

1 **Dynamic characteristics and Functional Analysis Provide new insights into the**
2 **role of *GauERF105* in resistance against Verticillium wilt in Cotton**

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20 **Abstract:**

21 Verticillium wilt is the most devastating disease of cotton and it results in huge yield
22 losses every year in the fields. The underlying mechanisms of VW in cotton are not
23 well explored yet. In the current approach we used the transcriptome data from *G.*
24 *australe* in response to Verticillium wilt attack to mine the ERF TFs and prove their
25 potential role in resistance against VW attack in cotton. We identified 23 ERFs in
26 total, and on the basis of expression at different time points i.e., 24h, 48h and 72h post
27 inoculation and selected *GauERF105* for further validation. We performed VIGS in
28 cotton and over expression in Arabidopsis respectively. Moreover, DAB and trypan
29 staining also suggests that the impact of disease was more in the wildtype as
30 compared to transgene lines. On the basis of our results, we confirmed that
31 *GauERF105* is the key candidate and playing a key role for defending cotton against
32 VW attack. Current finding might be helpful for generating resistance germplasm in
33 cotton and it will be beneficial to recover the yield losses in field.

34 **Keywords:** Verticillium wilt, Cotton, ERF, VIGS, Overexpression

35 1. Introduction

36 Cotton is an economic crop, while verticillium wilt severely restricts cotton
37 production (T. Li, Zhang, Jiang, Li, & Dhar, 2021). Cotton is one of the species of
38 Malvaceae with high economic value and wide geographical distribution. There are a
39 total of 53 species of cotton (Kunbo, Wendel, & Jinping, 2018), of which 46 diploid
40 cotton species divided into 8 genome groups of A, B, C, D, E, F, G, and K. The
41 remaining 7 tetraploid cotton species (AD)₁-(AD)₇ belong to one allopolyploid
42 genome(Beasley, 1942; Fryxell, 1992; Phillips, 1966). The A, B, E and F cotton type
43 genomes are distributed in Asia and Africa, the C, G and K cotton type genomes are
44 distributed in Australia, and the D and AD cotton type genomes are distributed in the
45 America.

46 Verticillium wilt can infect the vascular bundles of cotton plant. Verticillium wilt
47 was first discovered in the United States in 1915, and then introduced to China in
48 1935, and successively broke out in major cotton production areas (Jian, Lu, Xiu,
49 Wang, & Zhang, 2004). *Verticillium dahliae* is the pathogen of cotton verticillium
50 wilt, which is mainly divided into *Verticillium dahlia* and *Verticillium albo-atrum*.
51 *Verticillium dahliae* can cause the wilt of a variety of plants. In addition to cotton,
52 fruits, and vegetables such as potatoes, tomatoes, grapes, and some woody plants can
53 be attacked by *Verticillium dahliae* (Barbara, 2003). The main host of Verticillium
54 black and white are alfalfa, hops, soybeans, tomatoes, potatoes, and some weeds
55 (Chen, Lee, & Robb, 2004; Ligoxigakis, Vakalounakis, & Thanassoulopoulos, 2002).
56 After investigations, the diseases in cotton fields in China are mainly caused by
57 *Verticillium dahliae* (Yinhua et al., 2014).

58 Cotton verticillium wilt is one of the soil-borne fungal vascular disease, which
59 leads to yearly yield losses of over 30% and a severe economic loss of roughly 250–
60 310 million dollars for China (Gong et al., 2017). Though, it is hard to control
61 pathogenic harm to cotton plants, despite the fact that many attempts were made,
62 among them are the use of fungicides as well as cultural methods (Mohamed &
63 Akladios, 2017; Wei & Yu, 2018). Generally, protecting plants from pathogenic
64 damage, disease-resistant cultivars must be widely planted. Although certain

65 genes/proteins/TFs were characterized in cotton plant protection against pathogens,
66 several effective candidate genes were updated for their use in disease-resistant
67 breeding (Cai et al., 2009; Gong et al., 2017; Zeng, Chen, Luo, & Tian, 2016). Thus,
68 molecular mechanisms of plant resistance against the *V. dahliae* as well as the
69 functional analysis of genes linked to defense must be explored deeply.

70 The ERF transcription factor contains an AP2 domain, which is composed of a
71 transcription regulatory domain, a DNA domain, and a nuclear localization sequence
72 (NLS). In addition, some ERF family members contain oligomerization sites and
73 phosphoric acid modification sites to regulate gene expression. At the N-terminus of
74 the ERF domain is a basic hydrophilic region, which contains 3 β -sheet structures, in
75 which the 14th alanine and 19th aspartic acid residues in the second β -sheet helps in
76 binding ERF transcription factors to different cis-acting elements (Stockinger,
77 Gilmour, & Thomashow, 1997). DRE / CRT (Dehydration response element, DRE;
78 C-repeat, CRT) and GCC-box are the main cis-acting elements that ERF binds with.
79 Among them, DRE/CRT is mainly related to abiotic stress, and GCC-box is mainly
80 involved in regulating biotic stress. The promoter region of many drought
81 response-related genes contains a large amount of DRE, and its core sequence is
82 TACCGACAT (Kizis & Pagès, 2002). The core sequence TGGCCGAC of CRT
83 element is mainly present in low temperature response genes. It is precisely because
84 the cis-elements of DRE/CRT have the core sequence CCGAC, which is usually
85 related to temperature, salt, and drought, so they are usually referred to as the
86 cis-acting elements of DRE/CRT (Fujimoto, Ohta, Usui, Shinshi, & Ohme-Takagi,
87 2000; Kizis & Pagès, 2002). GCC-box has a conservative AGCCGCC sequence,
88 which is generally present in the promoter regions of many disease-related protein
89 genes. ERF can respond to some biological stress responses by combining with
90 GCC-box (Meng et al., 2010; H.-J. Yang et al., 2002).

91 Among the signal pathways that plants respond to biological stress, the ethylene
92 signaling pathway plays an important role, and many disease-resistant genes are
93 induced and regulated by this signal pathway (C. Yang, Lu, Ma, Chen, & Zhang,
94 2015). Overexpression of *AtERF1* directly activates the expression of plant defensins

95 (PDF1.2) and improves plant resistance to pathogens (Berrocal - Lobo, Molina, &
96 Solano, 2002; Lorenzo, Piqueras, Sánchez-Serrano, & Solano, 2003). The T-DNA
97 insertion mutant of *AtERF14* increases the susceptibility of Arabidopsis to *Fusarium*
98 *oxysporum* infection (Oñate-Sánchez, Anderson, Young, & Singh, 2007). *AtERF96*
99 positively regulates the resistance of Arabidopsis to necrotic pathogens by enhancing
100 the expression of PDF1.2a, PR-3, PR-4, and ORA59 (Catinot et al., 2015). The
101 overexpression of *GmERF5* in soybean increases its resistance to *Phytophthora sojae*,
102 and it can positively regulate the expression of PR genes after being induced by
103 *Phytophthora sojae* (L. Dong et al., 2015). Zang et al. found that overexpression of
104 *ZmERF105* can increase the resistance of maize to *S. sphaerocephala*, while the
105 *erf105* mutant strain showed the opposite phenotype; after *ZmERF105* overexpression
106 strains were infected with *S. phalacca*, *ZmPR1a*, *ZmPR2*, *ZmPR5*, *ZmPR10.1*. The
107 expression of disease-related genes such as *ZmPR10.2* is enhanced, on the contrary,
108 the expression of PR gene is reduced in the ERF105 mutant line (Zang et al., 2020).
109 Meng et al. cloned two ERF transcription factor members *EREB1* and *EREB2* from
110 sea island cotton, and *Verticillium dahliae* can induce the expression of these two
111 genes (Meng et al., 2010). Guo et al. used the SSH method to enrich some
112 differentially expressed genes related to defense response and cloned the gene
113 *GbERF1-like* from sea island cotton. The study showed that the overexpression of
114 *GbERF1-like* activated the synthesis of lignin-related genes, and enhanced cotton and
115 pseudo-resistance of Arabidopsis to Verticillium wilt (Guo et al., 2016).

116 Here we, cloned *GauERF105* gene from the *G. australe* and then verified the gene
117 function in cotton via VIGS and overexpression in Arabidopsis in response to VW
118 attack. This research will provide a basis for further mining of excellent disease
119 resistance genes in wild cotton, in-depth research on the molecular mechanism of
120 cotton resistance to Verticillium wilt and provide new genetic resources for cotton
121 disease resistance breeding.

123 2. Materials and methods

124 2.1 Plant material, *Verticillium dahliae* strains and gene selection

125 The Zhongzhimian 2 (disease-resistant upland cotton variety) , Diploid wild cotton
126 (*G. australe*) and the Colombian ecotype *Arabidopsis* were provided by the Cotton
127 Research Institute of the Chinese Academy of Agricultural Sciences, Anyang, China.
128 The cotton seedlings are grown in a growth box with a light/dark cycle of 16/8h and a
129 temperature of 27°C (day)/23°C (night). The wild-type *Arabidopsis thaliana* (COL-0)
130 was cultured under a light/dark cycle of 16/8h at a constant temperature of 22°C. The
131 highly invasive strain of *V. dahliae* (LX2-1) was used for disease resistance
132 identification, and the preparation of conidia suspension (10^7 conidia mL⁻¹ for cotton,
133 10^6 and 10^3 conidia for *Arabidopsis*) and inoculation are as described above [1]. From
134 previously available RNA-Seq data(Q. Dong et al., 2019), we searched all the ERF
135 family genes and on the basis of expression we selected *GauERF105* for further
136 experiments.

137 2.2 Gene cloning and Phylogenetic Analysis

138 RNAprep Pure Plant Plus Kit (TIANGEN BIOTECH, Beijing, China) was used to
139 extract the sample RNA, and the quality of the sample was checked by agarose gel
140 electrophoresis and spectrophotometer. TranScript-All-in-One First-Strand cDNA
141 Synthesis SuperMix (TransGen, Beijing, China) reverse transcription kit was used to
142 obtain the cDNA. Design primers based on the CDS sequence of *GauERF105*, use *G.*
143 *australe* cDNA as a template, and use P505 high-fidelity polymerase (Vazyme,
144 Nanjing, China) to amplify the target gene. Download the amino acid sequences of
145 other cotton ERF members from the NCBI website. DNAMAN software was used for
146 multiple sequence alignment, and MEGA-X was used to construct a phylogenetic
147 tree.

