Hydrogen sulphide alleviates *Fusarium* Head Blight in wheat seedlings

Yuanyuan Yao [1, 2], **Wenjie Kan** [1], **Pengfei Su** [1, 2], **Yan Zhu** [1, 2], **Wenling Zhong** [1, 2], **Jinfeng Xi** [1, 2], **Dacheng Wang** [1], **Caiguo Tang** [1], **Lifang Wu** [Corresp. 1, 2, 3]

[1] Hefei Institutes of Physical Science, Chinese Academy of Sciences, Hefei, China

[2] University of Science and Technology of China, Hefei, China

[3] Zhongke Taihe Experimental Station, Taihe, China

Corresponding Author: Lifang Wu
Email address: lfwu@ipp.ac.cn

Hydrogen sulphide (H_2S), a crucial gas signal molecule, has been reported to be involved in various processes related to development and adversity responses in plants. However, the effects and regulatory mechanism of H_2S in controlling *F usarium* head blight (FHB) in wheat have not been clarified. In this study, we first reported that H_2S released by low concentrations of sodium hydrosulphide (NaHS) could significantly alleviate the FHB symptoms caused by *Fusarium graminearum* (*F. graminearum*) in wheat. We also used coleoptile inoculation to investigate the related physiological and molecular mechanism. The results revealed that FHB resistance was strongly enhanced by the H_2S released by NaHS, and 0.3 mM was confirmed as the optimal concentration. H_2S treatment dramatically reduced the levels of hydrogen peroxide (H_2O_2) and malondialdehyde (MDA) while enhancing the activities of antioxidant enzymes. Meanwhile, the relative expressions levels of defence-related genes, including *PR1.1*, *PR2*, *PR3*, and *PR4*, were all dramatically upregulated. Our results also showed that H_2S was toxic to *F. graminearum* by inhibiting mycelial growth and spore germination. Taken together, the findings demonstrated the potential value of H_2S in mitigating the adverse effects induced by *F. graminearum* and advanced the current knowledge regarding the molecular mechanisms in wheat.

Hydrogen Sulphide Alleviates *Fusarium* Head Blight in Wheat Seedlings

Yuanyuan Yao[1,2], Wenjie Kan[1], Pengfei Su[1,2], Yan Zhu[1,2], Wenling Zhong[1,2], Jinfeng Xi[1,2], Dacheng Wang[1], Caiguo Tang[1*], Lifang Wu[1,2,3*]

[1]The center for Ion Beam Bioengineering & Green Agriculture, Hefei Institutes of Physical Science, Chinese Academy of Sciences, Hefei 230031, Anhui, China
[2]University of Science and Technology of China, Hefei 230026, Anhui, China
[3]Zhongke Taihe Experimental Station, Taihe 236626, Anhui, China

[*]Corresponding Author:
 Caiguo Tang[1]
350 Shushanhu Road, Hefei 230031, Anhui, P. R. China
E-mali: cgtang@ipp.ac.cn
Lifang Wu[1,2,3]
350 Shushanhu Road, Hefei 230031, Anhui, P. R. China
E-mail: lfwu@ipp.ac.cn

Abstract

30 Hydrogen sulphide (H_2S), a crucial gas signal molecule, has been reported to be involved in
31 various processes related to development and adversity responses in plants. However, the effects
32 and regulatory mechanism of H_2S in controlling *Fusarium* head blight (FHB) in wheat have not
33 been clarified. In this study, we first reported that H_2S released by low concentrations of sodium
34 hydrosulphide (NaHS) could significantly alleviate the FHB symptoms caused by *Fusarium*
35 *graminearum* (*F. graminearum*) in wheat. We also used coleoptile inoculation to investigate
36 the related physiological and molecular mechanism. The results revealed that FHB resistance
37 was strongly enhanced by the H_2S released by NaHS, and 0.3 mM was confirmed as the optimal
38 concentration. H_2S treatment dramatically reduced the levels of hydrogen peroxide (H_2O_2) and
39 malondialdehyde (MDA) while enhancing the activities of antioxidant enzymes. Meanwhile, the
40 relative expressions levels of defence-related genes, including *PR1.1*, *PR2*, *PR3*, and *PR4*, were
41 all dramatically upregulated. Our results also showed that H_2S was toxic to *F. graminearum* by
42 inhibiting mycelial growth and spore germination. Taken together, the findings demonstrated the
43 potential value of H_2S in mitigating the adverse effects induced by *F. graminearum* and
44 advanced the current knowledge regarding the molecular mechanisms in wheat.
45
46 **Keywords:** Hydrogen sulphide; Wheat; *Fusarium graminearum*; Resistance
47

Introduction

49 Wheat is an important grain crop whose production safety has global economic significance.
50 However, many factors, including abiotic and biotic stresses, can reduce wheat yield and quality
51 (Ghimire et al., 2020). Wheat *Fusarium* head blight (FHB), is a devastating disease in wheat that
52 is mainly caused by *Fusarium graminearum* (*F. graminearum*) (Qiu, Xu & Shi, 2019) and is also
53 known as "wheat cancer." In the growing season, it can infect the spike, stem base, and root of
54 the grain under long-term wet and warm environments (McMullen et al., 2012). FHB presents as
55 bleaching or necrotic browning of spike tissue, ultimately producing shrunken or discoloured
56 grains (Buhrow et al., 2016). The resulting loss of production and quality causes significant
57 economic losses worldwide every year (McMullen, Bergstrom & Gallenberg, 1997).
58   Various methods have been proposed to control wheat FHB, which mainly involve breeding
59 FHB-resistant cultivars and application of chemical fungicides. Although tens of thousands of
60 wheat accessions have been screened worldwide, no completely resistant germplasm has been
61 found (Wang et al., 2020). Currently, more than 200 FHB resistance QTLs have been mapped,
62 whereas only the genes *Fhb1* and *Fhb7* had been cloned, and are expected to provide genetic
63 resources for global wheat production and food safety (Ghimire et al., 2020). *Fhb1* from Sumai 3

64 has been recognised to offer the best chance of enhancing the resistance of FHB, and had been

65 widely used as a parent in hybrid crosses (Su et al., 2019). Nevertheless, its resistance

66 mechanism remains elusive (Hao et al., 2020). Wang et al. (2020) found that *Fhb7* from

67 *Thinopyrum elongatum* conferred resistance to FHB without a yield penalty in wheat. Notably,

68 since resistance, agronomic traits, and quality traits are all controlled by multiple genes, the

69 application of new resistance candidates in production requires comprehensive evaluation (Jia et

70 al., 2018). In addition, the use of fungicides has been widely attempted to control FHB

71 (Mesterházy, Bartók & Lamper, 2003). However, this approach has been shown various

72 deficiencies, including inadequate effectiveness and the widespread existence of resistance to

73 some fungicides (Duan et al., 2014). The first carbendazim (MBC)-resistant (MBC^R) field isolate

74 of *F. graminearum* was discovered by Zhou, Ye & Liu (1994). In addition, Becher et al. (2010)

75 reported the emergence of quantitative azole resistance in *F. graminearum*. Thus, development

76 of new and efficient methods for pathogen control is extremely important.

77 H_2S, which plays various roles in regulating plant physiology, has recently been recognised as

78 the third gaseous messenger after nitric oxide (NO) and carbon monoxide (CO) (Wang, 2002).

79 The high permeability of small gaseous molecules facilitates signal transduction through the

80 membrane (Wang, 2002). Available experimental information shows that exogenous applications

81 of H_2S can alleviate the negative effects of numerous abiotic stresses, including salinity (Deng et

82 al., 2016), drought (Ma et al., 2016), heavy metal (Zhang et al., 2008; Zhang et al., 2010a; Zhang

83 et al., 2010b), temperature (Yang et al., 2016), and water (Shan, 2011) stresses. However, there

84 were few studies on the regulation of biotic stress resistance by H_2S in plants.

85 In this study, we first reported that exogenous application of low concentrations of H_2S could

86 effectively control FHB in wheat. Then, by evaluating the H_2O_2 and MDA contents, activities

87 of antioxidant enzymes, relative expressions levels of defence-related genes, and antibacterial

88 effects, we performed a preliminary exploration of the underlying mechanisms by which H_2S

89 alleviated FHB in wheat seedlings. H_2S is easy to obtain, inexpensive and shows multiple-

90 effects, and exogenous H_2S treatment may be a prospective strategy to control wheat FHB.

Materials & Methods

Regents and media

Sodium hydrosulphide (NaHS) (Sinopharm Chemical Reagent Co., Ltd., Shanghai, China)

served as the donor for H_2S. Carboxymethyl cellulose (CMC) medium (15 g/L carboxymethyl

cellulose, 1 g/L yeast extract, and 1 g/L NH_4NO_3) was used for liquid cultures of fungi.

Plant materials and treatments

The local virulent *F. graminearum* Schw., was kindly provided by Professor Xiue Wang,

Nanjing Agricultural University, was used for FHB evaluation. *F. graminearum* inoculum was

99   prepared in accordance with the method described by Buhrow et al. (2016). *F. graminearum*

100 conidia collected from PDA agar plates were transferred into liquid medium (CMC) and

101 cultivated at 28°C for 5 d with shaking at 200 rpm. Then conidial suspension was filtrated

102 through six layers of gauze and centrifuged at 6500 rpm for 6 min, and the spore concentration

103 was adjusted to 3.2×10^7 conidia/mL (0.1% Tween 80) with a haemocytometer and light

104 microscopy.

105   Two-day-old seedlings of the FHB-susceptible wheat variety 'Bainong 207' were tested for

106 coleoptile infection analysis by using the method described by Wu et al. (2005) with minor

107 alterations. Two days post-sowing (dps), the top 1-2 mm of the coleoptiles was cut off, and 2 μL

108 of *F. graminearum* inoculum was added to the top of the remaining seedlings. Coleoptiles

109 inoculated with 0.1% Tween-80 served as controls. Then, the inoculated seedlings were grown in

110 controlled environmental conditions (temperature: 25°C; photoperiod: 16 h light/8 h dark;

111 relative humidity: 95%).

