomonas oryzae* treatment in GEO submission
645 GSE36272.

646 **Supplemental Table 4:** List of primers used in this study.

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647 **Supplemental Table 5:** Accession numbers of the genes mentioned in this study.

648 **Supplemental Figure S1:** Transcriptome profiling of rice leaves after treatment with LipA.

649 **Supplemental Fig. S2:** Overexpression of *OsWAKL21.2* induces rice immune responses.

650 **Supplemental Fig. S3:** Methodology for downregulation of *OsWAKL21.2* in rice seedlings
651 using Virus Induced Gene Silencing (VIGS).

652 **Supplemental Fig. S4:** Downregulation of *OsWAKL21.2*.

653 **Supplemental Fig. S5:** VIGS mediated transient downregulation of *OsWAKL21.2* does not
654 have significant effect on expression of predicted off-targets genes.

655 **Supplemental Fig. S6:** qRT-PCR and Western blot validation for ectopically expressing
656 *Os*WAKL21.2 transgenic Arabidopsis plants.

657 **Supplemental Fig. S7:** Biochemical characterization of OsWAKL21.2.

658 **Supplemental Fig. S8:** Biochemical activities of purified kinase domain of mutant versions of
659 OsWAKL21.2.

660 **Supplemental Fig. S9:** qRT-PCR and Western blot validation of expression of mutant versions
661 of *OsWAKL21.2* by transient transformation in rice and ectopic expression in Arabidopsis
662 transgenic lines.

663 **Supplemental Fig. S10:** Treatment with GC inhibitor attenuates *OsWAKL21.2* induced callose
664 deposition in transgenic Arabidopsis leaves.

665 **Supplemental Fig. S11:** Ectopic expression of *OsWAKL21.2* in Arabidopsis enhances *in*
666 *planta* cGMP level by its GC activity.

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879 **Figure 1: Expression of *OsWAKL21.2* is enhanced after treatment of rice leaves either**
880 **with LipA or *Xoo*.**

881 (A) Venn diagram indicating number of genes that are differentially expressed after 2hr and
882 12hr of LipA treatment.
883 (B) Three splice variants of OsWAKL21 as shown in Rice-MSU database.
884 (C) qRT-PCR analysis of the expression of all three splice variants of OsWAKL21 after 2hr
885 and 12hr of LipA treatment, and after 24hr of *Xoo* treatment in rice leaves. Relative expression
886 was calculated in leaves treated with LipA or *Xoo* with respect to leaves treated with buffer.
887 Asterisk (*) represents significant difference in fold change with $p<0.05$.
888 (D) qRT-PCR analysis of expression of *OsWAKL21.2* in rice leaves after 24hr of treatment
889 with either *Xoo*, LipA mutant of *Xoo* (*Xoo* LipA-) or LipA complementing clone of *Xoo* (*Xoo*
890 LipA-CC). a and b above the bars indicate significant difference with $p<0.05$.
891 In C and D, 12-14 days old leaves were infiltrated with either LipA (0.5mg/ml) or *Xoo* (O.D.
892 1.0). Each bar represents average value and error bar denotes standard error (SE) of at least
893 three different experiments. *OsActin1* was used as internal control for qRT-PCR. The relative
894 fold change was calculated by using $2^{-\Delta\Delta Ct}$ method.
895

896 **Figure 2: Overexpression of *OsWAKL21.2* in rice leaves enhances plant immune**
897 **responses.**
898 (A) Callose deposition in rice leaves after treatment with various Agrobacterium constructs or
899 controls. The image shown is representative image of one viewing area for each category. Scale
900 bar represents 100μm. The numbers denotes: 1-0.1% DMSO, 2-20μM β-estradiol (Est), 3,4-
901 Agrobacterium containing pMDC7 (Empty vector-EV) without (3) or with (4) inducer (Est),
902 5,6- Agrobacterium containing pMDC7::OsWAKL21.2 without (5) or with (6) inducer (Est),
903 7-LipA.

904 (B) Quantification of callose deposition in rice leaves after treatment with various
905 Agrobacterium constructs or controls. Bar diagram showing the quantification of number of
906 callose deposits per area in rice leaves. Number of callose deposits in 8 such viewing areas (as
907 shown in A) per leaf were considered. Each bar represents the average and error bar represents
908 SE of 10-15 leaves per treatment in one set of experiment. Similar results were obtained in
909 three independent experiments.
910 (C) Lesion length caused by *Xoo* in rice leaves when mid-vein of the leaves were previously
911 treated with various Agrobacterium constructs or controls. Mid-veins of rice leaves of 60 day
912 old plants were injected with either MQ, Agrobacterium carrying empty vector or
913 *OsWAKL21.2* and also with (20μM β-estradiol) or without (0.1% DMSO) inducer. After 24hr,
914 the leaves were pin prick inoculated with *Xoo*, 1cm above the point of Agrobacterium injection.
915 Lesion length was measured after 10 days of infection (supplemental figure 1C). Each bar
916 indicates average and error bar represents SE of >20 leaf per treatment in one set of experiment.
917 Similar results were obtained in three independent experiments.
918 (D) Relative expression of key defence related genes after transient overexpression of
919 *OsWAKL21.2* in rice leaves. Each bar represents average fold change and the error bars indicate
920 SE in three independent experiments (n=12 in each experiment). For each gene, transcript level
921 of uninduced condition (treatment with Agrobacterium carrying *OsWAKL21.2* with 0.1%
922 DMSO) was considered as 1 and was compared to induced condition (treatment with
923 Agrobacterium carrying *OsWAKL21.2* with 20μM estradiol). *OsActin1* was used as internal

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924 control for qRT-PCR. The relative fold change was calculated by using 2-ΔΔCt method.
925 In A and B, 12-14 days old rice leaves were infiltrated with either MQ, Agrobacterium carrying
926 empty vector or vector containing *OsWAKL21.2* and also with (20µM β-estradiol) or without
927 (0.1% DMSO) inducer. In B and C, asterisk (*) represents significant difference with $p<0.05$.
928 MQ (MilliQ or water) treatment indicate control without any Agrobacterium treatment. In A,
929 B and C, Leaves treated with LipA were used as positive control.

930

931

932 **Figure 3: Downregulation of *OsWAKL21.2* attenuates LipA induced immune responses**
933 **in rice.**
934 (A) Categorization of number of callose deposits in three different groups: low, medium and
935 high. The image shown is representative image of one viewing area for each group. 8 such
936 areas per leaf were viewed for categorization.
937 (B) Fraction of leaves showing low, medium or high callose deposition after LipA treatment.
938 (C) Fraction of leaves showing callose deposits post LipA infiltration that were previously
939 treated with either MQ (mock treatment), Agrobacterium containing VIGS-EV or WAK-RNAi
940 constructs [WAKL-RNAi 1-300 (WRi 1-300), WAKL-RNAi 451-600 (WRi 451-600 or
941 WAKL-RNAi 1-600 (WRi 1-600)] in 12-14 days old rice leaves.
942 (D) qRT-PCR analysis of *OsWAKL21.2* transcript levels in leaves showing either low or high
943 callose deposits (H: High callose, L: Low callose). Each bar represents average fold change
944 and error bar indicates SE observed in three biological replicates. For each sample, 4-5 leaves
945 showing respective callose phenotype were used for RNA isolation. Transcript level in mock
946 (MQ) treated leaves was considered as 1 and fold change in Agrobacterium treated leaves was
947 calculated with respect to it.
948 (E) Lesion length caused by *Xoo* in mid-veins of 60 days old rice leaves that were pre-treated
949 with either buffer and LipA alone or along with Agrobacterium strains [WAKL-RNAi 1-300
950 (WRi 1-300) or WALK-RNAi 1-600 (WRi 1-600)]. Each bar represents average lesion length
951 and error bar show SE of at least 20 leaves in one experiment. Similar results were obtained in
952 three independent experiments.