148 2.3 Cotton VIGS and quantification of disease resistance

149 Virus induced gene silencing (VIGS) was performed according to the procedure
150 described previously by (Q. Dong et al., 2019). A 432 bp *GhERF105* fragment was
151 amplified and inserted between the BamHI and EcoRI sites of the tobacco Rattle virus
152 (TRV) binary vector pTRV2. Phytoene desaturase (PDS) gene was used as a marker

153 to detect the reliability of silencing. These experiments were repeated three times
154 independently, using more than 35 plants for each treatment. At 25dpi, the seedlings
155 symptoms are divided into five levels: 0, 1, 2, 3, and 4 according to the symptoms on
156 the leaves (Z. K. Li et al., 2019). The calculation of Disease index (DI) was as
157 follows: $DI = [(\sum \text{disease grades} \times \text{number of infected plants}) / (\text{total number of scored plants} \times 4)]$
158 $\times 100$ (Cai et al., 2020).

159 **2.4 Generation of transgenic Arabidopsis lines**

160 We used the method of homologous recombination to link the gene with ‘BamHI’ and
161 ‘SacI’ restriction sites with the overexpression vector PBI121 to obtain the expression
162 vectorPBI121-GauERF105, which was then transformed into *Agrobacterium*
163 *tumefaciens* GV3101. Transgenic *Arabidopsis thaliana* plants were obtained using the
164 flower soaking method (Clough & Bent, 1998). The transgenic lines (T0, T1 and T2
165 seeds) were screened on half-strength MS medium with kanamycin added. The T3
166 transgenic lines were identified and characterized by qRT-PCR and then used in
167 subsequent experiments. The 20-day-old Arabidopsis plants were inoculated with *V.*
168 *dahliae*. Twenty days after inoculation (20Dpi), the symptoms were scored.
169 According to the degree of leaf yellowing, the degree of resistance to VW is graded
170 from 0 to 4. The calculation method of the disease index was kept same as above.

171 **2.5 Histochemical staining of cotton stem lignin**

172 The Wiesner method (Speer, 1987) was used to analyze the lignin histochemical
173 staining of cotton. The parts of cotton cotyledon nodes of wild-type, TRV: 00 and
174 TRV: *GhERF105* plants were sectioned by hand. Dip the slices with Wiesner reagent
175 [3% (w/v) phloroglucinol in dd solution, solubilized with absolute ethanol] for 5
176 minutes, wash twice with distilled water, acidify with 6% hydrochloric acid solution
177 for 5 minutes, and wash away residual after hydrochloric acid treatment, place it on a
178 glass slide to observe and take pictures under a stereo microscope.

179 **2.6 Fungal recovery assay of cotton stems after *V. dahliae* inoculation**

180 We performed the fungal recovery assay as described earlier by (Song & Thomma,
181 2018). We randomly took cotton plants treated with Verticillium wilt. We used the
182 stem sections that were above the cotyledons and placed them in a sterilized triangular

183 flask and use disinfectant to disinfect the surface of the cotton stems for 7 minutes and
184 sterilize them immediately after disinfection. Wash the stem with ddH₂O, rinse 3
185 times for 5min each time. Place the samples in a petri dish containing PDA with
186 cephalosporin and incubate at 25°C in the dark for 3-5 days then observe the fungal
187 growth.

188 **2.7 DAB staining**

189 The 3,3'-diaminobiphenyl (DAB) staining method as described by (Gao et al., 2013)
190 for estimating the production and accumulation of hydrogen peroxide in the leaves.
191 After 72 hours of inoculation with *Verticillium dahliae*, Arabidopsis leaves were
192 taken, rinsed with distilled water, and then dried with filter paper. Add the leaves in a
193 2mL centrifuge tube, take an appropriate amount of DAB staining solution for
194 staining, and store in the dark at room temperature for 8h. Remove the staining
195 solution, add 95% ethanol to remove chlorophyll, keep changing ethanol for 2-3 days.
196 Use sterilize water to wash the leaves before taking pictures.

197 **2.8 Trypan blue staining**

198 The true leaves of the *GauERF105* Arabidopsis experimental group and the wild-type
199 Arabidopsis WT blank control group were respectively inoculated for 72 hours and
200 soaked in trypan blue dye solution (10 mL lactic acid, 10 mL glycerin, 10 g phenol,
201 10 mg Trypan blue, 10 mL of distilled water), in a boiling water bath for 2 minutes,
202 decolorize in chloral hydrate (2.5 g/mL) after cooling, replace the decolorizing
203 solution every day, decolorize for 3 days, then wash with sterile water, take pictures
204 and record.

205 **2.9 Expression analysis of defense marker genes**

206 Using cotton and *Arabidopsis thaliana* inoculated with *Verticillium dahliae* as
207 materials, leaf tissues were obtained at 48hpi and 72hpi respectively, and then quickly
208 frozen in liquid nitrogen to extract total RNA. These defensive marker genes in
209 Arabidopsis and cotton were detected using specific primers of some disease-related
210 proteins (PRs) described by (Guo et al., 2016). Each sample has 3 biological
211 replicates and 3 technical replicates.

212 **2.10 qRT-PCR analysis**

213 According to the above method, the total RNA of the plant was extracted and reverse
214 transcribed into cDNA. PCR amplification was used SYBR®qPCR Master Mix
215 (Vazyme, Nanjing, China) The cotton GhUBQ7 gene and the Arabidopsis
216 AtACTIN gene were used as internal reference genes for qRT-PCR analysis,
217 Calculate the relative expression of genes according to the $2^{-\Delta\Delta ct}$ method. The
218 qRT-PCR assay were performed as described previously

219

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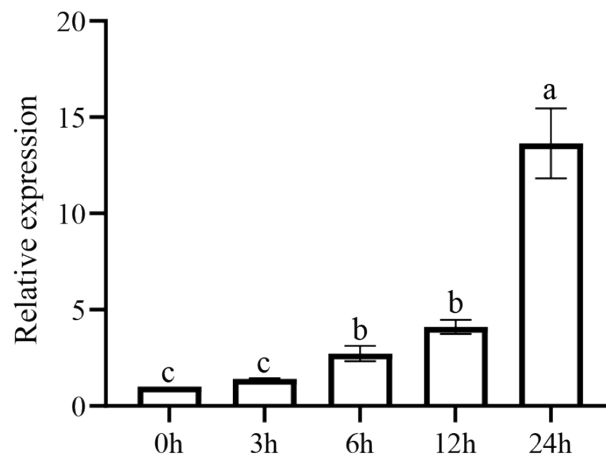
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222

245 **Figure 1.** Cloning and Sequence Analysis of *GauERF105* A- Gene structure analysis of *GauERF105*
246 (*Gau_G00013999*), B- Multiple sequence alignment of *GauERF105*, C- Prediction of conserved
247 domain for *GauERF105*, D- Phylogenetic analysis of *GauERF105*

248 3.2- Expression analysis of *GauERF105* under *Verticillium dahliae* stress

249 Expression patterns of *GauERF105* have been evaluated in the leaves of *Gossypium*
250 *australe* at 0h, 3h, 6h, 12h and, 24h after applying *Verticillium dahliae* in order to
251 confirm the role of selected gene in response to *Verticillium dahliae*. It was observed
252 that after inoculation, the expression level of *GauERF105* gradually increased from
253 0h to 24h post inoculation (Figure 4). Highest gene expression was observed at 24h
254 post inoculation. The results showed that *GauERF105* plays a critical role in response
255 to *Verticillium dahlia* attack and it might be the key candidate involved in
256 *Verticillium* wilt resistance.



257
258 **Figure 2:** Expression pattern of *GauERF105* via RT-qPCR at 0, 3, 6, 12 and 24 hours post *Verticillium*
259 *dahlia* inoculation.

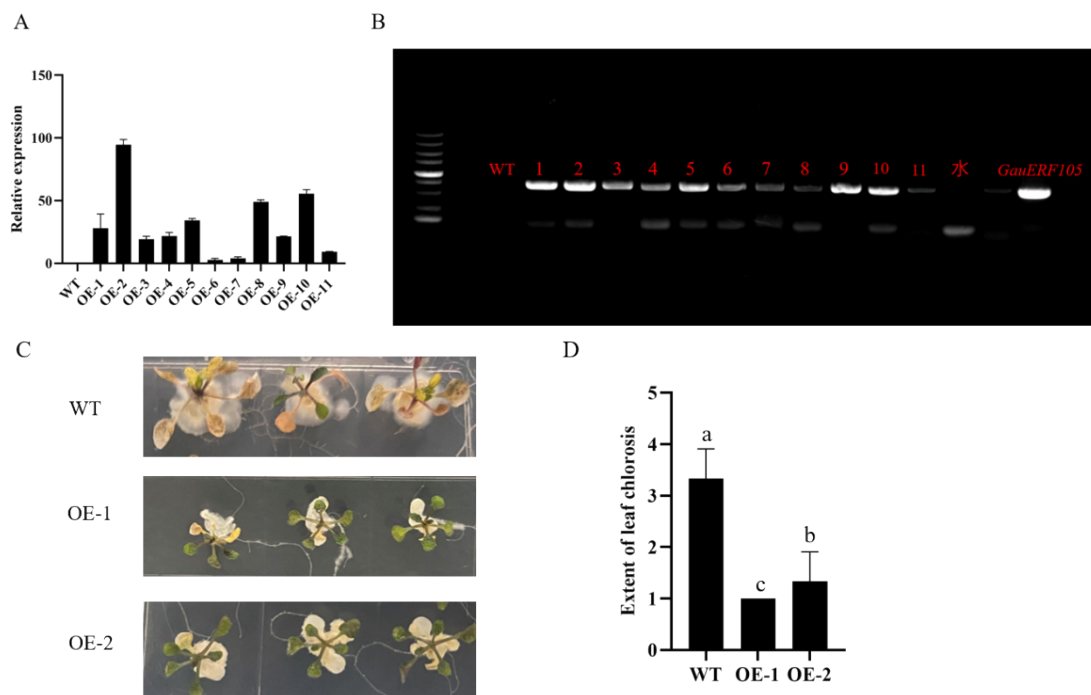
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261 3.3- Overexpression of *GauERF105* enhances the resistance of *Arabidopsis* in 262 response to *Verticillium* wilt attack

263 To validate the role of *GauERF105* in *Verticillium* wilt resistance we constructed an
264 overexpression vector “PBI121-*GauERF105*” and transform into *Arabidopsis*
265 *thaliana*. *Arabidopsis thaliana* was infiltrated using floral method. We selected
266 positive seedlings and screened them on the MS solid medium containing kanamycin
267 until the T₃ generation where homozygous lines were screened. Samples from eleven

268 positive T₃ generation plants were collected to analyze the expression levels of
269 *GauERF105*. Based on the results of RT-qPCR and agarose gel electrophoresis, we
270 selected OE1 and OE2 for subsequent experiments (**Figure 3A, B**).