112   NaHS was used as the H_2S donor according to the method described by Hosoki, Matsuki &

113 Kimura. (1997). In order not to affect the growth of plants, we first determined the application

114 concentration range of NaHS. Two-day-old wheat seedlings were culture in 0.0 (control), 0.1,

115 0.3, 0.5, and 1.0 mM NaHS respectively for 3 days, then the growth index was investigated on 9

116 dps (days post sowing). To explore the potential functions of H_2S in FHB resistance of wheat,

117 the seedlings were pre-treated with 0, 0.1, 0.3, and 0.5 mmol/L (mM) NaHS for 6 h; exposed to

118 *F. graminearum* inoculation; and then continuously cultured in NaHS at the pre-treatment

119 concentrations. After continuous treatment for 3 days, the treatment was stopped, and sterile

120 distilled water was used to irrigate the wheat. The optimal NaHS concentration, that effectively

121 relived the FHB symptoms, was determined according to the disease index combined with

122 growth indexes in wheat seedlings. To confirm the hypothesis that NaHS-mediated disease

123 suppression may be attributable to the production of H_2S or HS^-, various sodium salts were used

124 as controls for H_2S donors, in accordance with the method described by Zhang et al. (2008) with

125 some modifications. Wheat was treated in the same way as in the aforementioned experiment

126 with water (CK), 0.3 mM NaHS (the optimal concentration), Na_2SO_4, Na_2SO_3, $NaHSO_4$,

127 $NaHSO_3$, or NaAC. All measurements were collected with three replicates.

128 **Disease assessment**

129 On 7 days post-inoculation (dpi), visible necrosis symptoms induced by *F. graminearum* on the

130 wheat seedling stems were scored using the disease scoring system described by Nicholson et al.

131 (1998). Each disease index assessement was performed by taking 15 coleoptiles per replicate.

132   Lesion colour grade:

133   Grade 0: no disease.

134   Grade 1: very slight brown necrosis.

135   Grade 2: slight/moderate brown necrosis.

136   Grade 3: extensive brown necrosis.

137   Grade 4: extensive black necrosis.

138   Disease index (DI) $= \dfrac{\sum(lesion\ colour\ grade\ \times\ lesion\ length)}{total\ numbles\ of\ plant}$

139   Note: lesion length (mm).

140   **Determination of the wheat growth index**

141   Wheat growth indexes were determined to evaluate the effects of different concentrations of

142   NaHS on wheat growth. Each growth index was assessed by taking 15 seedlings per replicate.

143   Subsequently, the dry weights of the samples were measured after 72 h in an oven at 65°C.

144   **Relative quantification of *F. graminearum* DNA on the inoculated stem base of wheat**

145   A real-time quantitative PCR (RT-qPCR) assay was performed for relative quantification of *F.*

146   *graminearum* based on specific primers such as Fg16F/Fg16R according to Nicholson et al.

147   (1998). The inoculated plant sheath and frozen mycelium (PDA: potato dextrose agar, control)

148   were finely ground with a grinder for 2 min to a fine powder. Then, fungal DNA was extracted

149   from 100 mg of samples with the HP Fungal DNA Mini Kit (Omega, Shanghai, China).

150   Before qPCR, the quality of the qPCR primers was evaluated by PCR to determine whether

151   they could yield target fragment and if the product was unique. qPCR was performed on a Roche

152   LighterCycler 96 qPCR machine based on the protocol previously described by Tang et al.

153   (2020).

154   **Measurement of endogenous H$_2$S in wheat**

155   H$_2$S content data were collected as previously described by Sekiya et al. (1982) with some

156   modifications. Wheat leaves (0.3 g) were sampled and snap-frozen in liquid nitrogen. The

157   samples were ground and treated with 1.5 mL of Tris hydrochloride (Tris-HCl) buffer (pH 8.0),

158   after which the supernatant was collected and mixed with a zinc acetate trap in a test tube after

159   centrifugation. After adding 0.1 mL of 20 mM dimethyl-p-phenylenediamine dissolved in 7.2 M

160   HCl, the test tube was sealed quickly. Then, 0.1 mL of 30 mM ferric chloride in 1.2 M HCl was

161   injected into the test tube, and the mixture was incubated for 30 min at 25°C. Measurement of

162   H$_2$S was performed using a UV-VIS spectrophotometer (ScanDrop, Analytikjena, Germany) at a

163   wavelength of 667 nm. The blank was prepared with unused zinc acetate solution by using the

164   same procedures.

165   **Determination of the H$_2$O$_2$ and MDA contents**

166   The hydrogen peroxide (H$_2$O$_2$) and malondialdehyde (MDA) contents were measured in

167   accordance with the instructions of the H$_2$O$_2$ kit and MDA assay kit (Jiancheng Bioengineering

168   Institute, Nanjing, China), respectively.

169   **Measurement of SOD, CAT, APX, and POD activities**

170 The activities of catalase (CAT), superoxide dismutase (SOD), ascorbate peroxidase (APX), and

171 guaiacol-dependent peroxidase (POD) were assayed by the spectrophotometric method

172 according to the kits provided by Jiancheng Bioengineering Institute.

173 **RNA isolation and RT-qPCR**

174 Total RNA was isolated from samples according to the instructions provided with the Plant RNA

175 Rapid Extraction Kit (Mei5 Biotechnology, Beijing). The quantity and quality of total RNA were

176 tested by gel electrophoresis and spectrophotometry. Genes selected for further analysis included

177 *PR1.1*, *PR2*, *PR3*, and *PR4*, with the internal control being the glyceraldehyde-3-phosphate

178 dehydrogenase gene from *T. aestivum* (*TaGAPDH*). The primer sequences are listed in Table S1.

179 All procedures for RT-qPCR were conducted as mentioned above.

180 **Evaluation of H_2S- induced FHB resistance in wheat heads**

181 To further evaluate the FHB resistance regulation by H_2S in wheat, wheat plants at the flowering

182 stage were selected and transplanted under controlled environmental conditions. The wheat was

183 subjected to the treatment described above with an optimum concentration of NaHS (0.3 mM).

184 During this period, four spikes per plant were inoculated with 10 μL of spore suspension

185 ($3.2×10^7$ conidia/mL) of *F. graminearum* by using a syringe. Each treatment was repeated with

186 four spikes, represent disease phenotype of wheat spikes were observed at 4 dpi.

187 **Effect of H_2S on fungal growth**

188 The spore suspension of *F. graminearum* was prepared in accordance with the methods

189 described above. Four aliquots of the spore suspension (4 μL) were placed on 9-cm diameter

190 Petri dishes maintained in sealed 1-L containers (Hu et al., 2014). NaHS solutions, at

191 concentrations of 0, 0.01, 0.075, 0.1, and 0.3 mM were used to fumigate *F. graminearum* at

192 relative humidity of 90%-95% at the bottom of the sealed containers. Spore germination (24 h)

193 and hyphal diameter (3 d) were counted according to the method described by Fu et al. (2014).

194 **Statistical analysis**

195 All data were analysed by IBM SPSS 19.0 and graphed with Origin 8.5. The experimental data

196 values, which were expressed as the mean ± standard error of the mean, were the average of the

197 measurements obtained with triplicate independent assays. The data were evaluated by one-way

198 ANOVA at a 95% confidence level followed by Tukey's test, where differences were considered

199 significant at $P < 0.05$. The data collected from RT-qPCR were analysed using the $2^{-\Delta\Delta C_T}$

200 method.

201 **Results**

202 **Induction of endogenous H_2S content after infection with *F. graminearum***

203 To explore the role of endogenous H_2S in the response to *F. graminearum*, the endogenous H_2S
204 content in wheat was examined. After infection with *F. graminearum*, the endogenous H_2S
205 content increased quickly in comparison with that in the control, and the H_2S content in infected
206 wheat at 12 h was approximately twice that in the control ($p < 0.05$), and it peaked at 24 h
207 (Figure 1). As the disease symptoms increased, the H_2S content in wheat decreased. This result
208 showed that endogenous H_2S was strongly induced by *F. graminearum*, and might play an
209 important role in resistance to *F. graminearum* in wheat.

210 **Effects of NaHS treatments on the resistance of wheat to *F. graminearum***

211 To determine the toxic effects of the H_2S donor NaHS on wheat growth under normal conditions,
212 wheat was watered with solutions containing 0, 0.1, 0.3, 0.5, and 1.0 mM NaHS for 3 d. As
213 shown in Table 1, low concentrations of NaHS (0-0.5 mM) had no significant effect on the
214 growth parameters of wheat, whereas the 1.0 mM NaHS concentration was observed to inhibit
215 wheat growth to a certain extent, especially root length.

216 To explore the effect of external application of H_2S on FHB pathogenicity, a standard method
217 involving inoculation of seedlings (wheat coleoptiles), which has been proven to be a reliable
218 prescreening method for FHB resistance (Li et al., 2019; Shin et al., 2014; Soresi et al., 2015;
219 Wu et al., 2005), was used to assess FHB severity. Photographs of representative wheat seedlings
220 on 7 dpi were taken (Figure 2a). After coleoptile inoculation, the leaves of plants without NaHS
221 application turned yellow with observable fungal hyphae. In addition, a large number of hyphae
222 and brown lesions could be observed in the diseased stems. Figure 2a showed that the application
223 of NaHS at different concentrations (0.0, 0.1, 0.3, and 0.5 mM) reduced the disease symptoms to
224 varying degrees. In comparison with the untreated plants, those treated with 0.5 mM NaHS
225 subgroup showed the maximum decrease (85.85%) in the disease index ($p < 0.001$), followed by
226 an 85.60% decline in the 0.3 mM NaHS subgroup ($p < 0.001$) (Figure 2b). Furthermore, RT-
227 qPCR analysis showed a significantly lower quantity of *F. graminearum* in the sheath of the
228 NaHS/infected groups in comparison with the non-NaHS/infected group (Figure 2c),
229 especially at concentrations of either 0.3 or 0.5 mM ($p < 0.01$). These findings suggest that
230 NaHS could ameliorate the negative effects of *F. graminearum* stress.