953 (F) Expression level of *OsWAKL21.2* in rice leaves after 24hr of injection with either buffer
954 and LipA alone or along with Agrobacterium strains [WAKL-RNAi 1-300 (WRi 1-300) or
955 WAKL-RNAi 1-600 (WRi 1-600)]. Each bar represents average of three independent
956 experiments, $n>10$ in each experiment. Transcript level of buffer injected leaves was
957 considered as 1 and fold change in Agrobacterium with Buffer/LipA treated leaves was
958 calculated with respect to it.
959 In B, C, D and F, each bar represents the average and error bar denotes the SE of three different
960 biological replicates. Each sample denotes the ratio of leaves showing respective phenotype in
961 at least 40 leaves. In C, D, E and F small letters (a, b and c) above the bars indicates significant
962 difference with $p<0.05$. In D and F, *OsActin1* was used as internal control for qRT-PCR and
963 the relative fold change was calculated by using $2^{-\Delta\Delta Ct}$ method.
964

965

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966 **Figure 4: Ectopic expression of *OsWAKL21.2* in Arabidopsis induces immune responses.**
967 (A) Callose deposition in leaves of wild type (Col-0) or *OsWAKL21.2* transgenic Arabidopsis
968 lines following with 20µM β-estradiol (inducer) or 0.1% DMSO (control). Numbers denotes:
969 1,2- Col-0 treated with DMSO (1) or Est (2), 3,4- *OsWAKL21.2* transgenic line 7 treated with
970 DMSO (3) or Est (4), 5,6- *OsWAKL21*.2 transgenic line 14 treated with DMSO (5) or Est (6).
971 (B) Quantification of number of callose deposits in wild type Columbia (Col-0) and two
972 different Arabidopsis *OsWAKL21.2* transgenic lines after treatment with control or inducer.
973 Leaves were treated with either 20µM β-estradiol (inducer) or 0.1% DMSO (control). Each bar
974 represents the average and error bar represents SE of three different leaves for each treatment
975 in an experiment.
976 (C) Effect of ectopic expression of *OsWAKL21.2* on growth of *Pst* DC3000 after subsequent
977 infection. Leaves were treated with either 20µM β-estradiol (inducer) or 0.1% DMSO (control),
978 12hr before *Pst* infection. Each bar represents average and error bar represents SE of five leaves
979 for each treatment in an experiment.
980 (D) Effect of ectopic expression of *OsWAKL21.2* in transgenic Arabidopsis lines on the
981 expression of SA or JA pathway responsive genes. Expression in 0.1% DMSO treated leaves
982 was considered as 1 and relative expression in 20µM estradiol treated leaves was calculated
983 with respect to it. Each bar represents the average of three independent experiments for each
984 line. For each sample, RNA was isolated from 3 leaves for every treatment. *AtActin2* was used
985 as internal control for qRT-PCR. The relative fold change was calculated by using $2^{-\Delta\Delta Ct}$
986 method.
987

988 Transgenic or wild type plant leaves were treated with 0.1% DMSO (Control) or 20µM
989 estradiol (inducer). 12hr later leaves were either collected for callose deposition or
990 transcript/protein analysis or were infected with *Pst* DC3000. Similar results were obtained in
991 three independent experiments for A, B and C. Asterisk (*) represents significant difference
992 with $p<0.05$.

993

994

995 **Figure 5: Biochemical characterization and localization of *OsWAKL21.2***
996 (A) Domain architecture of OsWAKL21.2 using SMART tool (http://smart.embl-
997 heidelberg.de/) (SP: signal peptide, GUB: galacturonan binding domain, EGF: epidermal
998 growth factor like repeat, TM: transmembrane region, STYKc: serine threonine kinase domain,
999 GC: guanylate cyclase motif).
1000 (B) OsWAKL21.2-EGFP localize on the cell membrane in onion peel after transient
1001 expression. OsWAKL21.2-EGFP was transiently transformed to onion peel cells using
1002 Agrobacterium and peels were visualized after 2 days under epiflourescence microscope. The
1003 experiment was repeated three times and similar results were obtained.
1004 (C) Kinase assay: Kinase domain of OsWAKL21 cloned and purified from E. coli show
1005 autophosphorylation activity. 50µg of affinity purified recombinant protein was used for assay
1006 with or without radiolabelled ATP. After 1hr, denatured sample was loaded on 10% SDS-
1007 PAGE gel. The gel was further subjected to autoradiography and CBB staining. The experiment
1008 was repeated three times and similar results were obtained.
1009 (D) Guanylate cyclase assay: 50µg (in 50 µl) of affinity purified recombinant protein was used

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1010 for GC assay with or without GTP. After 1hr, 5 μl of the sample was directly used for cGMP
1011 quantification. Only GTP and GC buffer + GTP were used as controls. Each bar indicate
1012 average and error bar represents SE of three independent experiments. Small letters (a and b)
1013 above the bars indicate significant difference with $p<0.05$.
1014

1015

1016

1017 **Figure 6: Kinase activity of OsWAKL21.2 is required for induction of immune responses**
1018 **in rice but not in Arabidopsis.**
1019 (A) Quantification of callose deposition after transient overexpression of either wild type
1020 *OsWAKL21.2* (WAK-wt) or kinase deficient mutant of *OsWAKL21.2* (*OsWAKL21.2*-kd or
1021 WAK-kd) in rice leaves. Each bar represents average and error bar represents SE of at least 12
1022 leaves per treatment in an experiment
1023 (B) Lesion lengths after 10 days of *Xoo* pin prick inoculation when *OsWAKL21.2* or
1024 *OsWAKL21.2*-kd was transiently overexpressed prior to infection by *Xoo*. Each bar represents
1025 average and error bar represents SE of lesion length in 20-30 leaves in an experiment.
1026 (C) Relative expression of key defence related genes after transient overexpression of either
1027 *OsWAKL21.2* or *OsWAKL21.2*-kd in rice leaves. For each gene, transcript level of uninduced
1028 condition (treatment with Agrobacterium carrying WAK-wt or WAK-kd with 0.1% DMSO)
1029 was considered as 1 and was compared to induced condition (treatment with Agrobacterium
1030 carrying WAK-wt or WAK-kd with 20μM estradiol). Each bar represents average fold change
1031 and error bars indicate SE in three independent experiments (n=12 in each experiment).
1032 (D) Quantification of callose deposition in leaves of four different *OsWAKL21.2*-kd
1033 Arabidopsis transgenic lines (lines 1, 4, 11 and 12) treated with either 20μM β-estradiol
1034 (inducer) or 0.1% DMSO (control).. Each bar represents average and error bar represents SE
1035 of three leaves in an experiment.
1036 (E) Effect of ectopic expression of *OsWAKL21.2*-kd on growth of *Pst* DC3000 after subsequent
1037 infection. Leaves of wild type *OsWAKL21.2* (WAK-wt) and two different *OsWAKL21.2*-kd
1038 Arabidopsis transgenic lines (lines 1 and 11) were infiltrated with either 20μM β-estradiol
1039 (inducer) or 0.1% DMSO (control) and were subsequently inoculated with *Pst* DC3000,12hr
1040 post infiltration. Each bar represents average and error bar represents SE of five leaves in each
1041 sample.
1042 (F) Effect of ectopic expression of *OsWAKL21.2*-kd on expression of key defence related
1043 *OsWAKL21.2* induced genes in transgenic Arabidopsis lines. Expression in 0.1% DMSO
1044 treated leaves was considered as 1 and relative expression in 20μM estradiol treated leaves was
1045 calculated with respect to it. Each bar represents average fold change and error bars indicate
1046 SE in three independent experiments (n=3 in each experiment).
1047 In C and F, *OsActin1* and *AtActin2* were used respectively as internal control for qRT-PCR.
1048 The relative fold change was calculated by using $2^{-\Delta\Delta Ct}$ method. Similar results were obtained
1049 in three different experiments in A, B, D and E. Asterisk (*) represents significant difference
1050 with $p<0.05$.