271 The seeds of OE1 and OE2 lines were spot planted on MS medium, a small
272 amount of *Verticillium dahliae* was added to MS medium, and the growth of plants
273 was observed (**Figure 3C**). Results indicated that WT showed a more sensitive
274 phenotype to *Verticillium dahliae* as compared to the transgenic lines “OE1 and
275 OE2”. In addition, the degree of WT plants resistance towards verticillium wilt was
276 higher than that of transgenic lines, which indicates that the overexpression of
277 *GauERF105* gene enhances the plant's resistance to Verticillium wilt. We also planted
278 wild-type Arabidopsis and transgenic lines in nutrient soil and observed the symptoms
279 after inoculation. After overexpressing the *GauERF105* gene in Arabidopsis, the plant
280 ability to resist Verticillium wilt was significantly enhanced, which is consistent with
281 the phenotype of Arabidopsis grown on MS plates. The disease index was further
282 counted by using the formula already mentioned in the methodology section, and a
283 quantitative experiment of *Verticillium dahliae* was carried out, we observed results
284 were consistent with that of phenotypic observations (**Figure 3D**).



285

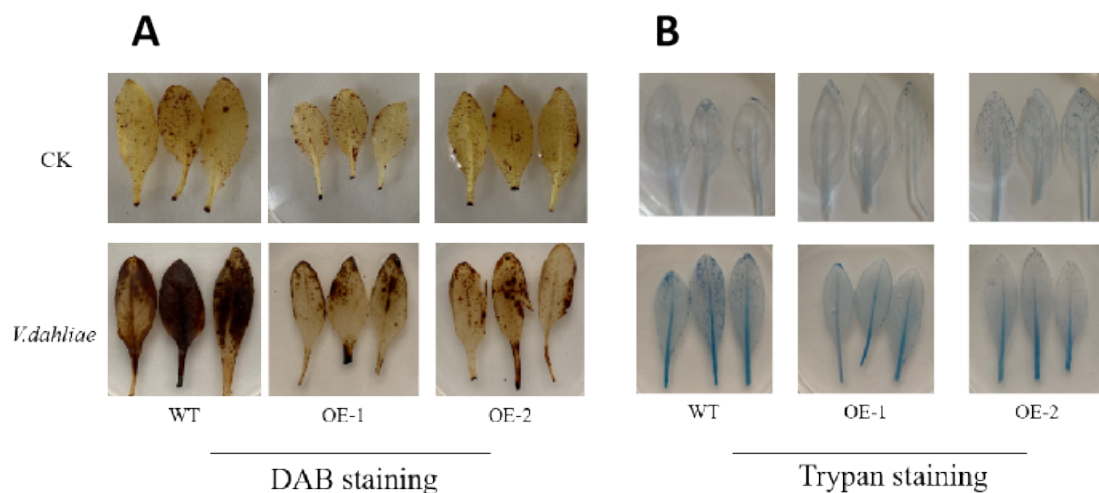
286 **Figure 3:** Overexpression of *GauERF105* enhances the resistance of Arabidopsis in response to

287 Verticillium wilt attack **A-** Relative expression of *GauERF105* in the overexpressed lines, **B-**
288 Polymerase chain reaction (PCR) to confirm 639 bp coding sequence (CDS) integration in
289 transformed T2 generation, number 1–11 transgenic lines, **C-** Transgenic lines and wild type
290 under normal and diseased conditions, **D-** Extent of leaf chlorosis in wildtype and overexpressed
291 lines after fungal inoculation.

292 3.4- DAB and trypan blue staining

293 The accumulation of ROS represents the oxidative damage which occurs due to the
294 stress caused by *Verticillium dahliae* attack. After 72 hours of inoculation leaves were
295 taken for DAB staining. Wild-type *Arabidopsis* and transgenic lines both started to
296 accumulate ROS but the leaves of WT plants are affected more as compared to
297 transgenic lines, indicating that the overexpression of *GauERF105* reduced the
298 damage caused by *Verticillium dahliae* attack.

299 Due to the damage of *Verticillium dahliae*, a large number of dead cells were
300 produced in the plants. Therefore, the wild-type plants are stained darker. At the same
301 time, the staining area of the wild-type *Arabidopsis thaliana* after inoculation was
302 significantly larger than that of the transgenic *Arabidopsis thaliana*, which indicated
303 that the damage of *Verticillium dahliae* to plants was greatly reduced after the
304 *GauERF105* gene was overexpressed **Figure 4**.



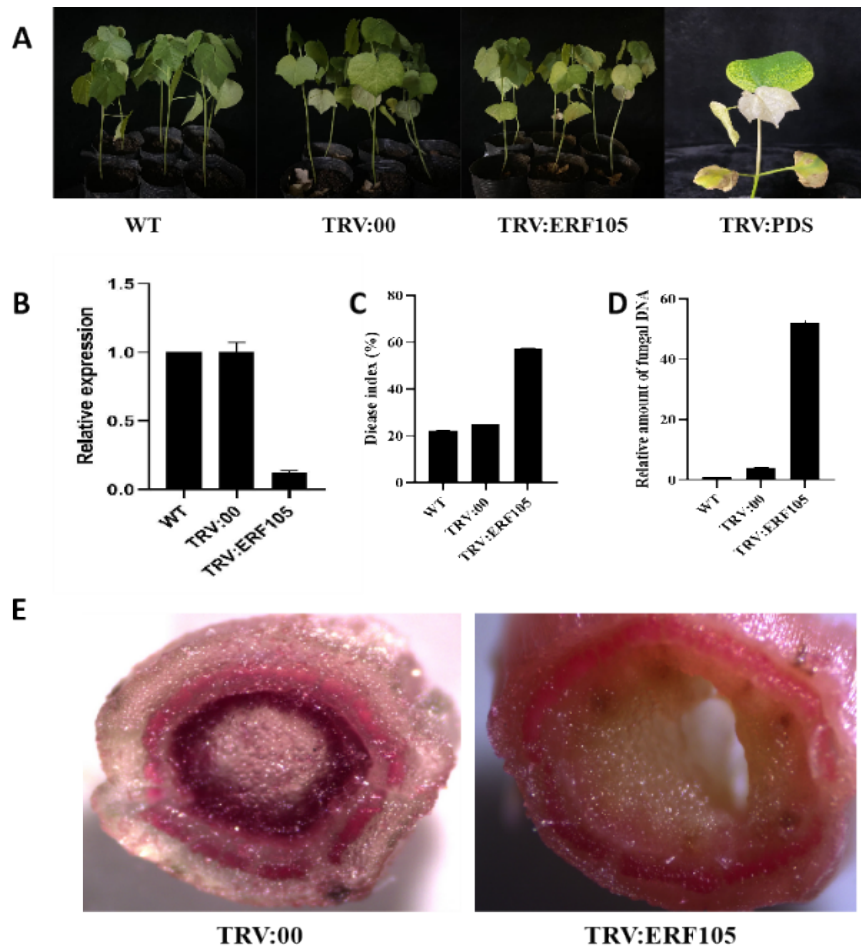
305

306 **Figure 4:** DAB and trypan blue staining **A-** DAB staining to estimate the damage on *Arabidopsis*
307 leaves after fungal inoculation, **B-** DAB staining to estimate the damage on *Arabidopsis* leaves after
308 fungal inoculation

309 **3.5- Silencing of *GauERF105* gene decreases the resistance against *Verticillium***
310 **wilt in cotton**

311 In order to verify the function of *GauERF105* in response to *Verticillium* wilt attack
312 in cotton, virus-induced gene silencing was used to silence the homologous gene
313 *GhERF105* in upland cotton (**Figure 5**). About 13 days after VIGS, the cotton leaves
314 were injected with TRV:PDS bacteria, chlorosis started and an albino phenotype was
315 observed, which proved that the VIGS system was established successfully and the
316 results were accurate for further experiment (**Figure 5A**). The qRT-PCR results
317 showed that the expression level of *GhERF105* gene was significantly lower in
318 silenced plants as compared to WT and TRV:00, indicating that *GhERF105* gene is
319 accurately silent.

320 Further, wildtype plants, empty vector plants and silent plants were inoculated with
321 *Verticillium dahliae*, and the phenotype was observed after 25 days of inoculation
322 (**Figure 5B**). Compared with the control plants, the leaves of the silent plants turned
323 yellow, wilted, and even fell off, and the disease index of the silent plants was also
324 significantly higher. The degree of infection in silent plants was severe as compared
325 to control plants (**Figure 5C**). In addition, the leaves of WT, TRV:00 and
326 TRV:GhERF105 plants were quantified for *Verticillium dahliae*. The expression level
327 of *Verticillium dahliae* in the silenced target gene plants were significantly higher
328 than that of the control plants, which was consistent with the results of the previous
329 disease and disease index investigation (**Figure 5D**). We sterilized the cotton stems
330 after inoculation and cultured them in a PDA solid medium. The number of
331 *Verticillium dahliae* in the TRV:00 plants were significantly smaller than that of the
332 TRV:ERF105 plant, indicating that *Verticillium dahliae*. This indicates that silencing
333 of *GhERF105* gene weakens the plant's resistance to *Verticillium* wilt attack and
334 make the plant more vulnerable to damage. Cotton lignin dying results showed that
335 silent plants have inhibition of lignin as compared to wildtype and non-silent plants
336 (**Figure 5E**). Thus, proving the role of *GhERF105* in VW resistance in cotton.