231 As shown in Table 2, the non-NaHS/infected group showed a notable decrease in plant height,
232 fresh weight and dry weight, whereas exogenous NaHS inhibited the decline in wheat seedling
233 growth indexes under *F. graminearum* stress. Moreover, an increase in the concentration of
234 NaHS from 0.3 mM to 0.5 mM led to reductions in plant height, root length, and fresh weight.
235 On the basis of these results, we chose a working concentration of 0.3 mM NaHS for the
236 subsequent analyses.

237 To further verify the resistance regulation of H_2S to FHB in wheat, spike inoculated wheat was
238 treated with 0.3 mM NaHS (Figure 2d). The result showed that NaHS significantly reduced FHB

239 severity in spike, which was consistent with the phenomenon in coleoptile. On the basis of these
240 results, we could hypothesise that the application of NaHS may confer resistance to FHB in
241 wheat and result in suppression of fungal propagation.

242 **Protective roles of H_2S in amelioration of _F. graminearum_ stress in wheat**

243 To investigate the effect of H_2S in alleviating disease symptoms induced by NaHS in wheat
244 inoculated with _F. graminearum_, 0.3 mM Na_2SO_4, Na_2SO_3, $NaHSO_4$, $NaHSO_3$, and NaAC were
245 applied in the same experimental system. As shown in the data in Figure 3a, the control groups
246 with Na^+ or sulphur-containing components did not show reduced the disease severity as the
247 NaHS group did, which indicated that H_2S or HS^-, rather than other derivatives, promoted the
248 role of NaHS in heightening FHB resistance in wheat.

249 The endogenous H_2S level in wheat seedlings was measured under various treatment
250 conditions (Figure 3b). Marked modulation of endogenous H_2S content in the results showed that
251 NaHS pre-treatment caused a change in the H_2S content of wheat seedlings. Briefly, the H_2S
252 content in pre-treated plants was higher than that the control plants within 36 h, especially at 6'/0
253 h and 9 h, and almost tripled in comparison with the values in the control group ($p < 0.001$).
254 Meanwhile, the time-course of changes in the endogenous H_2S content in the control group in
255 response to _F. graminearum_ was consistent with the results in Figure 1. These results showed
256 that the increase in endogenous H_2S levels through exogenous application of NaHS could
257 contribute to resistance against _F. graminearum_ in wheat seedlings.

258 **Exogenous H_2S modulated oxidative stress in wheat seedlings under biotic stress conditions**

259 To further understand the mechanism underlying H_2S-induced resistance against _F._
260 _graminearum_, the H_2O_2 and MDA contents were determined. An H_2O_2 burst occurred in the
261 control group after infection, while the H_2O_2 content was notably reduced in the NaHS group
262 (Figure 4a). The H_2O_2 levels increased by approximately 59.6% in the control group ($p < 0.001$),
263 whereas NaHS treatment decreased the H_2O_2 content by 10.4% after 12 h of inoculation ($p <$
264 0.05).

265 MDA, a product of membrane lipid peroxidation, is one of the most commonly used indexes
266 of lipid peroxidation. The MDA content in the control wheat samples increased significantly up
267 to 36 h of inoculation, which was twice that of the treatment group ($p < 0.001$), and subsequently
268 decreased (Figure 4b). In addition, it was lower in the NaHS group than in the control group
269 during the entire treatment period. These results indicated that _F. graminearum_ could cause H_2O_2
270 accumulation and membrane damage in wheat seedlings, while exogenous application of H_2S
271 could relieve the oxidative stress caused by H_2O_2 and MDA.

272 **Effects of H_2S on the activities of the antioxidant enzymes in wheat grown under biotic**
273 **stress**

274 The major antioxidant enzymes include SOD, POD, APX, and CAT, which detoxify reactive

275   oxygen species (ROS) in various plant tissues. In our experiments, the activities of antioxidant
276   enzymes (SOD, POD, APX, and CAT) in the NaHS group were substantially higher than those
277   in the control group during most of the treatment period (Figure 5). SOD activity in the NaHS
278   group and control group increased sharply and reached the maximum level at 48 h, which was
279   22.09% higher than that in the control ($p < 0.01$), and then rapidly declined from 48 h to 72 h
280   (Figure 5a). Additionally, NaHS treatment activated POD activity, which was approximately
281   1.34 times higher than that in the control group at 36 h ($p < 0.01$). APX activity varied similarly
282   in the control and NaHS groups, while APX activity in the NaHS group was higher than that in
283   the control group during the entire treatment period (10'/4 and 36 h, $p < 0.05$), except for 72 h
284   (Figure 5c). Furthermore, there was no obvious difference in CAT activity between the NaHS
285   and control groups at the beginning. This gap narrowed from 0 to 12 h, after which CAT activity
286   in the NaHS group increased faster than that in the control group, and was 1.71 times the activity
287   in the control group at 72 h ($p < 0.01$) (Figure 5d). Unambiguously, these results demonstrated
288   that alleviation of oxidative stress by H_2S in wheat under FHB stress was closely related to
289   improvement of antioxidant system activities in wheat seedlings.

290   **Activation effects of H_2S on defence response genes**
291   Defence response genes, including genes encoding pathogenesis-related proteins (*PRs*), are
292   involved in the resistance response to many plant-pathogen interactions (Hammond-Kosack &
293   Jones, 1996; Pritsch et al., 2000; Pritsch et al., 2001; Stevens et al., 1996). To examine the
294   association between H_2S-induced resistance and *PR* gene expression in wheat, we conducted a
295   quantitative analysis for the *PR1.1*, *PR2*, *PR3* and *PR4* genes after NaHS application (Figure 6).
296   The results showed that H_2S upregulated the transcription levels of *PR1.1*, *PR2*, *PR3* and *PR4*,
297   which peaked at 12 h and 48 h, with the degree of between 2 and 4 times (*PR1.1*, *PR2*, and *PR3*,
298   peaked at 12 h, $p < 0.001$; *PR4*, peaked at 48 h, $p < 0.05$). Moreover, the relative expression
299   levels of *PRs* in the NaHS group were higher than those in the control group within 48 h. Thus,
300   our results demonstrated that exogenous H_2S improved disease resistance against *F.*
301   *graminearum* by advancing the accumulation of defence-related genes.

302   **Effects of H_2S on mycelial growth and spore germination**
303   To further understand the mechanism underlying the regulatory effects of H_2S in resistance to *F.*
304   *graminearum* in wheat, the effects of H_2S on mycelial growth and spore germination were
305   investigated (Figure 7). As shown in Figure 7, with increasing NaHS concentration, the mycelial
306   diameter of *F. graminearum* on PDA decreased, and mycelial growth was inhibited by 37.66%
307   even at 0.075 mM NaHS treatment ($p < 0.001$) (Figure 7b). At 0.3 mM NaHS, no signs of
308   mycelial growth were observed. Moreover, in our assessment of spore germination after 24-h
309   incubation, and most of the fungal spores germinated at 0.01 mM. However, treatment with 0.1
310   mM NaHS completely restrained spore germination of *F. graminearum* (Figure 7a, c). These

311 data showed that exposure to H_2S notably inhibited mycelial growth and spore germination of *F.*
312 *graminearum* in a dose-dependent manner, suggesting that H_2S had an effectively fungicidal role.

313 Discussion

314 Application of exogenous H_2S reduced FHB symptoms in wheat

315 *Fusarium* diseases resulted in severe economic losses for the production of cereal crops. Global
316 climate change has increased the contribution of weather conditions to epidemics of wheat FHB,
317 and control of this disease has proven to be exceedingly challenging (Zhang et al., 2014). H_2S is
318 an essential signalling molecule that exhibits positive effects on numerous physiological
319 functions, including those serving against environmental stress and pathogens. Treatments of
320 H_2S combined with silicon have been reported to alleviate the negative effects of drought stress
321 and rust infection in wheat (Naz et al., 2021). Our study demonstrated that exogenous H_2S
322 alleviated FHB symptom in wheat seedlings, enhancing the plant defense mechanism and
323 inhibiting fungal growth.

324 In this study, H_2S content was conspicuously induced after *F. graminearum* infection,
325 suggesting that H_2S may be partly responsible for the defence response to *F. graminearum* in
326 wheat (Figure 1). This result was supported by previous studies in *Arabidopsis* showing that the
327 endogenous H_2S content increased significantly after pathogen infection (Shi et al., 2015).
328 Nevertheless, previous to this study, the role of H_2S in wheat resistance to FHB was not clear.

329 Coleoptile inoculation method can be used as a rapid and reliable method for FHB
330 pathogenicity research, and it has been described in many studies in the literature (Wu et al.,
331 2005; Yang et al., 2018). Comparative pathogenic analyses indicated that the application of
332 NaHS effectively reduced the disease symptoms of wheat seedlings in a dose-dependent manner,
333 and at an optimal concentration of 0.3 mM, the FHB severity of wheat spikes was also alleviated
334 (Figure 2). NaHS is a commonly used H_2S donor in biological systems that decomposes into Na^+
335 and HS^- in solution, of which HS^- combines with H^+ to generate H_2S (Zhang et al., 2010). The
336 role of NaHS as an H_2S donor, in reducing the negative effects of *F. graminearum* in wheat was
337 supported by two sets of evidence. First, only NaHS could effectively reduce the FHB severity of
338 wheat in the solutions we tested. Moreover, as shown in Table 1, NaHS itself did not negatively
339 affect seedling growth at low concentrations, implying that NaHS counteracts the pathogen-
340 induced stresses in plants in a way that was independent of any "cross-tolerance." On the other
341 hand, wheat seedlings treated with NaHS showed markedly higher the levels of extractable H_2S
342 compared with the control (Figure 3). These data provided new insights, hinting at a possible
343 role of H_2S in the plant defence signalling of wheat against *F. graminearum*.