1051

1052 **Figure 7: Guanylate cyclase activity of OsWAKL21.2 is required for induction of immune**
1053 **responses in Arabidopsis but not in rice.**

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1054 (A) Quantification of callose deposition in leaves of two different Arabidopsis transgenic lines
1055 (lines 3 and 6) expressing GC deficient *OsWAKL21.2* (*OsWAKL21.2*-gcd or WAK-gcd) were
1056 treated with either 20µM β-estradiol (inducer) or 0.1% DMSO (control). Each bar represents
1057 average and error bar represents SE of three leaves in an experiment.
1058 (B) Effect of ectopic expression of *OsWAKL21.2*-gcd on growth of *Pst* DC3000 after
1059 subsequent infection. Leaves of wild type *OsWAKL21.2* (WAK-wt) and two different
1060 *OsWAKL21.2*-gcd Arabidopsis transgenic lines (lines 3 and 6) were infiltrated with either
1061 20µM β-estradiol (inducer) or 0.1% DMSO (control) and were subsequently inoculated with
1062 *Pst* DC3000, 12hr post infiltration. Each bar represents average and error bar represents SE of
1063 five leaves in each sample.
1064 (C) Effect of ectopic expression of *OsWAKL21.2*-gcd on expression of key defence related
1065 *OsWAKL21.2* induced genes in transgenic Arabidopsis lines. Expression in 0.1% DMSO
1066 treated leaves was considered as 1 and relative expression in 20µM estradiol treated leaves was
1067 calculated with respect to it. Each bar represents average fold change and error bars indicate
1068 SE in three independent experiments (n=3 in each experiment).
1069 (D) Quantification of callose deposition after transient overexpression of either wild type
1070 (WAK-wt) or WAK-gcd in rice leaves. Each bar represents average and error bar represents
1071 SE of at least 12 leaves per treatment in an experiment.
1072 (E) Lesion lengths after 10 days of *Xoo* pin prick inoculation when *OsWAKL21.2* or
1073 *OsWAKL21.2*-gcd was transiently overexpressed prior to infection by *Xoo*. Each bar represents
1074 average and error bar represents SE of lesion length in 20-30 leaves in an experiment.
1075 (F) Relative expression of key defence related genes after transient overexpression of either
1076 *OsWAKL21.2* or *OsWAKL21.2*-gcd in rice leaves. For each gene, transcript level of uninduced
1077 condition (treatment with Agrobacterium carrying WAK-wt or WAK-gcd with 0.1% DMSO)
1078 was considered as 1 and was compared to induced condition (treatment with Agrobacterium
1079 carrying WAK-wt or WAK-gcd with 20µM estradiol). Each bar represents average fold change
1080 and error bars indicate SE in three independent experiments (n=12 in each experiment).
1081

1082 In C and F, *AtActin2* and *OsActin1* were used respectively as internal control for qRT-PCR.
1083 The relative fold change was calculated by using $2^{-\Delta\Delta Ct}$ method. Similar results were obtained
1084 in three different experiments in A, B, D and E. Asterisk (*) represents significant difference
1085 with $p<0.05$.

1086

1087 **Figure 8: *OsWAKL21.2* induces JA pathway in rice while activates SA pathway in**
1088 **Arabidopsis.**
1089 (A) Relative expression of ten JA pathway related genes after transient overexpression of
1090 *OsWAKL21.2* in rice leaves. These genes include three ZIM domain-containing proteins
1091 (LOC_Os03g08310, LOC_Os03g08330 and LOC_Os10g25230), two lipoxygenases
1092 (LOC_Os08g39840 and LOC_Os08g39850), one allene oxide synthase (AOS,
1093 LOC_Os03g55800), one basic helix loop helix transcription factor (RERJ1,
1094 LOC_Os04g23550), one ethylene-responsive transcription factor (ERF, LOC_Os02g43790),
1095 one chitinase (PR3, LOC_Os06g51050) and an AP2 domain-containing transcription factor
1096 (LOC_Os08g36920). For each gene, transcript level of uninduced condition (treatment with
1097 Agrobacterium carrying WAK-wt with 0.1% DMSO) was considered as 1 and was compared
1098 to induced condition (treatment with Agrobacterium carrying WAK-wt with 20µM estradiol).

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1099 Each bar represents average fold change and error bars indicate SE in three independent
1100 experiments (n=12 in each experiment). *OsActin1* was used as internal control.

1101 (B) Effect of ectopic expression of *OsWAKL21.2* on expression of SA pathway related genes
1102 in transgenic Arabidopsis lines. Expression in 0.1% DMSO treated leaves was considered as 1
1103 and relative expression in 20μM estradiol treated leaves was calculated with respect to it. Each
1104 bar represents average fold change and error bars indicate SE in three independent experiments
1105 (n=3 in each experiment). *AtActin2* was used as internal control for qRT-PCR.
1106 (C) Quantification of callose deposits in Arabidopsis crossing lines expressing *NahG* and
1107 *OsWAKL21.2* (line 2 and 3) or either one of those (*NahG*: line 12, WAK-wt: line 8). Leaves
1108 were treated with either 20μM β-estradiol (inducer) or 0.1% DMSO (control). Each bar
1109 represents average and error bar represents SE of three leaves in an experiment.

1110 (D) Modal depicting mechanistic role of OsWAKL21.2 in induction of immune responses in
1111 rice and Arabidopsis. OsWAKL21.2 likely perceive cell wall damage caused after LipA
1112 treatment in rice. Upon overexpression in rice, OsWAKL21.2 induces rice immune responses
1113 via its kinsase activity. Upon ectopic expression in Arabidopsis transgenic lines, OsWAKL21.2
1114 induce Arabidopsis immune responses by its guanylate cyclase activity.

1115 Asterisk (*) represents significant difference with $p<0.05$. In A and B the relative fold change
1116 was calculated by using $2^{-\Delta\Delta Ct}$ method.