337

338 **Figure 5:** Silencing of *GauERF105* gene decreases the resistance against *Verticillium* wilt in cotton **A-**

339 Representative images of WT, Positive Control, and VIGS plants, **B-** Relative expression of

340 *GhERF105* in WT, TRV:00 and TRV:GhERF105, **C-** Disease index (%) WT, TRV:00 and TRV:

341 GhERF105, **D-** Relative amount of fungal DNA in WT, TRV:00 and TRV:GhERF105, **E-**

342 Histochemical staining of cotton stem lignin. Bars show standard error. WT: Wild type, TRV:00

343 Positive control, TRV:GhERF105, the VIGS plants.

344 **3.6- Expression of disease-resistant marker genes in Cotton**

345 In order to further analyze the regulatory role of *GauERF105* gene in the process of

346 plant disease resistance, we further screened the expression of some disease-resistant

347 pathway related genes in Arabidopsis and cotton (**Figure 6 A, B, C**). The results showed

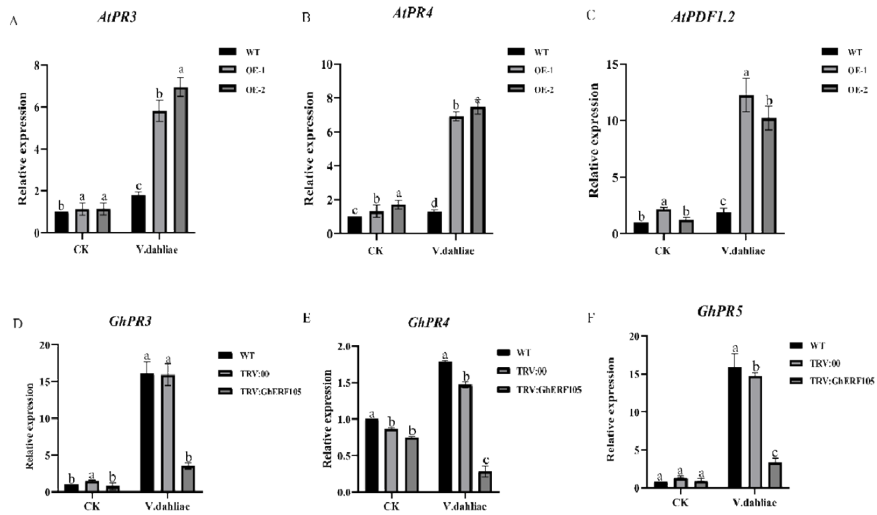
348 that when the plants were inoculated with *Verticillium dahliae*, the expression of PRs

349 increased; the expression of *AtPDF1.2*, *AtPR3* and *AtPR4* genes in the two

350 *GauERF105* gene-transformed transgene lines OE-1 and OE-2 lines was significantly

351 higher than that in the wildtype. In VIGS plants, the expression of PRs genes in the

352 silenced plants was significantly downregulated. It further illustrates that the
353 *GauERF105* gene can activate hormone-related pathways to participate in plant
354 disease resistance (Figure 6 D, E, F).



355

356 **Figure 6:** Expression of disease resistant marker genes in Transgenic Arabidopsis and Cotton.

357 **4- Discussion**

358 Plants opt a series of defense mechanisms after being invaded by pathogens. As one
359 of the largest transcription factor families in plants, ERFs participate in the regulation
360 of plant disease resistance.

361 ERF transcription factors positively activate the expression of genes related to plant
362 resistance to pathogens or regulate the accumulation of some secondary metabolites in
363 plants, thereby enhancing resistance to pests and diseases (SHAO, SHI, ZHANG, &
364 LANG, 2021). In the interaction network of plant immune response, SA and JA/ET
365 have cross-effects (Guo et al., 2016). Studies have shown that the SA pathway is
366 involved in regulating the defense of plants against living vegetative pathogens, and
367 JA and ET signal transduction are considered to be effective against pathogen attack.
368 Necrotrophic pathogens such as *B. cinerea* and *F. oxysporum* are more effective
369 (Derksen, Rampitsch, & Daayf, 2013). In this study, the *GauERF105* gene was
370 screened and cloned from *G. australe*. Here we overexpressed *GauERF105* in
371 Arabidopsis and checked the expression levels of AtPR3, AtPR4 and AtPDF1.2 genes
372 in the transgenic lines OE-1 and OE-2. We observed that, the expressions were
373 significantly higher in transgene lines as compared to wild-type Arabidopsis (**Figure**
374 **3**), which shows that when the expression level of *GauERF105* gene increases, the
375 expression level of its downstream genes also increases; DAB staining results show
376 that the staining degree of *GauERF105* in transgenic Arabidopsis leaves is lighter
377 than that of wild-type Arabidopsis. These result shows that overexpression of the
378 *GauERF105* gene reduces the oxidative damage of pathogens to plants and improves
379 the resistance of Arabidopsis against Verticillium wilt. PR1 and PR5 are the
380 downstream genes of the SA pathway, and PR3 and PR4 are the downstream genes of
381 the ET/JA pathway. The promoter regions of these disease related proteins have
382 GCC-box. ERF transcription factors activate the expression of downstream defense
383 genes by binding to GCC-box. So, as to enhance the plant's resistance to diseases and
384 insects attacks. After overexpression of the potato *StERF94* transcription factor, the
385 expression of PRs-related genes increased, thereby enhancing the potato's resistance
386 to *Fusarium oxysporum* (Charfeddine, Samet, Charfeddine, Bouaziz, & Bouzid,

387 2019). Wang et al. (Wang, Liu, & Wang, 2020) overexpressed the *VqERF112*,
388 *VqERF114* and *VqERF072* genes in Arabidopsis, and activated the SA signal-related
389 genes AtNPR1 and AtPR1 and JA/ET signal-related genes AtPDF1.2, AtLOX3,
390 AtPR3 and AtPR4, thereby enhancing the expression of Arabidopsis resistance to
391 *Pst-DC3000* and *B. cinerea*.

392 We used VIGS to verify the role of *GauERF105* homologous gene *GhERF105* in
393 upland cotton in the disease-resistant variety Zhongzhimian No. 2, after inoculation
394 with *Verticillium dahliae*. The silent plants turned yellow, wilted, or even died,
395 compared with the control plants. After silencing *GauERF105* gene, plants are more
396 sensitive to Verticillium wilt (**Figure 5**). Cotton disease index survey (**Figure 5C**),
397 lignin staining (**Figure 5E**), *Verticillium dahliae* recovery culture, and quantitative
398 experiment of *Verticillium dahliae* (**Figure 5D**) showed that the silencing of
399 *GauERF105* gene, weaken the defense ability of plants against pathogens. Compared
400 with the control, the expressions of GhPR3, GhPR4 and GhPR5 were significantly
401 downregulated in the silent plants (**Figure 6 D, E, F**), which indicated that when the
402 expression of *GhERF105* gene was interfered, the expression of its downstream genes
403 was inhibited, which weakened the plant's disease resistance. Compared with
404 non-inoculated plants, the expression levels of disease-related protein genes in cotton
405 or *Arabidopsis thaliana* were increased, indicating that the plants activated SA,
406 ET/JA, and other hormone transmission pathways after being subjected to biological
407 stress. , In order to participate in the defense response of plants.

408 The above results indicate that *GauERF105* acts as a positive regulator of plant
409 Verticillium wilt resistance in both the model plant Arabidopsis and cotton.

410

411 **4- Conclusions:**

412 Verticillium wilt attack on cotton are very severe in China and results in more
413 and more yield loss every year. Therefore, it is an utmost requirement to have
414 disease resistant cotton varieties. For that purpose, we need the candidate
415 genes responsible for disease resistant especially VW in cotton. Here, we
416 selected and screened that *GauERF105* gene from *Gossypium Australe* in
417 order to verify its potential role against verticillium wilt attack in cotton and
418 Arabidopsis. We performed overexpression experiments in Arabidopsis and
419 VIGS in cotton. Our results indicated that overexpression of *GauERF105*
420 increases the disease resistance ability in Arabidopsis and by silencing
421 *GauERF105*, results in a decrease in defense and resistance. RT-qPCR,
422 Trypan blue, DAB and lignin staining also validates our findings and hence it
423 is proved that *GauERF105* is a truer candidate gene for resistance against VW
424 attack in cotton. This gene can be used for further breeding programs to create
425 the disease resistance against VW attack.

426

427 **Authors' statement**

428 W.Y.Q, M.J.U, and Y.X conducted the experiment and wrote the manuscript.
429 R. O. M, T.G.M and M. L.S, Z.J assisted in data analysis. K.W., X.C, YH,
430 YW, Z.Z., and F.L. revised the manuscript. All authors reviewed and approved
431 the final manuscript.