344 H_2S enhanced the resistance of wheat to *F. graminearum* by enhancing the defense
345 mechanism

346   ROS, especially H_2O_2, are known to be important in plant-pathogen interactions. Low levels of

347   H_2O_2 are essential for peroxidase-dependent lignification, which hinders penetration by a

348   pathogen. However, necrotrophic pathogens induced H_2O_2 at toxic levels, which could lead to

349   cell death, and the pathogens could use these dead cells as an entry point into plants or obtain

350   nutrients from dead cells for further invasion and growth (Hückelhoven, 2007). Correspondingly,

351   during the process of infection and colonisation of grain hosts, *Fusarium* causes oxidative stress

352   in the host by secreting toxic levels of ROS including H_2O_2, which acts as weapons to attack

353   plants (Walter, Nicholson & Doohan, 2010). In this study, *F. graminearum*-infected wheat

354   showed a marked increase in H_2O_2 levels, and this increase was mitigated by treatment with

355   NaHS (Figure 4). Furthermore, considering the MDA content, control plants showed elevated

356   MDA levels after infection, which was likely connected with the remarkable accumulation of

357   H_2O_2, implied that rapid oxidative explosion destroyed membranes. Plants receiving NaHS

358   treatment showed significantly lower post-infection MDA levels in comparison with the control

359   group, indicating decreased ROS levels, and a reduced degree of membrane peroxidation. Taken

360   together, these data support the idea that H_2S appears to be an antioxidant signalling molecule

361   participating in the resistance against *F. graminearum* in wheat.

362   To further examine the role of NaHS in relieving ROS toxicity, the activities of various

363   antioxidant enzymes, including SOD, POD, APX, and CAT, were evaluated. As shown in Figure

364   5, the activities of SOD, POD, APX, and CAT were upgraded by NaHS treatment, indicating that

365   H_2S could enhance the activities of antioxidant enzymes to scavenge ROS, which was consistent

366   with findings showing H_2S attenuated the rot of freshly cut pears by enhancing the antioxidant

367   system to exterminate excessive ROS (Hu et al., 2014). *In vitro* assays showed that APX and

368   CAT were targets of persulfidation, which were positively modulated by persulfidation in the

369   presence of H_2S (Corpas, 2019). Moreover, exogenous H_2S treatment has also been shown to

370   relieve the ROS burst and cell damage induced by drought, salinity, temperature, and heavy

371   metals in plants (Corpas, 2019). The present data also speculated that the resistance of the FHB-

372   resistant variety 'Vulkan' was associated with antioxidative enzyme activity (Spanic et al., 2017).

373   These results may partly account for the effects of H_2S treatment in inducing resistance to *F.*

374   *graminearum* in wheat.

375   *PRs* are related to the development of systemic acquired resistance (SAR) against further

376   infection enforced by pathogeny. Additionally, published papers have described that the genes

377   encoding *PRs* in wheat were activated after *F. graminearum* attack (Pritsch et al., 2000; Pritsch

378   et al., 2001). However, physiological concentrations of H_2S have been shown to upregulate the

379   expression levels of disease-related genes (Shi et al., 2015). For example, the auxin signalling

380   pathway mediated by MIR393 includes MIR393a/b and its target genes (*TIR1*, *AFB1*, *AFB2*, and

381   *AFB3*), which were all transcriptionally regulated by H_2S (Shi et al., 2015). In this study, we

382 noted that, the *PR* gene transcript accumulation in NaHS-treated wheat was greater than that in
383 control plants (Figure 6a-d). Taken together, these results could indicate that H_2S displayed the
384 ability to enhance the expression of defence response genes, thereby possibly contributing to
385 greater disease resistance in wheat.

386 **The effects of H_2S on the growth of *F. graminearum in vitro***
387 We also studied the toxicity of H_2S to *F. graminearum*, and found that H_2S released by 0.3 mM
388 NaHS hindered the development of *F. graminearum* during the testing period (Figure 7a-c). Our
389 results suggested that H_2S exerted its antifungal effect by influencing various aspects of fungal
390 development, including inhibition of spore germination and retardation mycelial growth. Similar
391 effects of H_2S have been confirmed by Fu et al. (2014) and Hu et al. (2014).

392 **The potential application of H_2S in wheat FHB in the future.**
393 Our experiments showed that H_2S could be a potential superior candidate for controlling FHB.
394 NaHS has been widely used as the rapid H_2S donor that generates a large amount of H_2S over a
395 short time period (Sun et al., 2017). The rapid generation and loss of H_2S limit its application in
396 agriculture. Controlled release of H_2S could make it safer and more cost-effective to use. With
397 this in mind, Lee et al. (2011) previously reported that the slow-releasing H_2S donor, GYY4137,
398 exhibited novel anti-cancer effects *in vitro* and *in vivo*. Likewise, in the work of Liu et al. (2021),
399 they provided an infrared-light-responsive pesticide delivery system to intentionally regulate
400 imidacloprid release and enhance utilization efficiency. Hence, it would be valuable to develop a
401 delivery system that could be manipulated to achieve sustained release of H_2S from its donor
402 NaHS. In the future, reformulated NaHS could be applied to plants or sprayed directly into the
403 soil to inhibit the fungal, which might have an application value.

404 ## Conclusions

405 In conclusion, irrespective of the inoculation methods, the application of H_2S released by 0.3
406 mM NaHS could significantly alleviate FHB symptoms in wheat. H_2S enhanced the resistance of
407 wheat to *F. graminearum* by enhancing the defence mechanism (i) promotion of the activities of
408 antioxidant enzymes with decreasing the amount of H_2O_2 and MDA and (ii) induction of the
409 expression of disease response genes. On the other hand, H_2S was capable of inhibiting the
410 development of the pathogen. On the basis of these data, we have provided a theoretical basis for
411 H_2S-mediated resistance to *F. graminearum* in wheat.

412 ## Acknowledgments

413 We are grateful to Xian Shu, Chinese Academy of Sciences for his suggestions and efforts in
414 revising of the manuscript.

415 ## Author Contributions

416 YYY and CGT thoroughly discussed and designed this study. YYY and CGT were in charge of
417 the investigation, data collection and manuscript writing. WJK, PFS, YZ, WLZ, JFX and DCW
418 helped with data curation and formal analysis. LFW provided funding and managed the project.

Abbreviations

420 RT-qPCR, real-time quantitative PCR; H_2S, hydrogen sulfide; MDA, malondialdehyde; FHB,
421 *Fusarium* head blight; *F. graminearum*, *Fusarium graminearum*; ROS, reactive oxygen species;
422 H_2O_2, hydrogen peroxide; SA, salicylic acid; PRs, pathogenesis-related proteins; CMC,
423 Carboxymethyl cellulose; NaHS, sodium hydrosulfide; mM, mmol/L; DI, disease index; SOD,
424 superoxide dismutase; CAT, catalase; PDA, potato dextrose agar PDA; dps, days post sowing;
425 dpi, days post inoculation; APX, ascorbate peroxidase; POD, peroxidase.

Fundings

427 This work was supported by Major Special Project of Anhui Province, China
428 (202103a06020002), the Grant of the President Foundation of Hefei Institutes of Physical
429 Science of Chinese Academy of Sciences, China (YZJJ2020QN29).

Declaration of competing interest

431 Authors declare that there are no conflicts of interest.

Appendix A. Supplementary data

433 The following are the Supplementary data to this article:

434

435 **REFERENCE**

436 Becher, R., Hettwer, U., Karlovsky, P., Deising, H.B., Wirsel, S.G.R. 2010. Adaptation of *Fusarium*

437   *graminearum* to Tebuconazole Yielded Descendants Diverging for Levels of Fitness, Fungicide Resistance,

438   Virulence, and Mycotoxin Production. *Phytopathology®* 100: 444-453 Doi: 10.1094/PHYTO-100-5-0444.

439 Buhrow, L.M., Cram, D., Tulpan, D., Foroud, N.A., Loewen, M.C. 2016. Exogenous Abscisic Acid and

440   Gibberellic Acid Elicit Opposing Effects on *Fusarium graminearum* Infection in Wheat. *Phytopathology®*

441   106: 986-996 Doi: 10.1094/PHYTO-01-16-0033-R.

442 Corpas, F.J. 2019. Hydrogen Sulfide: A New Warrior against Abiotic Stress. *Trends in Plant Science* 24: 983-

443   988 Doi: 10.1016/j.tplants.2019.08.003.

444 Deng, Y.-Q., Bao, J., Yuan, F., Liang, X., Feng, Z.-T., Wang, B.-S. 2016. Exogenous hydrogen sulfide

445   alleviates salt stress in wheat seedlings by decreasing Na^+ content. *Plant Growth Regulation* 79: 391-399

446   Doi: 10.1007/s10725-015-0143-x.

447 Duan, Y., Zhang, X., Ge, C., Wang, Y., Cao, J., Jia, X., Wang, J., Zhou, M. 2014. Development and

448   application of loop-mediated isothermal amplification for detection of the F167Y mutation of

449   carbendazim-resistant isolates in *Fusarium graminearum*. *Scientific Reports* 4: 7094 Doi:

450   10.1038/srep07094.