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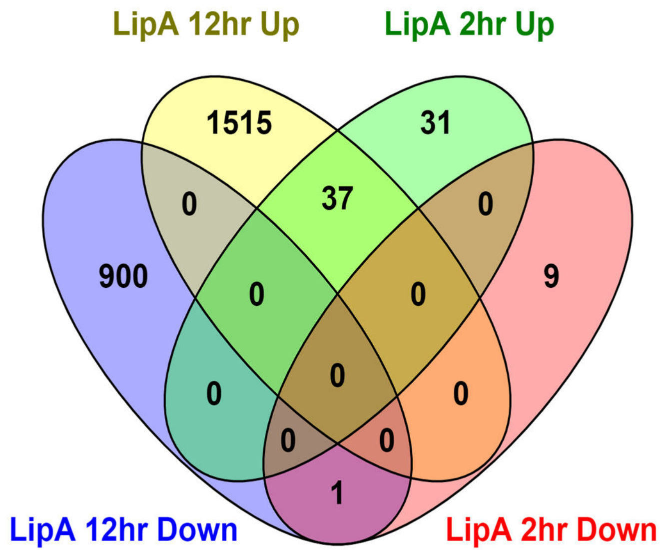

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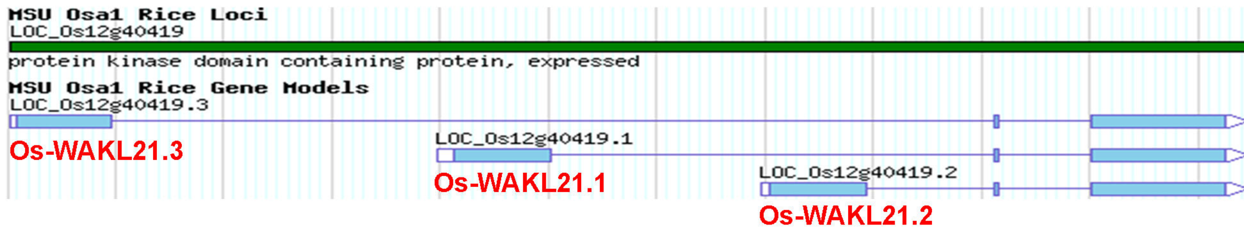

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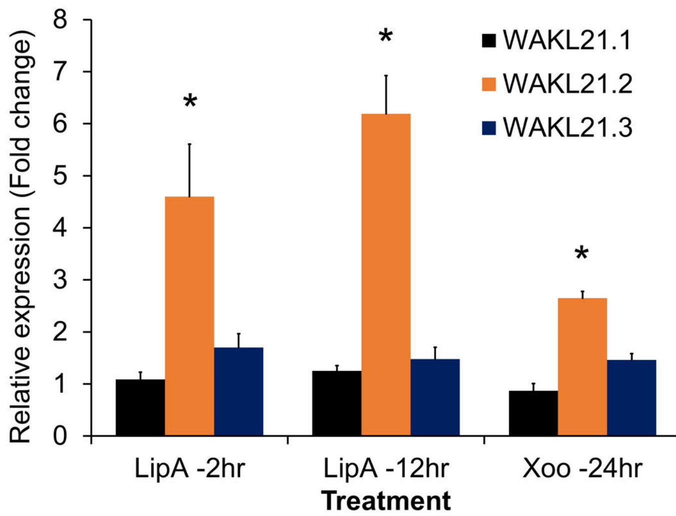

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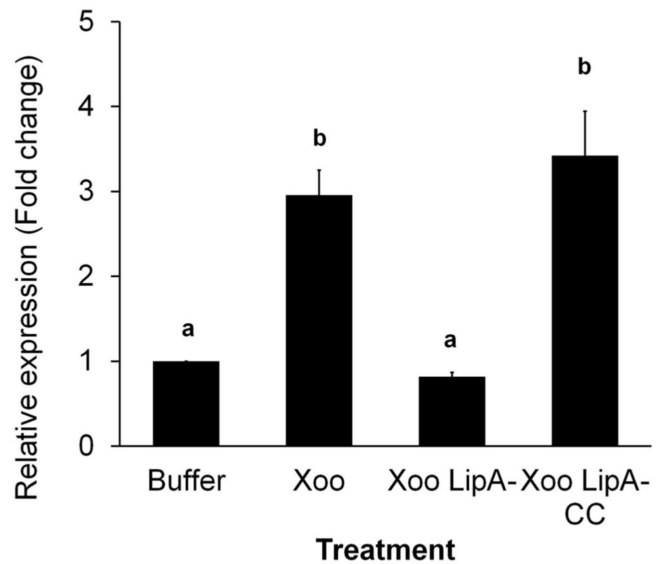

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Figure 2

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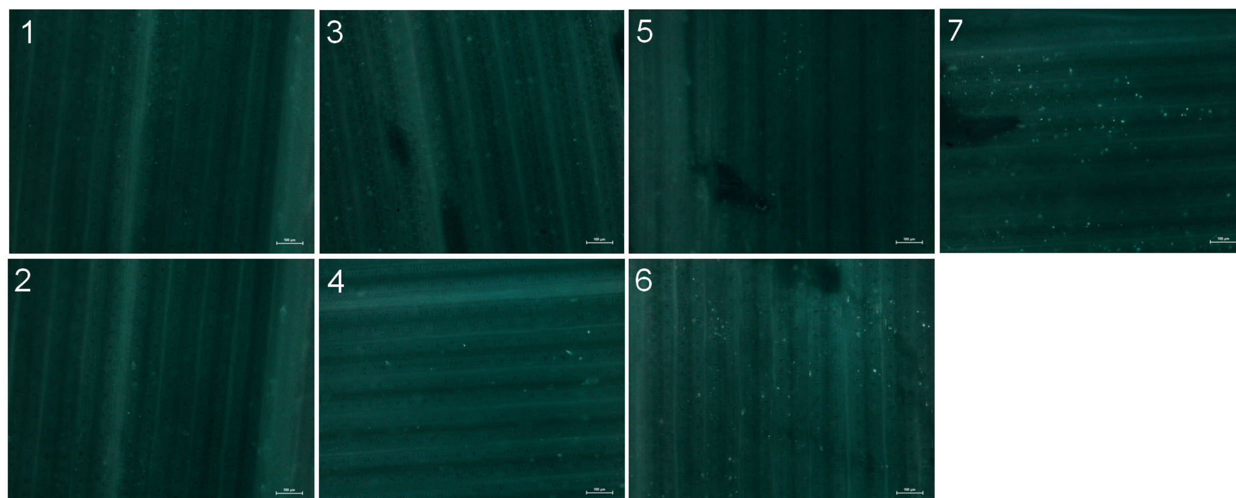