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436 **Conflict of Interest**

437 The authors declared that they have no competing interests

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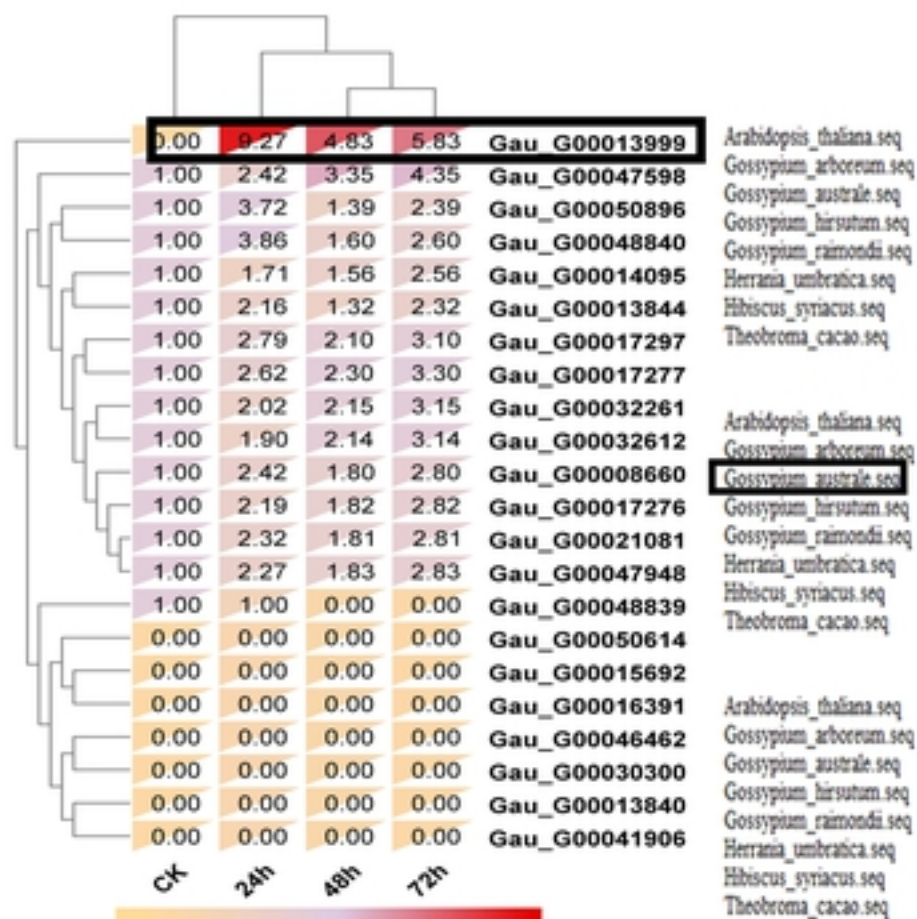
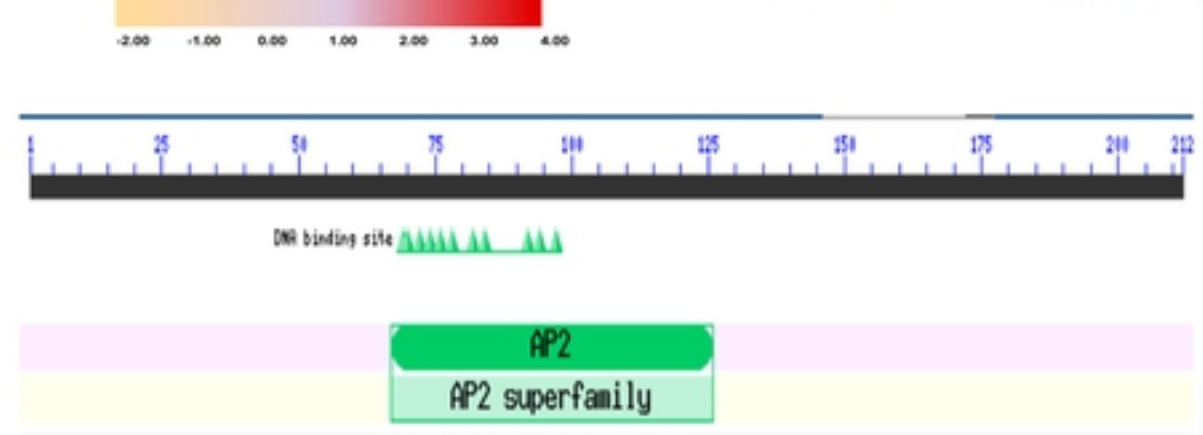
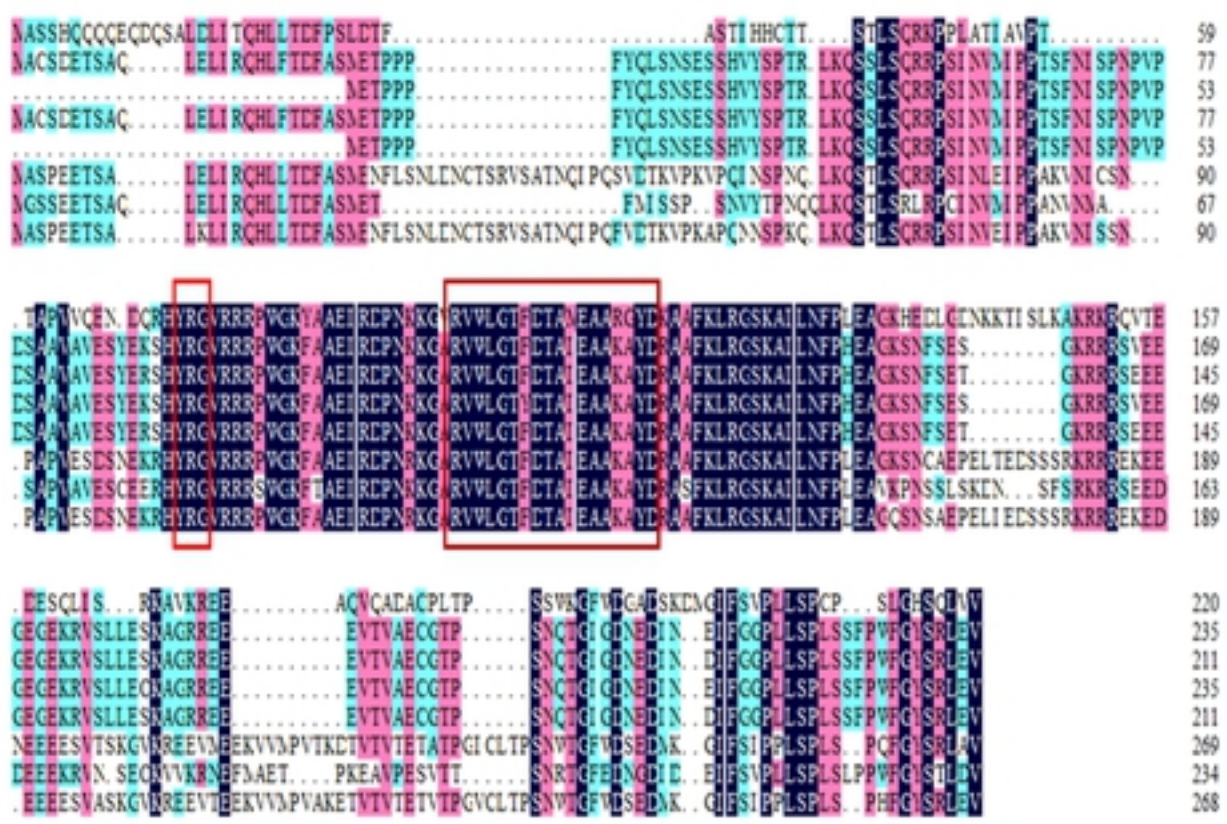
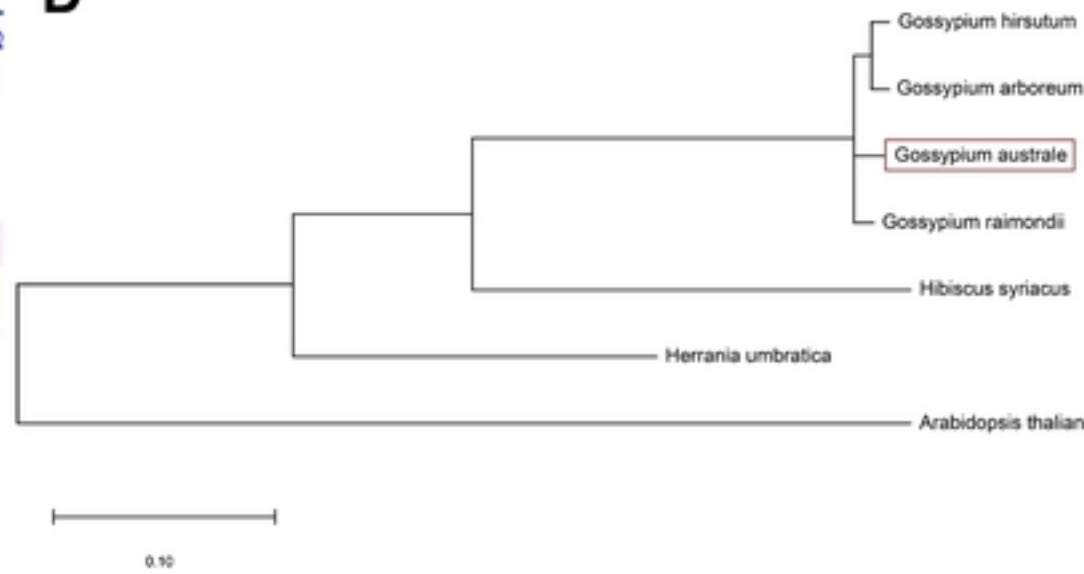
A**C****B****D**