451 Fu, L.H., Hu, K.D., Hu, L.Y., Li, Y.H., Hu, L.B., Yan, H., Liu, Y.S., Zhang, H. 2014. An antifungal role of

452   hydrogen sulfide on the postharvest pathogens *Aspergillus niger* and *Penicillium italicum*. *PLoS One* 9:

453   e104206 Doi: 10.1371/journal.pone.0104206.

454 Ghimire, B., Sapkota, S., Bahri, B.A., Martinez-Espinoza, A.D., Buck, J.W., Mergoum, M. 2020. *Fusarium*

455   Head Blight and Rust Diseases in Soft Red Winter Wheat in the Southeast United States: State of the Art,

456   Challenges and Future Perspective for Breeding. *Frontiers in Plant Science* 11: 1080 Doi:

457   10.3389/fpls.2020.01080.

458 Hammond-Kosack, K.E., Jones, J.D. 1996. Resistance gene-dependent plant defense responses. *The Plant Cell*

459   8: 1773-1791 Doi: 10.1105/tpc.8.10.1773.

460 Hao, Y., Rasheed, A., Zhu, Z., Wulff, B.B.H., He, Z. 2020. Harnessing Wheat *Fhb1* for *Fusarium* Resistance.

461   *Trends in Plant Science* 25: 1-3 Doi: 10.1016/j.tplants.2019.10.006.

462 Hosoki, R., Matsuki, N., Kimura, H. 1997. The Possible Role of Hydrogen Sulfide as an Endogenous Smooth

463   Muscle Relaxant in Synergy with Nitric Oxide. *Biochemical and Biophysical Research Communications*

464   237: 527-531 Doi: 10.1006/bbrc.1997.6878.

465 Hu, K.D., Wang, Q., Hu, L.Y., Gao, S.P., Wu, J., Li, Y.H., Zheng, J.L., Han, Y., Liu, Y.S., Zhang, H. 2014.

466   Hydrogen sulfide prolongs postharvest storage of fresh-cut pears (*Pyrus pyrifolia*) by alleviation of

467   oxidative damage and inhibition of fungal growth. *PLoS One* 9: e85524. Doi:

468   10.1371/journal.pone.0085524.

469 Hückelhoven, R. 2007. Cell Wall–Associated Mechanisms of Disease Resistance and Susceptibility. *Annual*

470   *Review of Phytopathology* 45: 101-127 Doi: 10.1146/annurev.phyto.45.062806.094325.

471   Jia, H., Zhou, J., Xue, S., Li, G., Yan, H., Ran, C., Zhang, Y., Shi, J., Jia, L., Wang, X., Luo, J., Ma, Z. 2018.

472   A journey to understand wheat *Fusarium* head blight resistance in the Chinese wheat landrace

473   Wangshuibai. *The Crop Journal* 6: 48-59 Doi:10.1016/j.cj.2017.09.006.

474   Lee, Z.W., Zhou, J., Chen, C.-S., Zhao, Y., Tan, C.-H., Li, L., Moore, P.K., Deng, L.-W. 2011. The Slow-

475   Releasing Hydrogen Sulfide Donor, GYY4137, Exhibits Novel Anti-Cancer Effects *In Vitro* and *In Vivo*.

476   *PLoS One* 6: e21077 Doi:10.1371/journal.pone.0021077.

477   Li, J., Duan, Y., Bian, C., Pan, X., Yao, C., Wang, J., Zhou, M. 2019. Effects of validamycin in controlling

478   *Fusarium* head blight caused by *Fusarium graminearum*: Inhibition of DON biosynthesis and induction of

479   host resistance. *Pesticide Biochemistry and Physiology* 153: 152-160 Doi: 10.1016/j.pestbp.2018.11.012.

480   Liu, B., Zhang, J., Chen, C., Wang, D., Tian, G., Zhang, G., Cai, D., Wu, Z. 2021. Infrared-Light-Responsive

481   Controlled-Release Pesticide Using Hollow Carbon Microspheres@Polyethylene Glycol/α-Cyclodextrin

482   Gel. *Journal of Agricultural and Food Chemistry* 69: 6981-6988 Doi: 10.1021/acs.jafc.1c01265.

483   Ma, D., Ding, H., Wang, C., Qin, H., Han, Q., Hou, J., Lu, H., Xie, Y., Guo, T. 2016. Alleviation of Drought

484   Stress by Hydrogen Sulfide Is Partially Related to the Abscisic Acid Signaling Pathway in Wheat. *PLoS*

485   *One* 11: e0163082 Doi: 10.1371/journal.pone.0163082.

486   McMullen, M., Bergstrom, G., De Wolf, E., Dill-Macky, R., Hershman, D., Shaner, G., Van Sanford, D. 2012.

487   A Unified Effort to Fight an Enemy of Wheat and Barley: *Fusarium* Head Blight. *Plant Disease* 96: 1712-

488   1728 Doi: 10.1094/PDIS-03-12-0291-FE.

489   McMullen, M., Jones, R., Gallenberg, D. 1997. Scab of Wheat and Barley: A Re-emerging Disease of

490   Devastating Impact. *Plant Disease* 81: 1340-1348 Doi:10.1094/PDIS.1997.81.12.1340.

491   Mesterházy, Á., Bartók, T., Lamper, C. 2003. Influence of Wheat Cultivar, Species of *Fusarium*, and Isolate

492   Aggressiveness on the Efficacy of Fungicides for Control of *Fusarium* Head Blight. *Plant Disease* 87:

493   1107-1115 Doi: 10.1094/PDIS.2003.87.9.1107.

494   Naz, R., Batool, S., Shahid, M., Keyani, R., Yasmin, H., Nosheen, A., Hassan, M.N., Mumtaz, S., Siddiqui,

495   M.H. 2021. Exogenous silicon and hydrogen sulfide alleviates the simultaneously occurring drought stress

496   and leaf rust infection in wheat. *Plant Physiology and Biochemistry* 166: 558-571 Doi:

497   10.1016/j.plaphy.2021.06.034.

498   Nicholson, P., Simpson, D.R., Weston, G., Rezanoor, H.N., Lees, A.K., Parry, D.W., Joyce, D. 1998.

499   Detection and quantification of *Fusarium culmorum* and *Fusarium graminearum* in cereals using PCR

500   assays. *Physiological and Molecular Plant Pathology* 53: 17-37 Doi:10.1006/pmpp.1998.0170.

501   Pritsch, C., Muehlbauer, G.J., Bushnell, W.R., Somers, D.A., Vance, C.P. 2000. Fungal Development and

502   Induction of Defense Response Genes During Early Infection of Wheat Spikes by *Fusarium graminearum*.

503   *Molecular Plant-Microbe Interactions®* 13: 159-169 Doi: 10.1094/MPMI.2000.13.2.159.

504   Pritsch, C., Vance, C.P., Bushnell, W.R., Somers, D.A., Hohn, T.M., Muehlbauer, G.J. 2001. Systemic

505   expression of defense response genes in wheat spikes as a response to *Fusarium graminearum* infection.

506   *Physiological and Molecular Plant Pathology* 58: 1-12 doi:10.1006/pmpp.2000.0308.

507 Qiu, J., Xu, J., Shi, J. 2019. *Fusarium* Toxins in Chinese Wheat since the 1980s. *Toxins* 11: 248 Doi:

508   10.3390/toxins11050248.

509 Sekiya, J., Schmidt, A., Wilson, L.G., Filner, P. 1982. Emission of Hydrogen Sulfide by Leaf Tissue in

510   Response to L-Cysteine 1. *Plant Physiology* 70: 430-436 Doi: 10.1104/pp.70.2.430.

511 Shan, C. J., Zhang, S. L., Li, D. F., Zhao, Y. Z., Tian, X. L., Zhao, X. L., Wu, Y.X., Wei, X.Y., Liu, R. Q.

512   2011. Effects of exogenous hydrogen sulfide on the ascorbate and glutathione metabolism in wheat

513   seedlings leaves under water stress. *Acta Physiologiae Plantarum* 33: 2533-2540 doi: 10.1007/s11738-

514   011-0746-4.

515 Shi, H., Ye, T., Han, N., Bian, H., Liu, X., Chan, Z. 2015. Hydrogen sulfide regulates abiotic stress tolerance

516   and biotic stress resistance in *Arabidopsis*. *Journal of Integrative Plant Biology* 57: 628-640 doi:

517   10.1111/jipb.12302.

518 Shin, S., Kim, K.-H., Kang, C.-S., Cho, K.-M., Park, C.S., Okagaki, R., Park, J.-C. 2014. A Simple Method for

519   the Assessment of *Fusarium* Head Blight Resistance in Korean Wheat Seedlings Inoculated with *Fusarium*

520   *graminearum*. *The Plant Pathology Journal* 30 25-32. Doi: 10.5423/PPJ.OA.06.2013.0059.

521 Soresi, D., Zappacosta, D., Garayalde, A., Miranda, R., Carrera, A. 2015. An in vitro assay for pre-screening

522   resistance to *Fusarium* head blight in durum wheat. *Phytopathologia Mediterranea* 54: 253-264 Doi:

523   10.14601/Phytopathol_Mediterr-14986.

524 Spanic, V., Viljevac Vuletic, M., Abicic, I., Marcek, T. 2017. Early response of wheat antioxidant system with

525   special reference to *Fusarium* head blight stress. *Plant Physiology and Biochemistry* 115: 34-43 Doi:

526   10.1016/j.plaphy.2017.03.010.

527 Stevens, C., Titarenko, E., Hargreaves, J.A., Gurr, S.J. 1996. Defence-related gene activation during an

528   incompatible interaction between *Stagonospora* (*Septoria*) *nodorum* and barley (*Hordeum vulgare* L.)

529   coleoptile cells. *Plant Molecular Biology* 31: 741-749 Doi: 10.1007/BF00019462.