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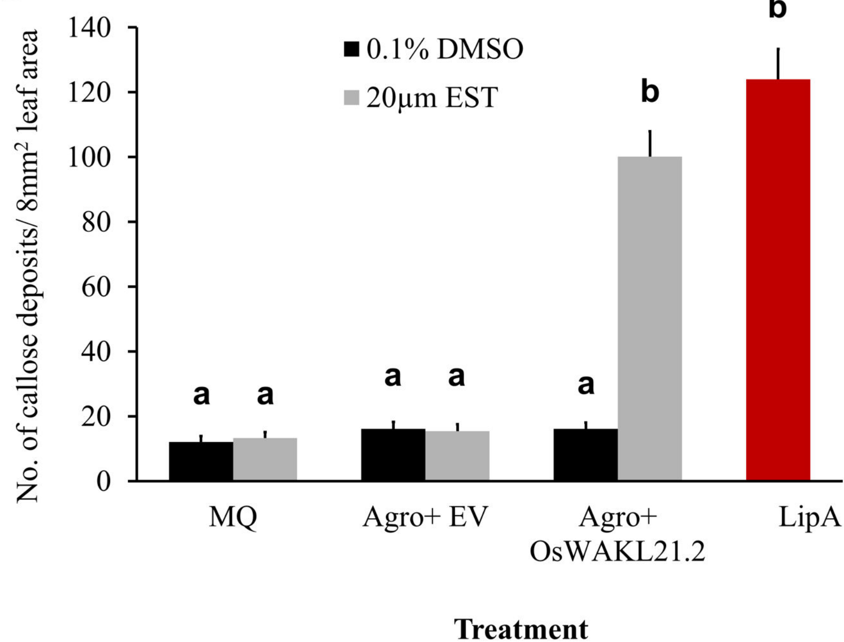

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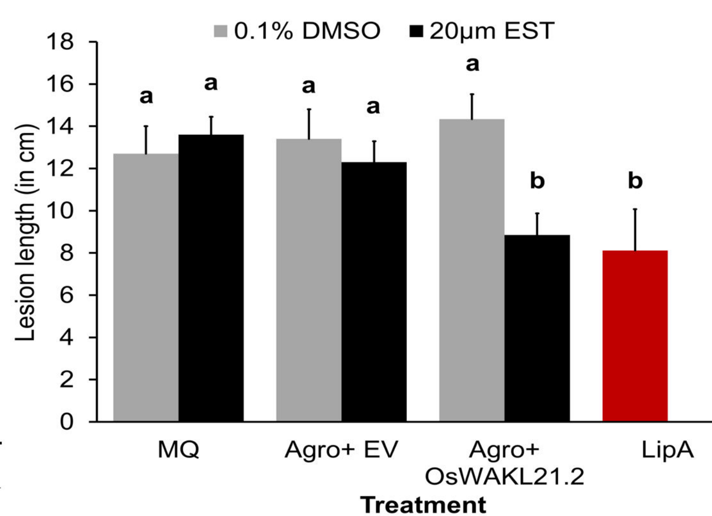

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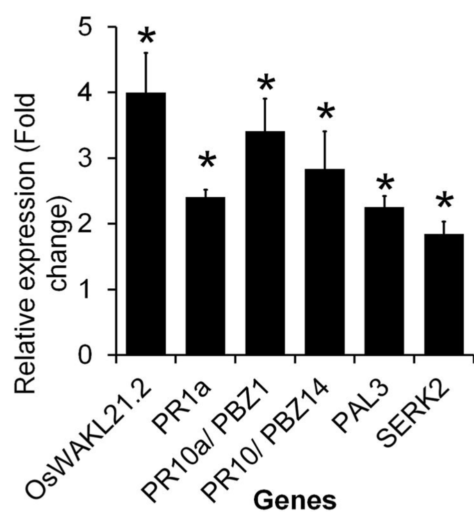

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Figure 3

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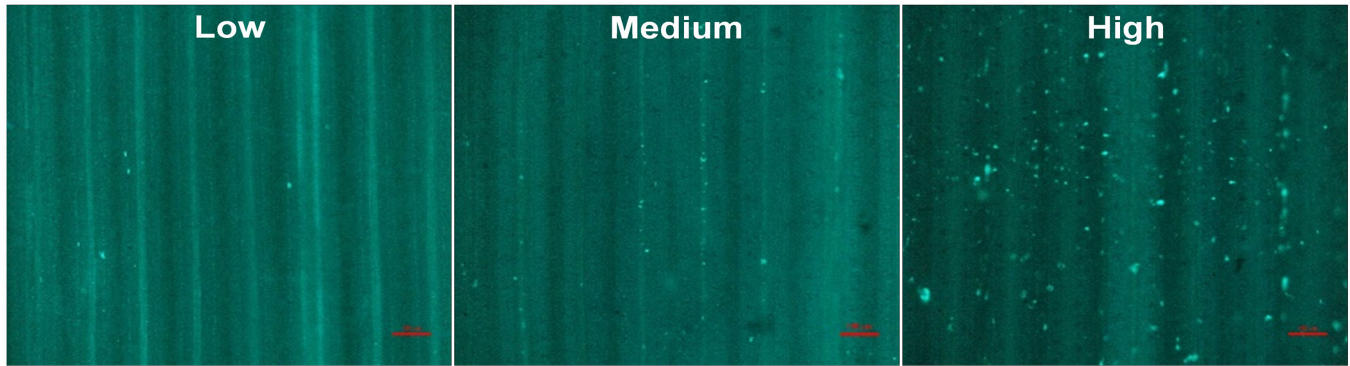

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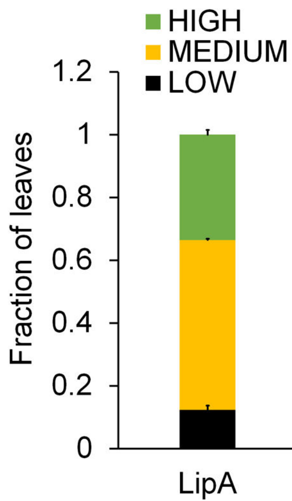

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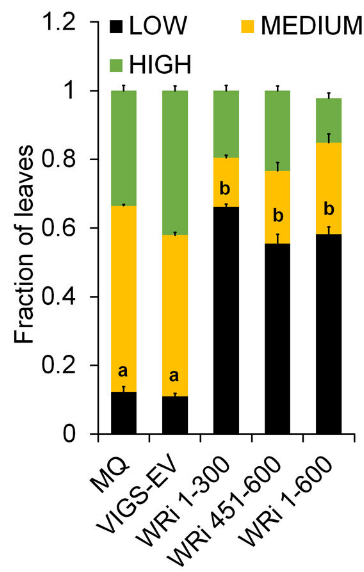

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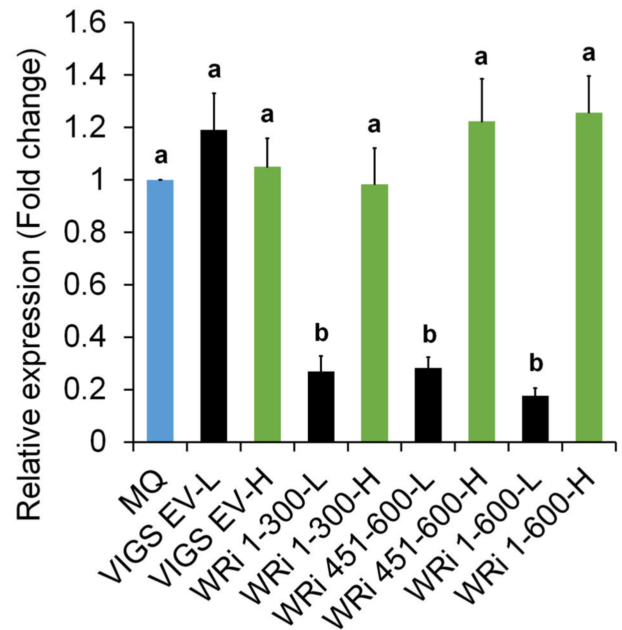