Figure 1

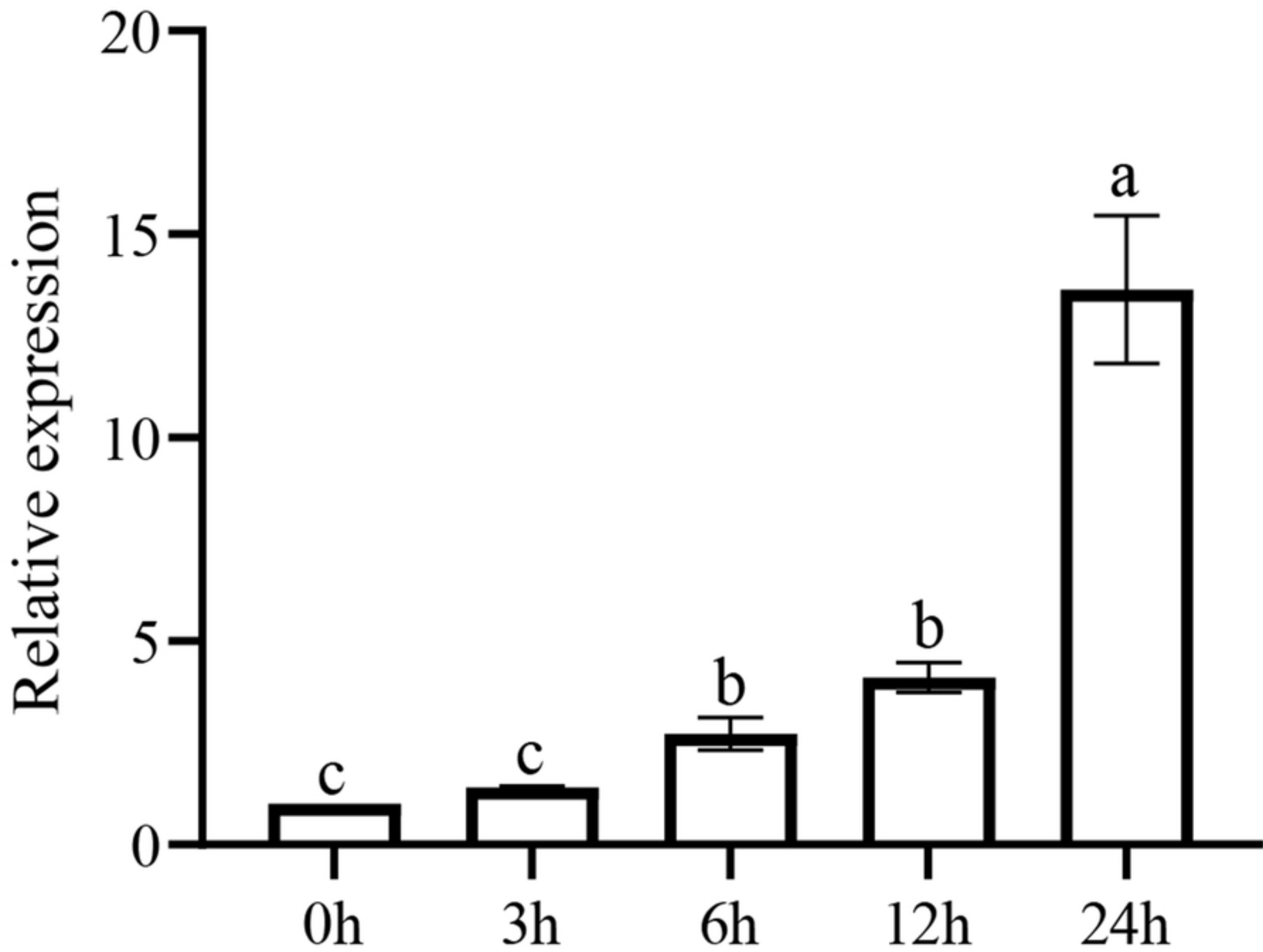


Figure 2

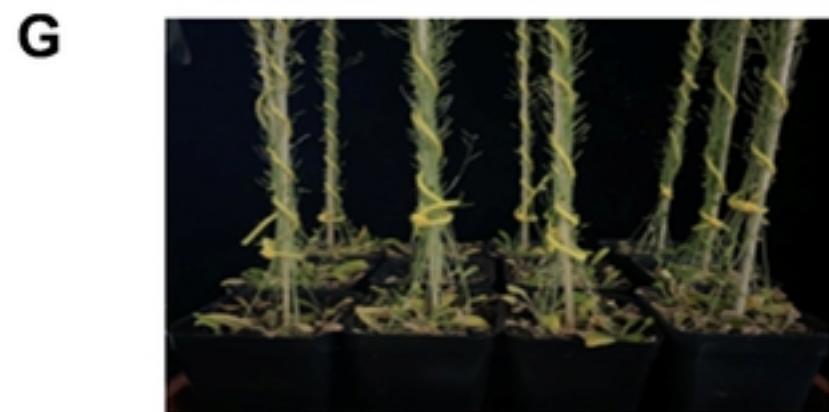
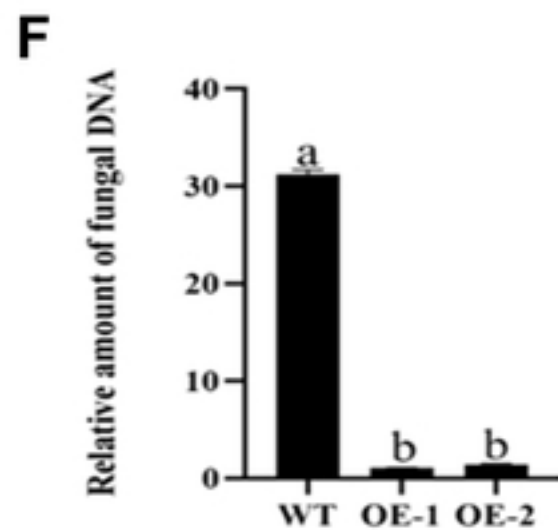
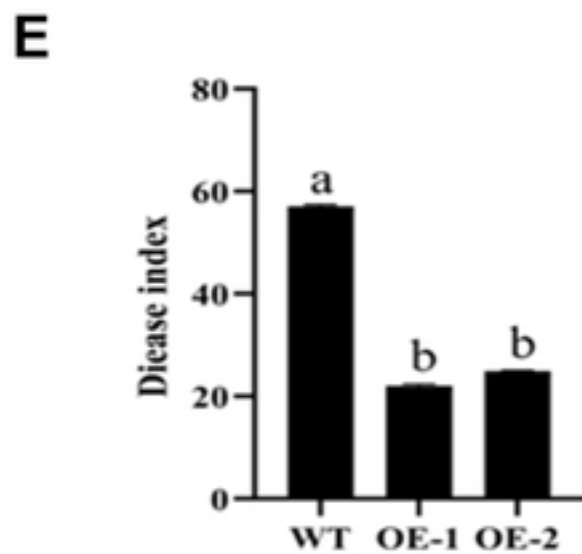
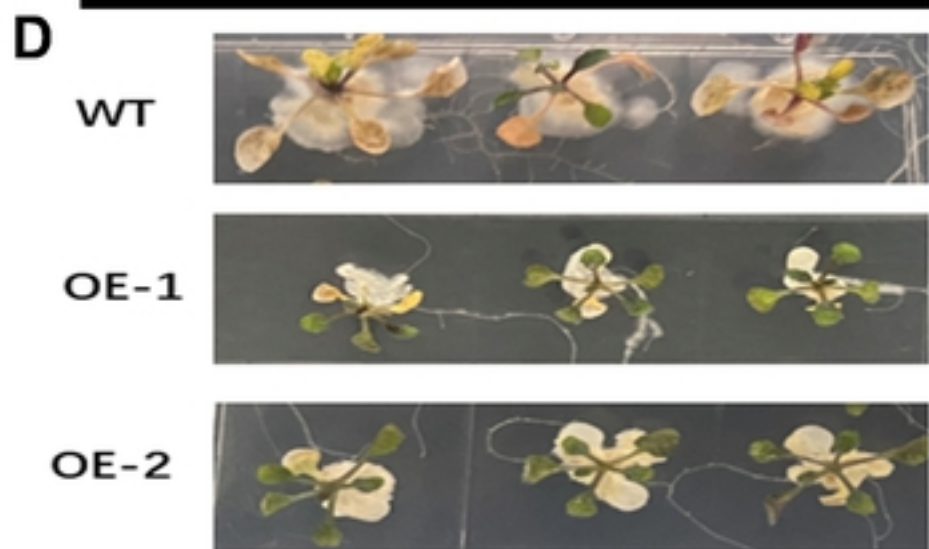
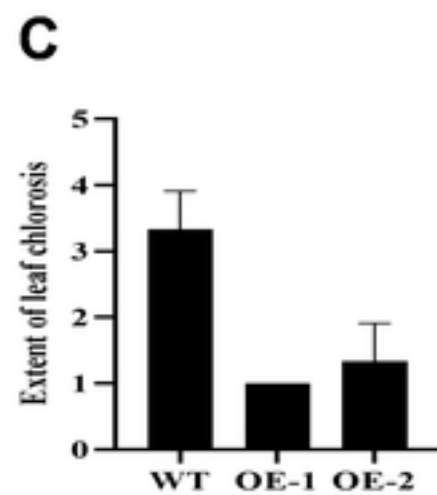