530 Su, Z., Bernardo, A., Tian, B., Chen, H., Wang, S., Ma, H., Cai, S., Liu, D., Zhang, D., Li, T., Trick, H., St.

531   Amand, P., Yu, J., Zhang, Z., Bai, G. 2019. A deletion mutation in *TaHRC* confers *Fhb1* resistance to

532   *Fusarium* head blight in wheat. *Nature Genetics* 51: 1099-1105 Doi: 10.1038/s41588-019-0425-8.

533 Sun, X., Wang, W., Dai, J., Jin, S., Huang, J., Guo, C., Wang, C., Pang, L., Wang, Y. 2017. A Long-Term and

534   Slow-Releasing Hydrogen Sulfide Donor Protects against Myocardial Ischemia/Reperfusion Injury.

535   *Scientific Reports* 7: 3541 Doi: 10.1038/s41598-017-03941-0.

536 Tang, C., Li, M., Cao, M., Lu, R., Zhang, H., Liu, C., Huang, S., Zhang, P., Hu, H., Zhao, W., Wu, L. 2020.

537   Transcriptome analysis suggests mechanisms for a novel flowering type: Cleistogamous wheat. *The Crop*

538   *Journal* 8: 313-326 Doi: 10.1016/j.cj.2019.08.009.

539 Walter, S., Nicholson, P., Doohan, F.M. 2010. Action and reaction of host and pathogen during *Fusarium* head

540   blight disease.   *New Phytologist* 185: 54-66 Doi: 10.1111/j.1469-8137.2009.03041.x.

541 Wang, H., Sun, S., Ge, W., Zhao, L., Hou, B., Wang, K., Lyu, Z., Chen, L., Xu, S., Guo, J., Li, M., Su, P., Li,

542   X., Wang, G., Bo, C., Fang, X., Zhuang, W., Cheng, X., Wu, J., Dong, L., Chen, W., Li, W., Xiao, G.,

543   Zhao, J., Hao, Y., Xu, Y., Gao, Y., Liu, W., Liu, Y., Yin, H., Li, J., Li, X., Zhao, Y., Wang, X., Ni, F., Ma,

544   X., Li, A., Xu, S.S., Bai, G., Nevo, E., Gao, C., Ohm, H., Kong, L. 2020. Horizontal gene transfer of *Fhb7*

545   from fungus underlies *Fusarium* head blight resistance in wheat. *Science* 368: eaba5435 Doi:

546   10.1126/science.aba5435.

547 Wang, R. 2002. Two's company, three's a crowd: can H$_2$S be the third endogenous gaseous transmitter? *The*

548   *FASEB Journal* 16: 1792-1798 Doi: 10.1096/fj.02-0211hyp.

549 Wu, A.B., Li, H.P., Zhao, C.S., Liao, Y.C. 2005. Comparative pathogenicity of *Fusarium graminearum*

550   isolates from China revealed by wheat coleoptile and floret inoculations. *Mycopathologia* 160: 75-83 Doi:

551   10.1007/s11046-005-1153-4.

552 Yang, M., Qin, B.P., Ma, X.L., Wang, P., Li, M.L., Chen, L.L., Chen, L.T., Sun, A.Q., Wang, Z.L., Yin, Y.P.

553   2016. Foliar application of sodium hydrosulfide (NaHS), a hydrogen sulfide (H$_2$S) donor, can protect

554   seedlings against heat stress in wheat (*Triticum aestivtrue* L.). *Journal of Intergrative Agricultrue* 15: 2745-

555   2758 Doi: 10.1016/S2095-3119(16)61358-8.

556 Yang, Y., Li, M.X., Duan, Y.B., Li, T., Shi, Y.Y., Zhao, D.L., Zhou, Z.H., Xin, W.J., Wu, J., Pan, X.Y., Li,

557   Y.J., Zhu, Y.Y., Zhou, M.G. 2018. A new point mutation in β2-tubulin confers resistance to carbendazim

558   in *Fusarium asiaticum*. *Pesticide Biochemstry and Physiology* 145: 15-21 Doi:

559   10.1016/j.pestbp.2017.12.006.

560 Zhang, H., Hu, L.Y., Hu, K.D., He, Y.D., Wang, S.H., Luo, J.P. 2008. Hydrogen Sulfide Promotes Wheat Seed

561   Germination and Alleviates Oxidative Damage against Copper Stress. *Journal of Integrative Plant Biology*

562   50: 1518-1529. Doi: 10.1111/j.1744-7909.2008.00769.x.

563 Zhang, H., Hu, L.Y., Li, P., Hu, K.D., Jiang, C.X., Luo, J.P. 2010a. Hydrogen sulfide alleviated chromium

564   toxicity in wheat. *Biologia Plantarum* 54: 743-747 Doi: 10.1007/s10535-010-0133-9.

565 Zhang, H., Tan, Z.Q., Hu, L.Y., Wang, S.H., Luo, J.P., Jones, R.L. 2010b. Hydrogen Sulfide Alleviates

566   Aluminum Toxicity in Germinating Wheat Seedlings. *Journal of Integrative Plant Biology* 52: 556-567

567   Doi: 10.1111/j.1744-7909.2010.00946.x.

568 Zhang, X., Halder, J., White, R.P., Hughes, D.J., Ye, Z., Wang, C., Xu, R., Gan, B., Fitt, B.D.L. 2014. Climate

569   change increases risk of *Fusarium* ear blight on wheat in central China. *Annals of Appllied Biology* 164:

570   384-395 Doi: 10.1111/aab.12107.

571 Zhou, M., Ye, Z., Liu, J. 1994. Progress of fungicide resistance research. *Journal of Nanjing Agricultral*

572   *University* 17: 33-41 Doi: 10.7685/j.issn.1000-2030.1994.03.005.

573

Effects of NaHS treatment on wheat growth index under normal conditions.

1 **Table 1.** Effects of NaHS treatment on wheat growth index under normal conditions.

Concentration of NaHS treatment (mM)	0.0	0.1	0.3	0.5	1.0
Plant height (cm)	12.033 ab (100)	12.544 a (104)	12.402 a (103)	11.969 ab (99.5)	11.120 b (92.4)
Root length (cm)	16.591 a (100)	16.278 a (98.1)	16.313 a (98.3)	14.313 a (86.3)	11.574 b (69.8)
Fresh weight (mg/plant)	260.484 a (100)	265.790 a (102)	268.873 a (103)	258.920 a (99.4)	229.436 b (88.1)
Dry weight (mg/plant)	31.456 a (100)	32.900 a (104)	33.209 a (105)	33.633 a (107)	31.553 a (100)

2 **Note:** Two-day-old wheat seedlings were culture in 0.0 (control), 0.1, 0.3, 0.5, and 1.0 mM NaHS

3 respectively for 3 days, then the growth index was investigated on 9 dps (days post sowing). The

4 data are presented as the mean $\pm$ SD (n=15); a, b and c mean significant differences at $P<0.05$ for

5 the same row; The data shown in brackets is the percentages of the control group.

Effect of exogenous NaHS on wheat growth parameters under stressed conditions.

1 **Table 2.** Effect of exogenous NaHS on wheat growth parameters under stressed conditions.

Concentration of NaHS treatment (mM)	CK	0.0	0.1	0.3	0.5
Plant height (cm)	12.103 a (100)	10.827 b (89.5)	11.173 ab (92.3)	11.357 ab (93.8)	10.893 b (90)
Root length (cm)	12.627 a (100)	11.400 a (90.3)	12.077 a (95.6)	11.903 a (94.3)	11.357 a (90)
Fresh weight (mg/plant)	283.109 ab (100)	256.769 b (90.7)	283.251 ab (100)	288.367 a (101.9)	274.209 ab (96.9)
Dry weight (mg/plant)	32.920 a (100)	28.949 b (87.9)	30.760 ab (93.4)	31.387 ab (95.3)	31.696 ab (96.3)

2 **Note:** The growth index was investigated on 7 dpi. CK:healthy control. The data are presented as

3 the mean ± SD (n = 15); a, b and c mean significant differences at $P < 0.05$ for the same row; The

4 data shown in brackets is the percentages of the control group.

figure captions

1 **Figure captions**

2 **Figure 1.** The content of H_2S in wheat seedlings at different time points (0, 12, 24, 36, 48, and

3 96 h) after infected with *F. graminearum*. Wheat uninfected with *F. graminearum* served as

4 control group. Inoculation was administered to two-day-old wheat. Data represent means ± S.D.

5 of three replicate samples. Different letters mean significance of difference between the

6 treatments according to Tukey's multiple range test at $P < 0.05$.

7 **Figure 2.** Effects of different concentrations of NaHS treatment on wheat disease resistance to *F.*

8 *graminearum*. (a) Disease phenotype of wheat seedlings on 7 dpi. (b) Disease score of wheat

9 seedlings on 7 dpi. (c) qPCR analysis showing levels of relative fungal DNA in wheat seedlings.

10 (d) Disease phenotype of wheat spike at 4 dpi. CK:healthy control that uninfected with *F.*

11 *graminearum*. Data represent means ± S.D. of three replicate samples. Different letters mean

12 significance of difference between the treatments according to Tukey's multiple range test at $P <$

13 0.05.