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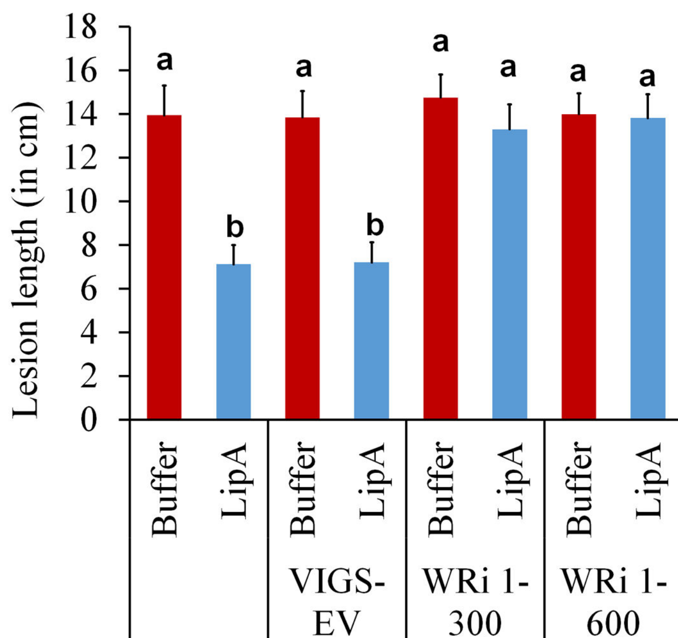

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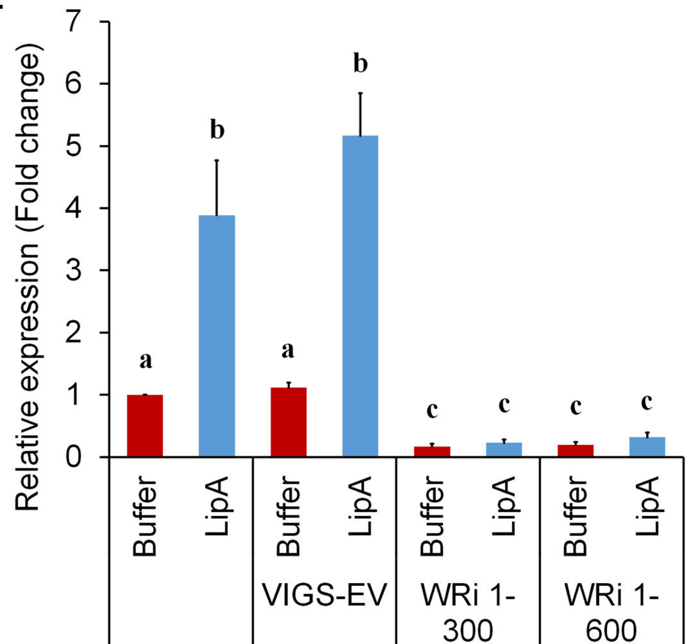