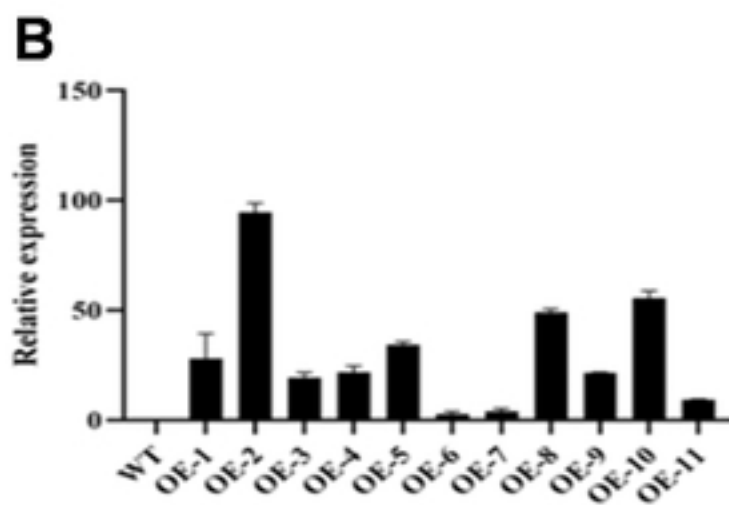
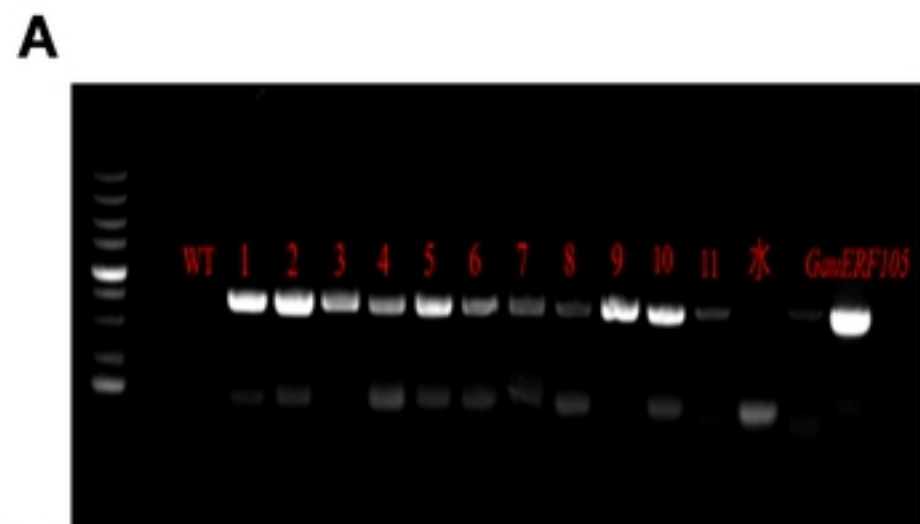
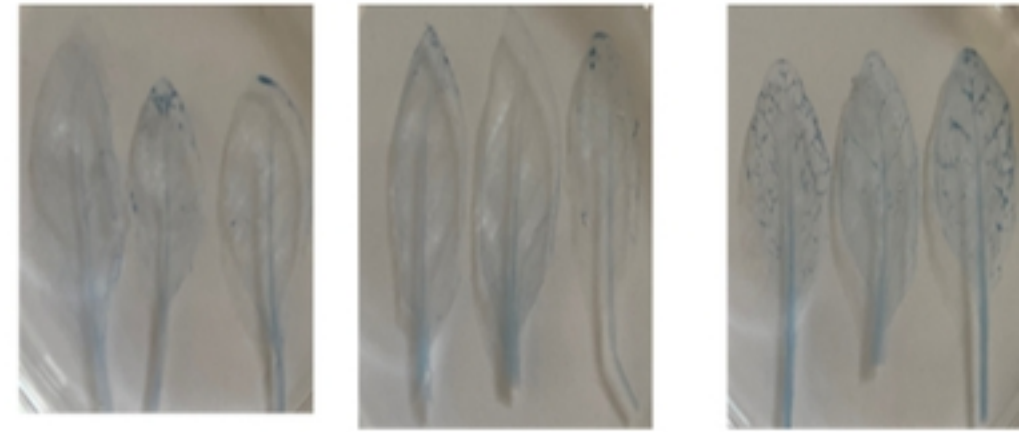
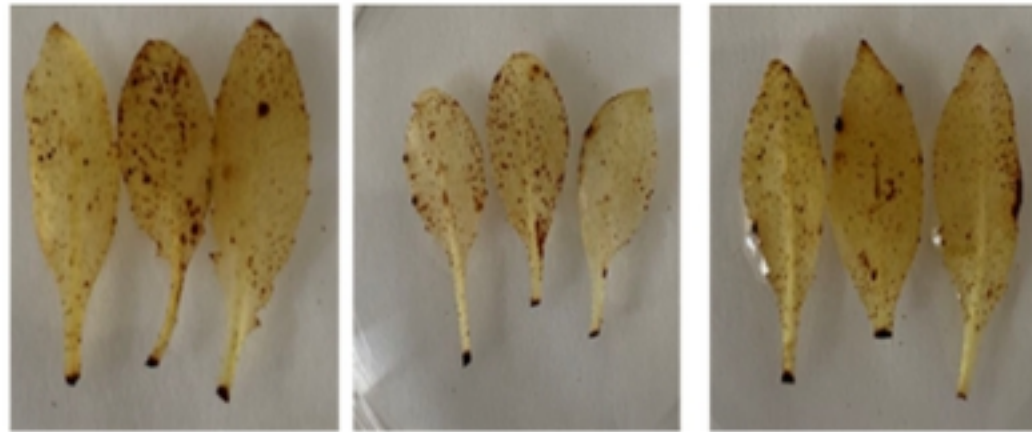
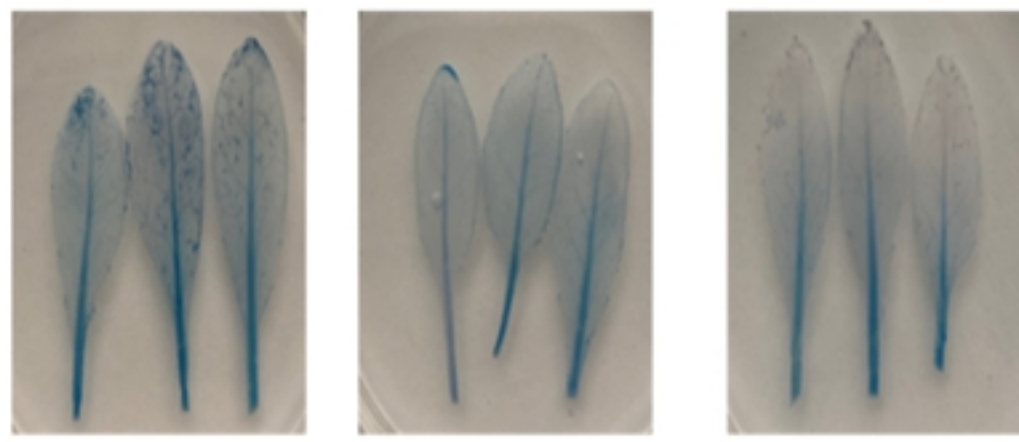
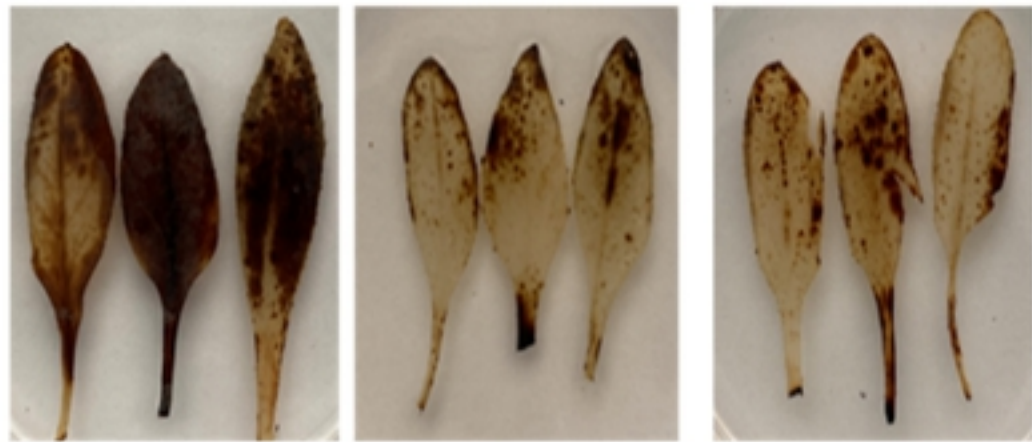