14 **Figure 3.** Effects of H_2S on wheat resistance to *F. graminearum* through NaHS treatment. (a)

15 The protective roles of NaHS on amelioration of FHB stress in wheat could be attributed to H_2S

16 or HS^-. Seedlings were pretreated with H_2O (CK), 0.3 mM NaHS, Na_2SO_4, Na_2SO_3, $NaHSO_4$,

17 $NaHSO_3$, and NaAC, respectively for 6 h, and subsequently subjected to FHB stress, then

18 watered after 3 days of each compound incubation, disease index on 7 dpi was investigated. (b)

19 Effect of NaHS treatment on endogenous H_2S content in wheat seedlings exposed to FHB stress.

20 After 6 h of pretreatment, seedlings were inoculated with *F. graminearum*. 0' represented

21 seedlings sample without NaHS treatment; 6'/0 represented the samples from the seedlings

22 pretreated with water (Control, CK) or NaHS (0.3 mM) after 6 h, and at the time point, subjected

23 to inoculation with *F. graminearum*; and 9, 18 and 36 as samples of the seedlings inoculated

24 with *F. graminearum* for 9, 18 and 36 h respectively. Data represent means ± S.D. of three

25 replicate samples. Different letters mean significance of difference between the treatments

26 according to Tukey's multiple range test at $P < 0.05$.

27 **Figure 4.** Effect of H_2S on the contents of hydrogen (H_2O_2) (a) and malonaldehyde (MDA) (b)

28 in wheat seedlings. 0' represented seedlings sample without NaHS treatment; 10'/4 represented

29 the samples from the seedlings pretreated with water (Control, CK) or NaHS (0.3 mM) after 10

30 h, and at the time point, 4 h post inoculation; and 12, 36 and 48 as samples of the seedlings

31 inoculated with *F. graminearum* for 12, 36 and 48 respectively. Data represent means ± S.D. of

32 three replicate samples.

33 **Figure 5.** Effects of H_2S on activities of antioxidant enzymes (a) SOD activity, (b) POD activity,

34 (c) APX activity, and (d) CAT activity. 0' represented seedlings sample without NaHS

35 treatment; 10'/4 represented the samples from the seedlings pretreated with water (Control, CK)

36   or NaHS (0.3 mM) after 10 h, and at the time point, 4 h post inoculation; and 12, 36, 48, and 72

37   as samples of the seedlings inoculated with *F. graminearum* for 12, 36, 48, and 72 h

38   respectively. Data represent means ± S.D. of three replicate samples.

39   **Figure 6.** Effects of H$_2$S treatment on defense-related gene (a) *PR1.1*, (b) *PR2*, (c) *PR3*, (d)*PR4*.

40   Significant differences between 0.3 mM NaHS treatment group and control group were

41   compared by Tukey's multiple range test. Significant difference at $P < 0.001$, $P < 0.01$ and $P<$

42   0.05 were marked as triple (***), double (**) and single (*), respectively. Data represent means

43   ± S.D. of three replicate samples.

44   **Figure 7.** H$_2$S effects development of *F. graminearum* on potato dextrose agar (PDA) (a)

45   Mycelium growth of *F. graminearum*, which were cultured on medium and subjected to different

46   concentrations of NaHS solutions for 3 days. Lower panel represents micro-morphology of *F.*

47   *graminearum*, which were cultured on medium and subjected to different concentrations of

48   NaHS solutions for 24 h. (b) Hyphae diameter of fungi clones (culture for 3 d). (c) Spore

49   germination of *F. graminearum* (culture 24 h). Data represent means ± S.D. of three replicate

50   samples. Different letters mean significance of difference between the treatments according to

51   Tukey's multiple range test at $P < 0.05$.

52

53

Figure 1

The content of H$_2$S in wheat at different time points (0, 12, 24, 36, 48, and 96 h) after infected by *F. graminearum*.
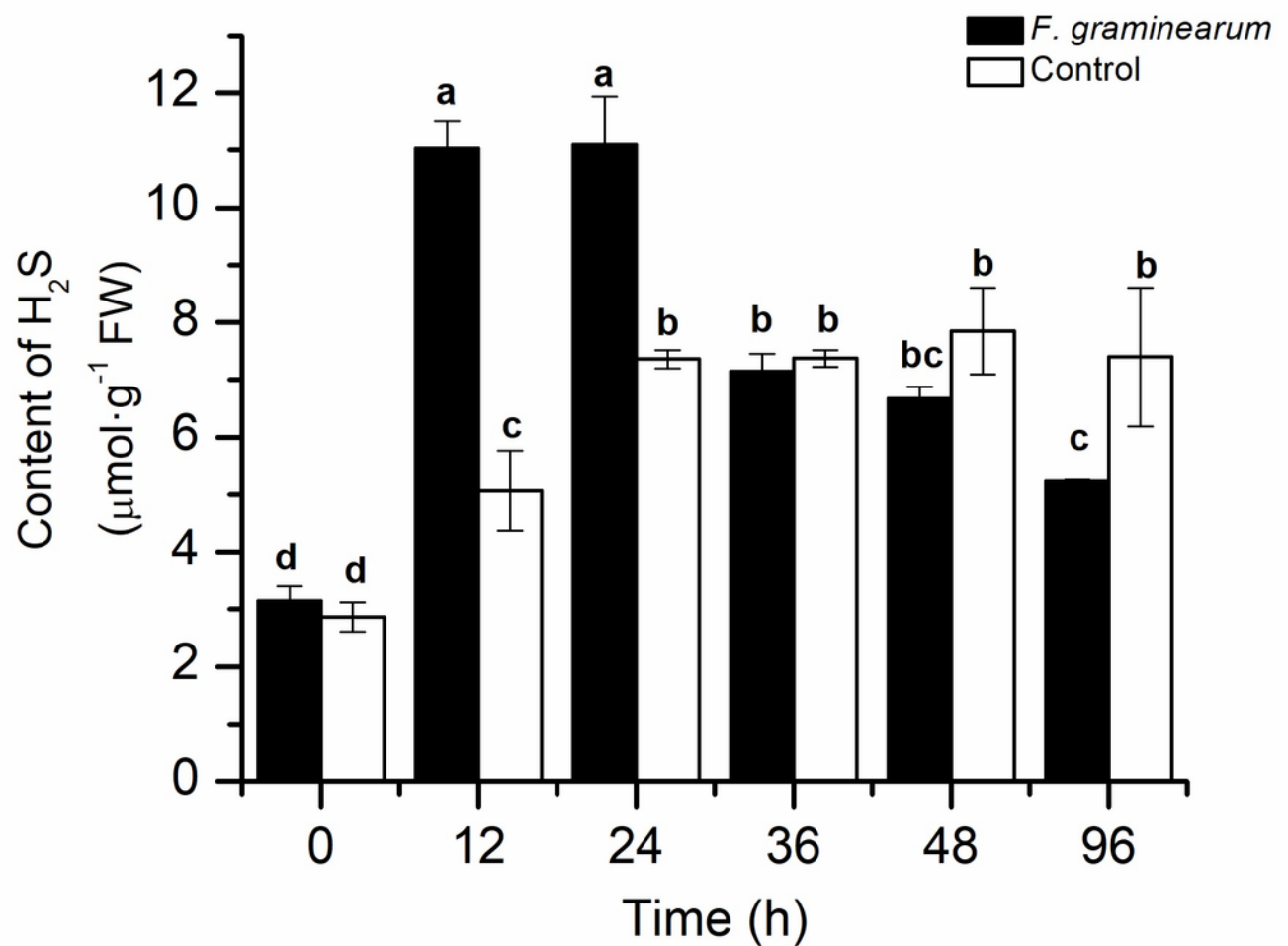
Figure 2

Effects of different concentrations of NaHS treatment on wheat disease resistance to *F. graminearum*.
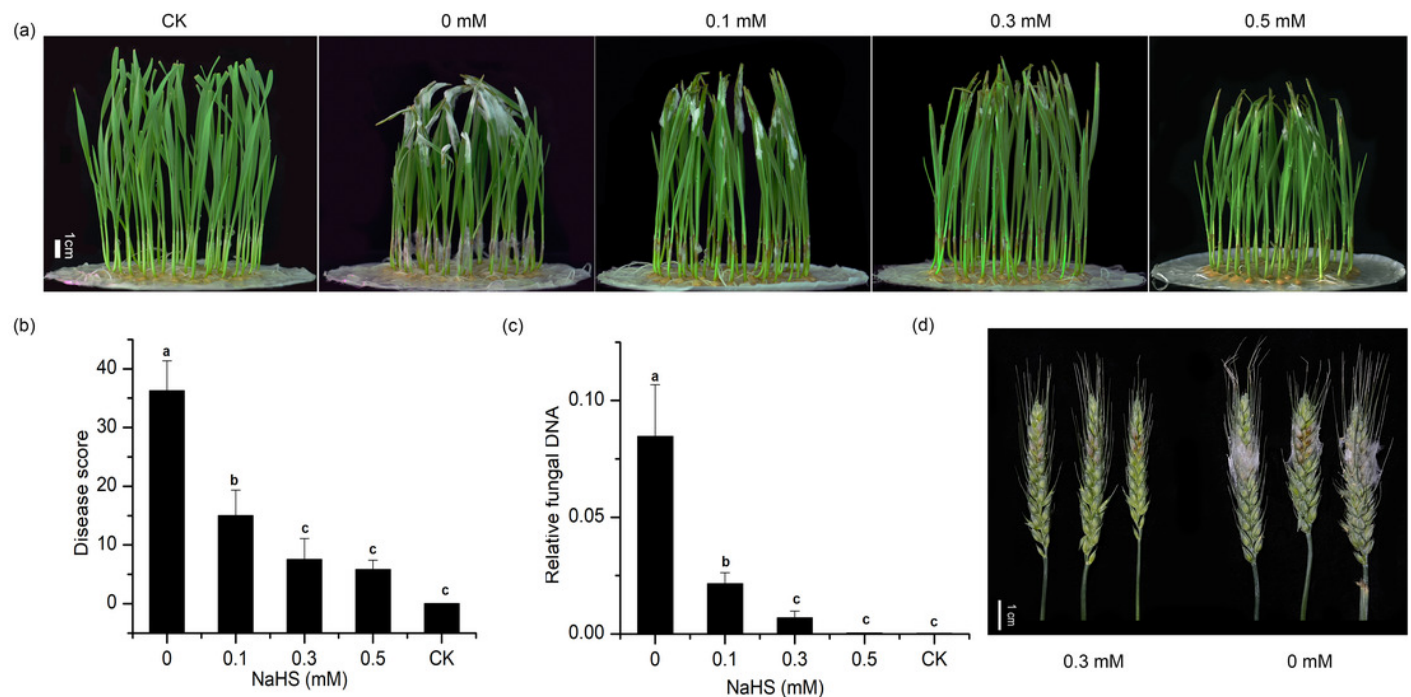
Figure 3