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Figure 4

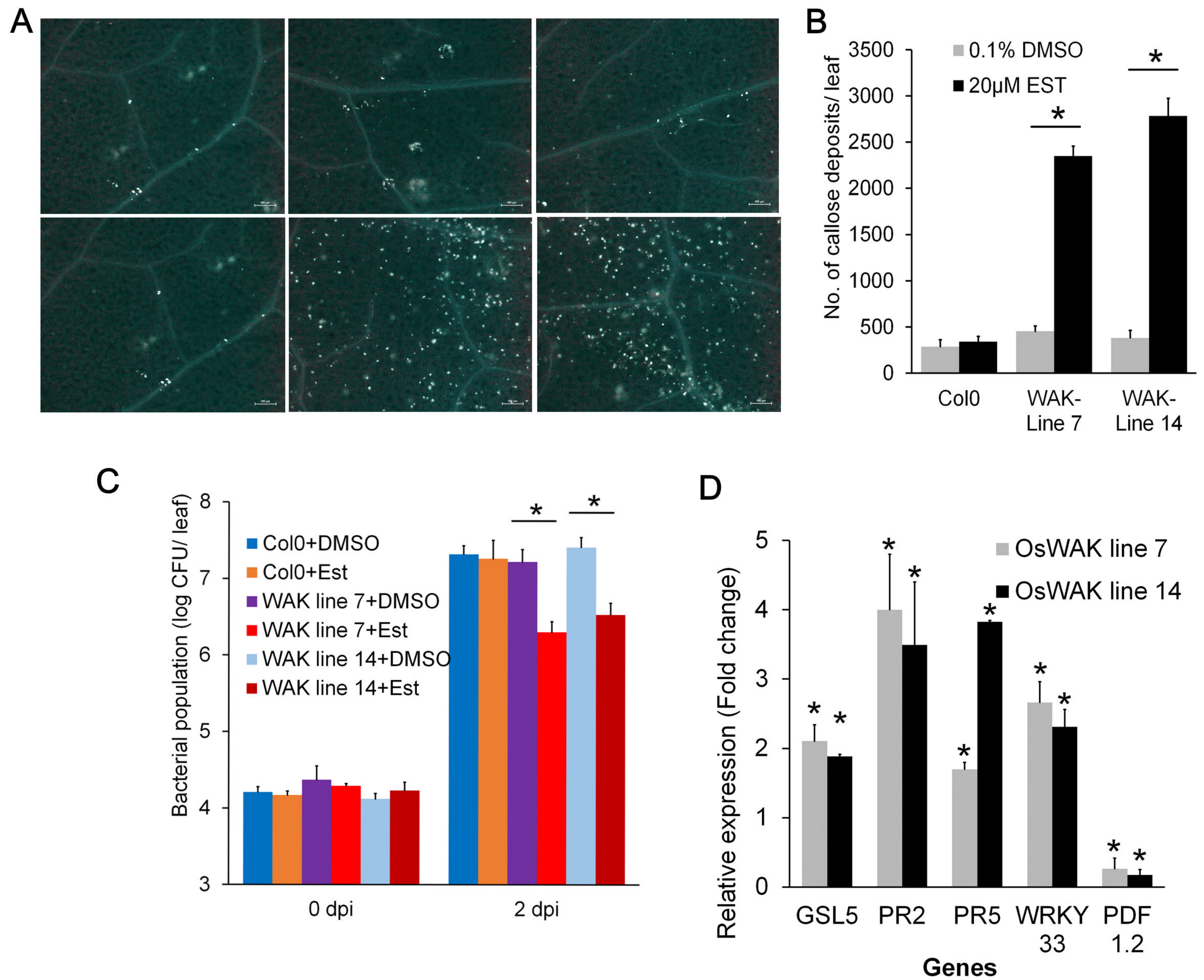

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Figure 5

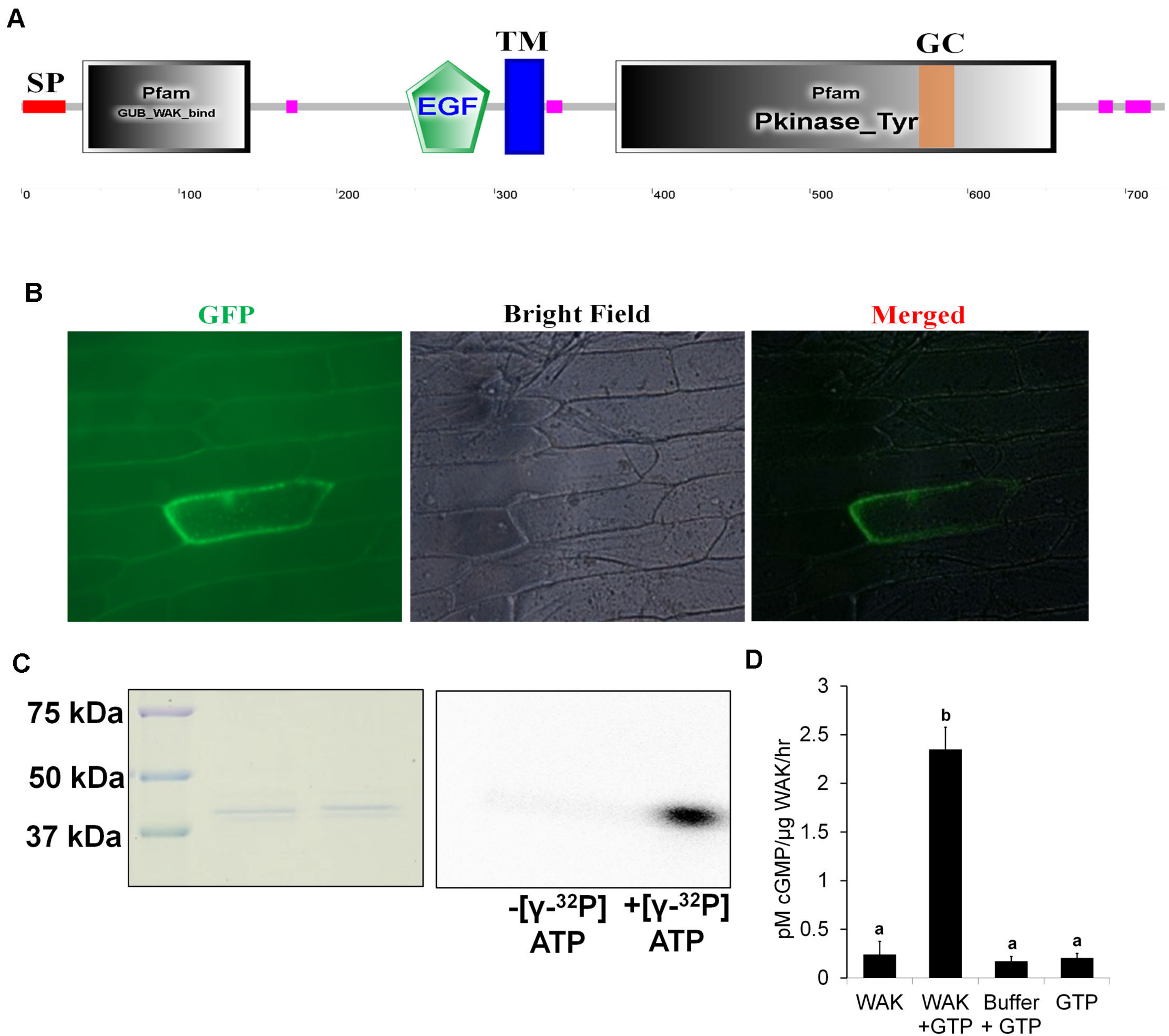

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Figure 6

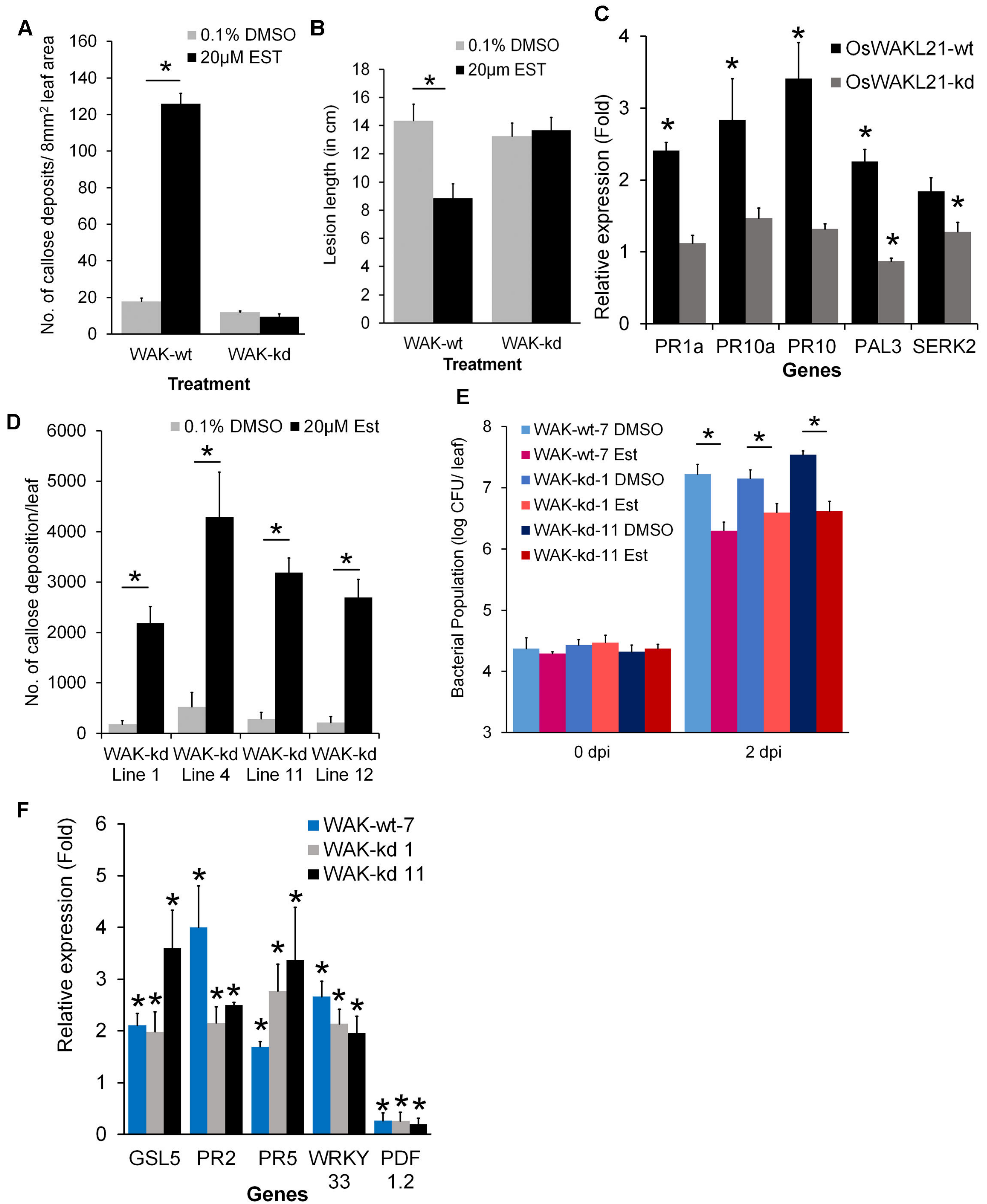

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Figure 7