Figure 3

A**B**

CK

*V.dahliae*

WT

OE-1

OE-2

WT

OE-1

OE-2

DAB staining

Trypan staining

Figure 4

A

bioRxiv preprint doi: <https://doi.org/10.1101/2021.12.30.474616>; this version posted January 11, 2022. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

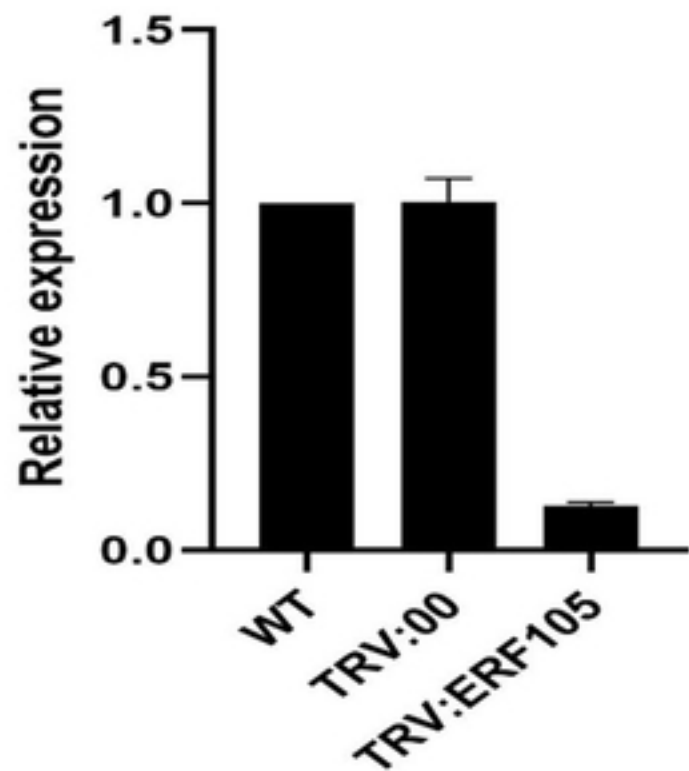
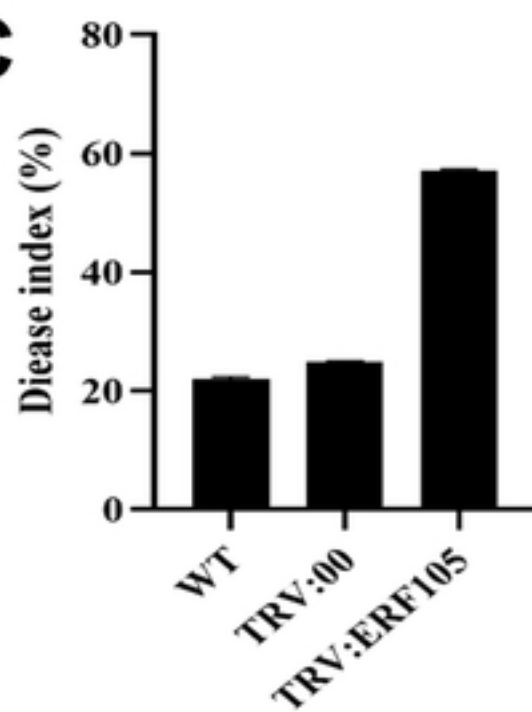
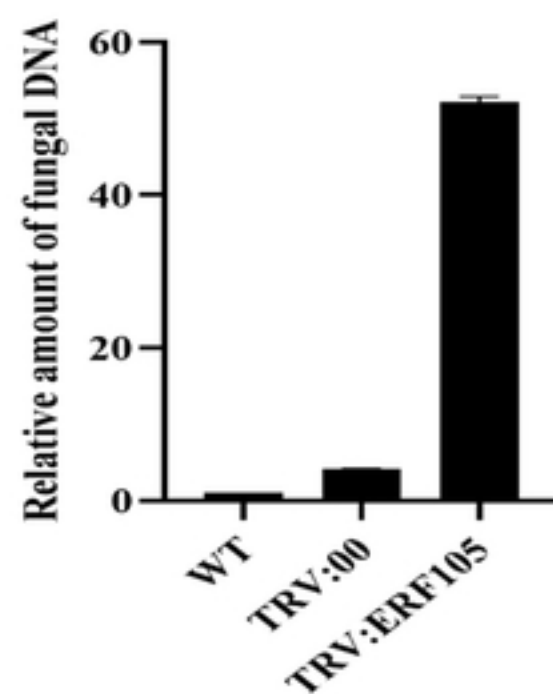
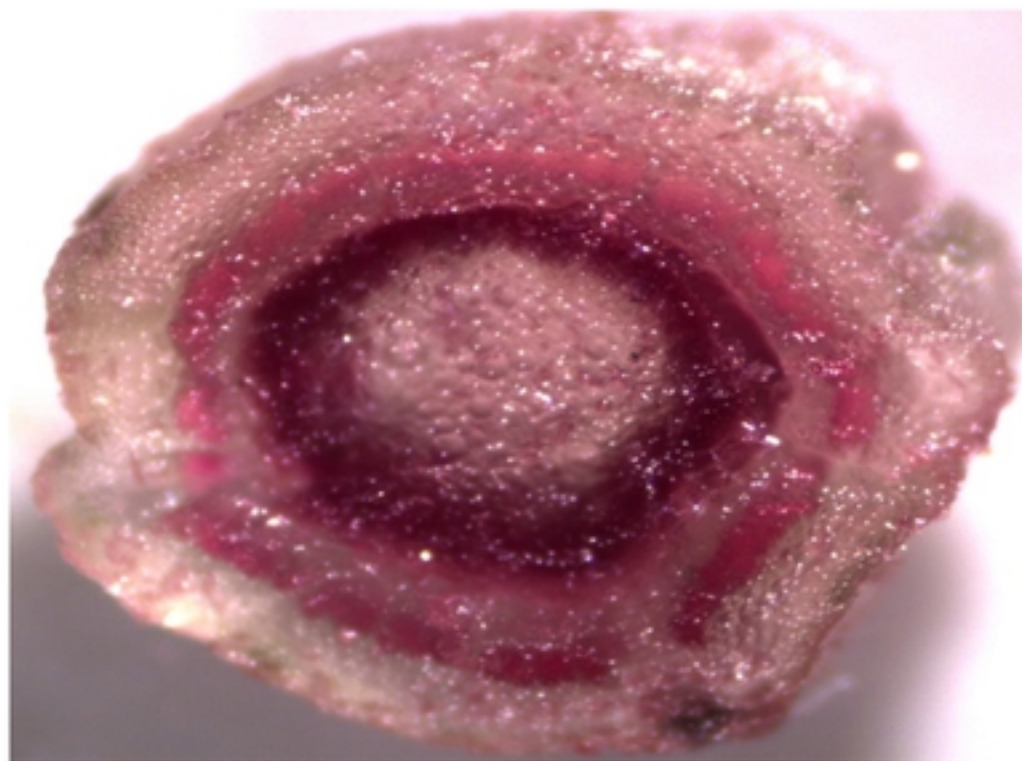
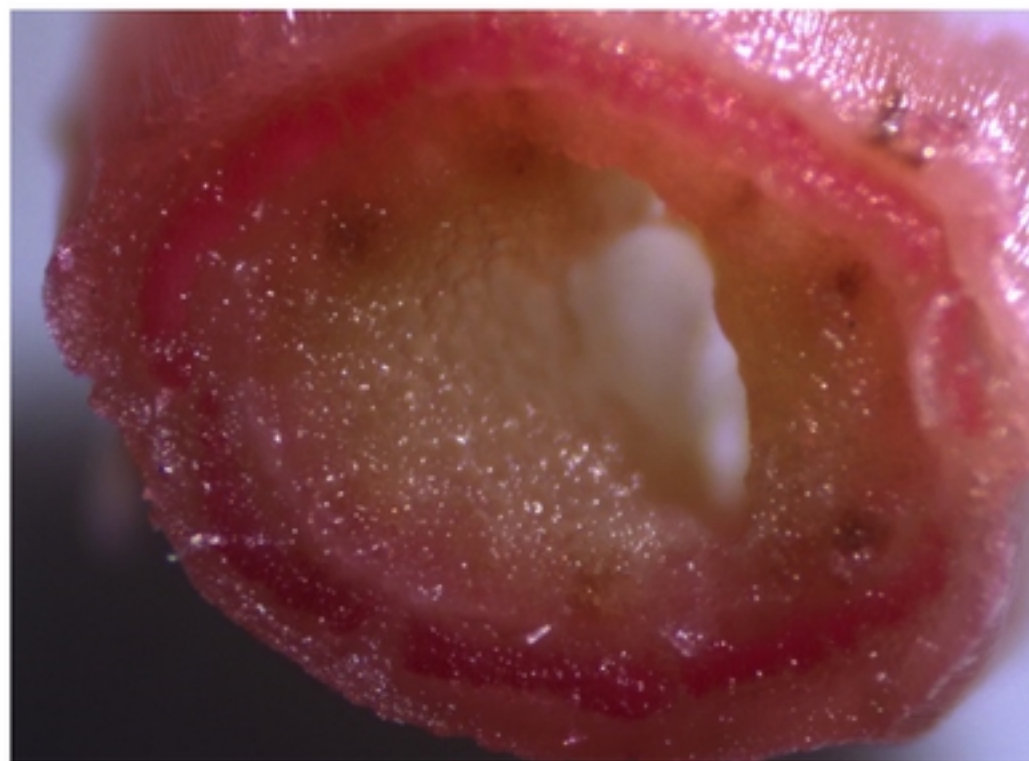
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Figure 5

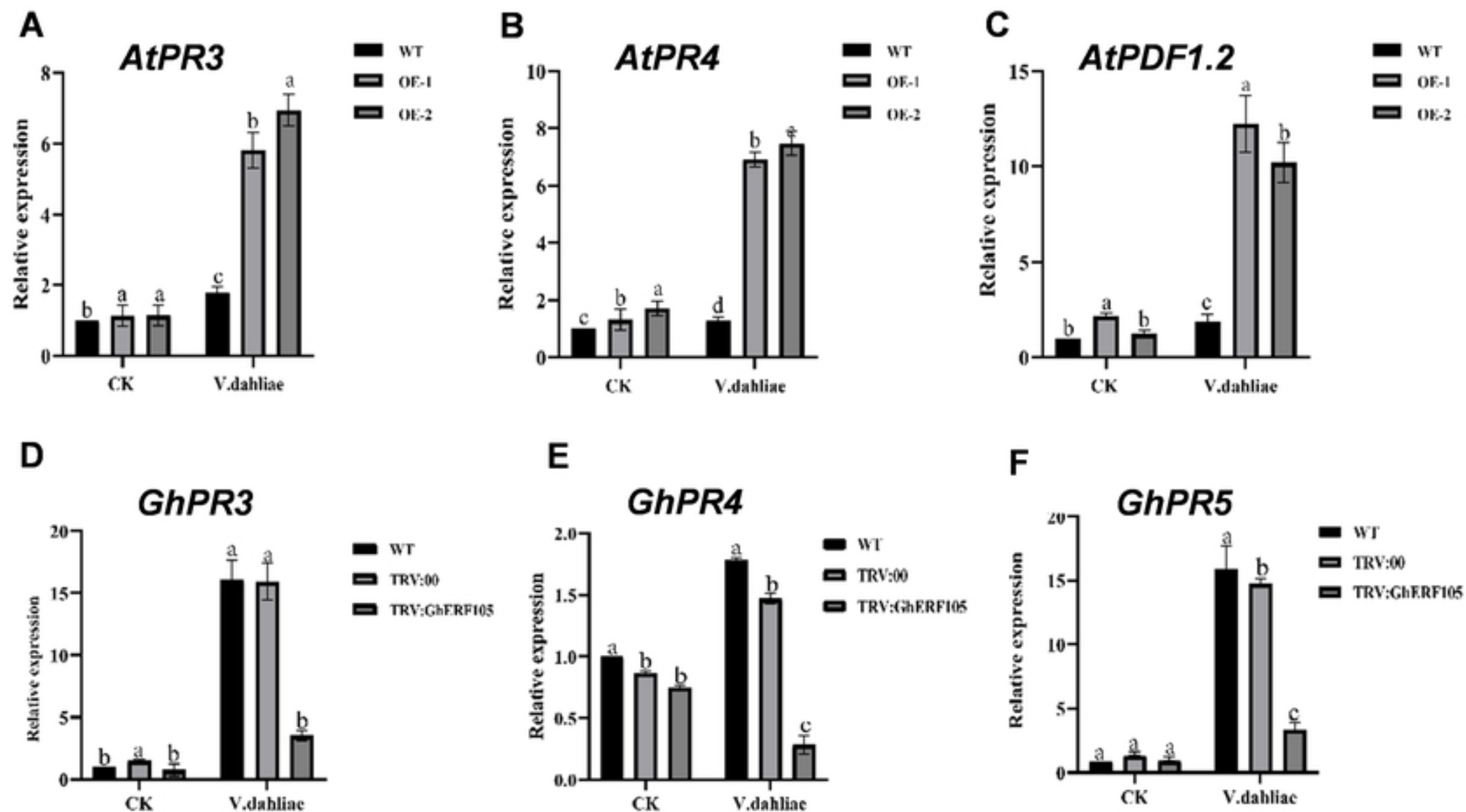


Figure 6