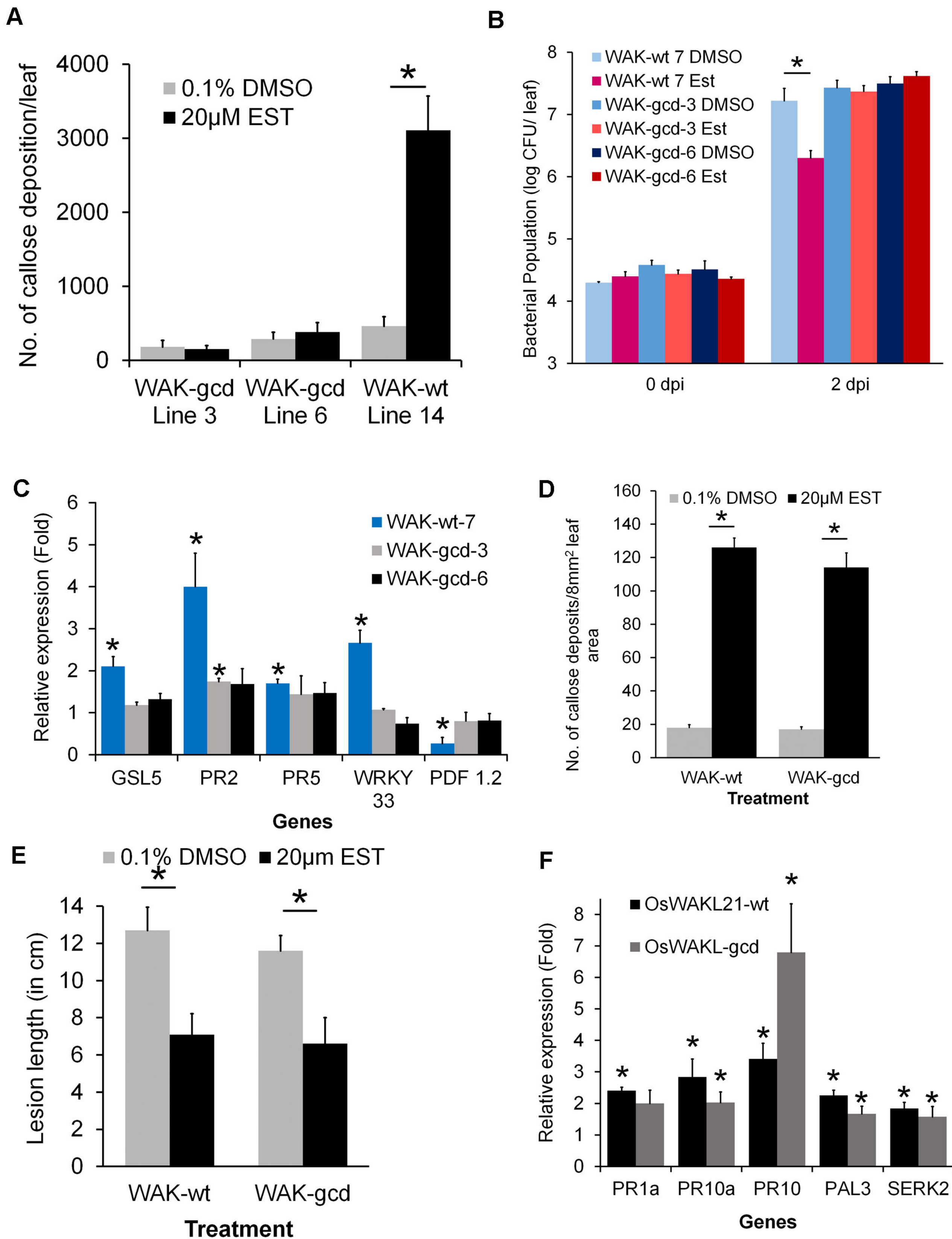

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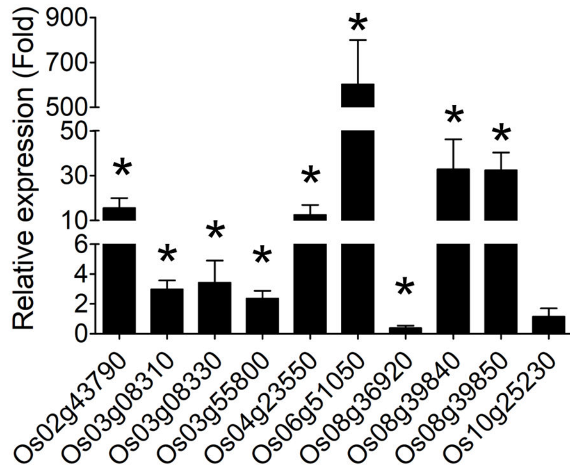
Figure 8

A



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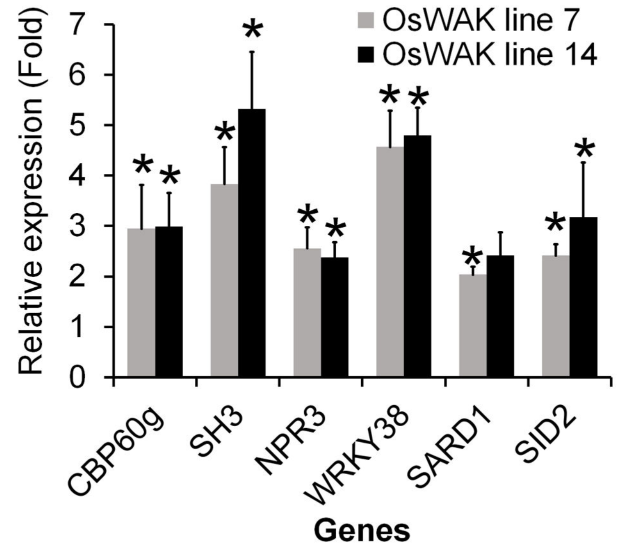

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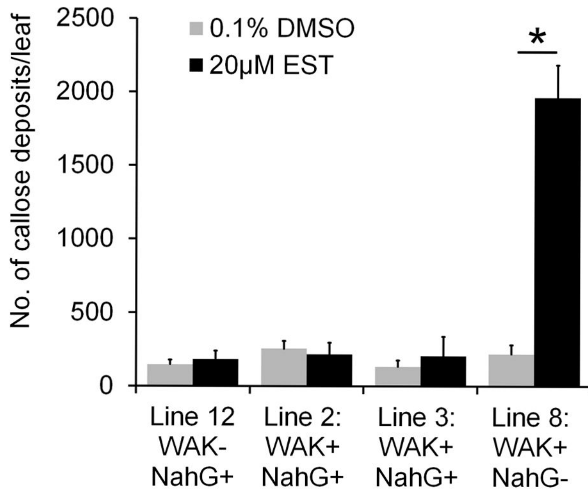

D