Effects of H$_2$S on wheat resistance to *F. graminearum* through NaHS treatment.
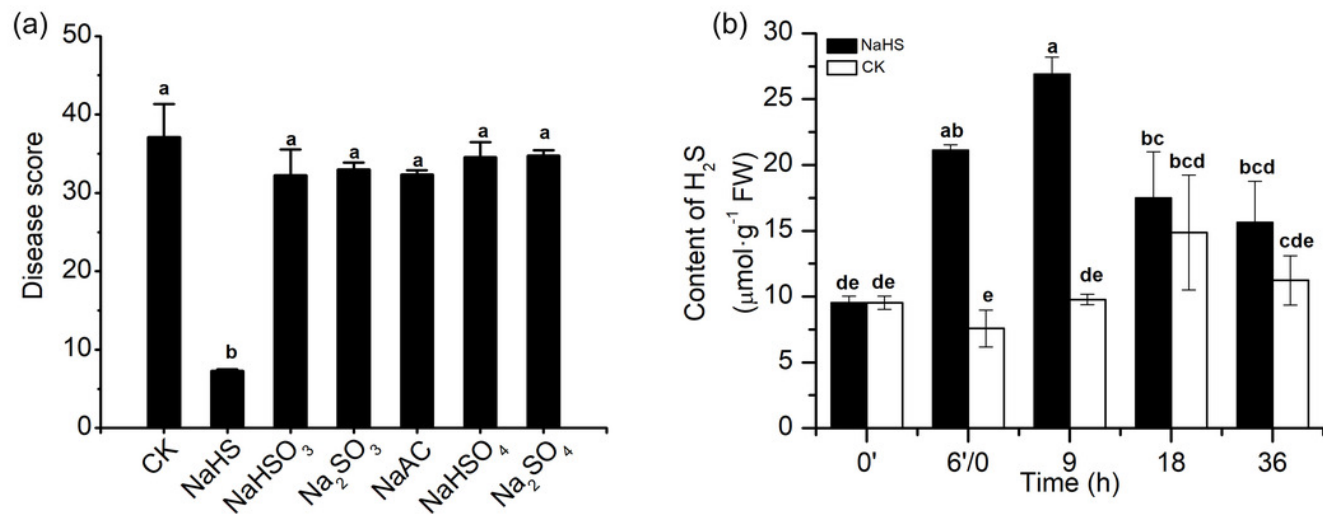
Figure 4

Effect of H$_2$S on the contents of hydrogen (H$_2$O$_2$) (a) and malonaldehyde (MDA) (b) in wheat seedlings.
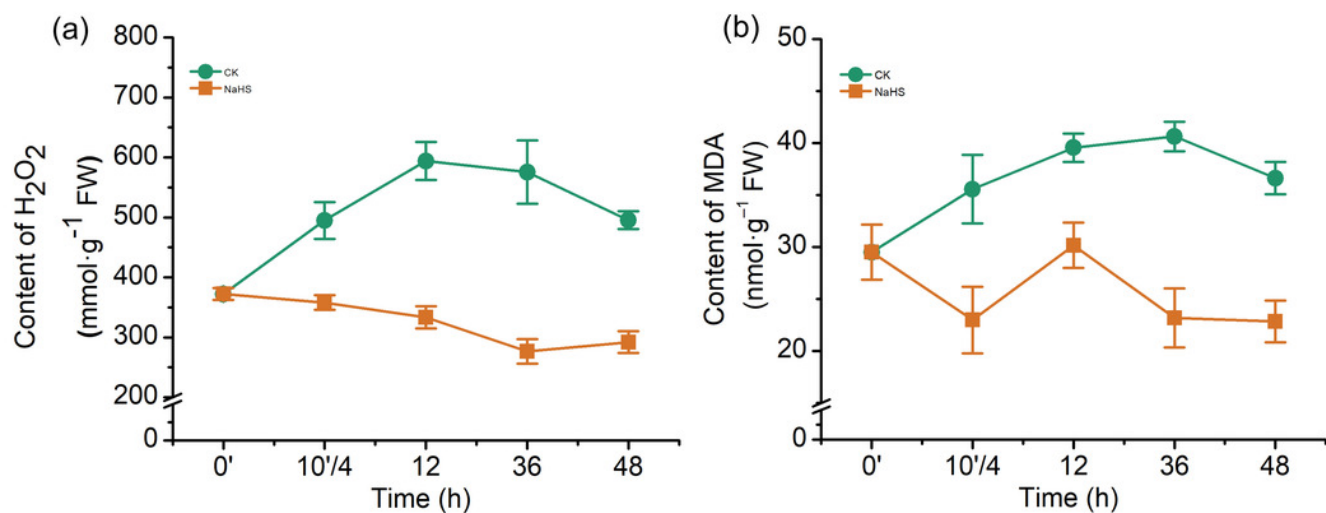
Figure 5

Effects of H$_2$S on activities of antioxidant enzymes (a) SOD activity, (b) POD activity, (c) APX activity, and (d) CAT activity.
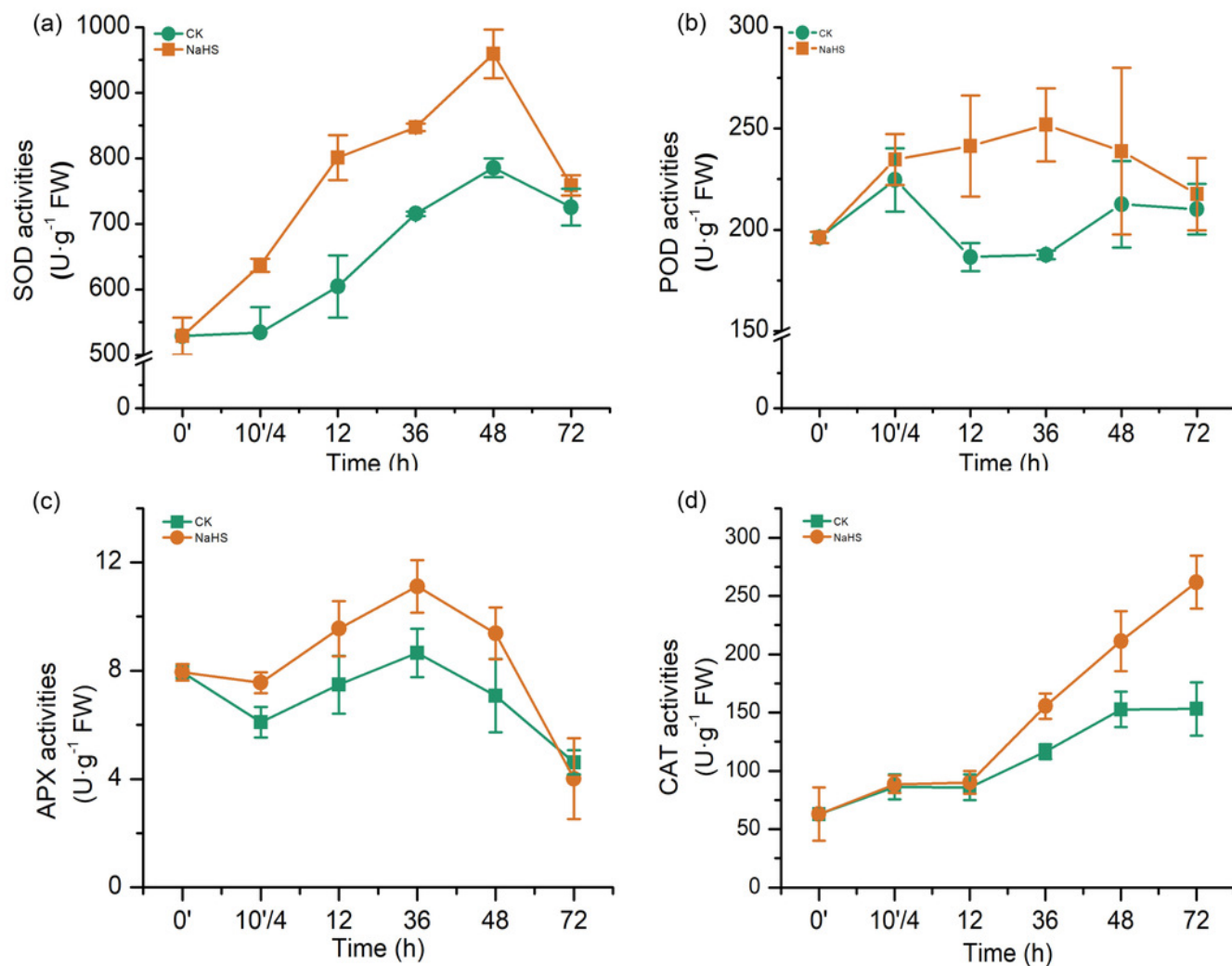
Figure 6

Effects of H$_2$S treatment on defense-related gene (a) *PR1.1*, (b) *PR2*, (c) *PR3*, (d)*PR4*.

Figure 7

H$_2$S effects development of *F. graminearum* on potato dextrose agar (PDA).

(a)

0 mM	0.01 mM	0.075 mM	0.1 mM	0.3 mM

1cm

30μm

(b)

Hyphae diameter (mm) vs NaHS (mM)

(c)

Spore germination of *F.graminearum* (%) vs NaHS (mM)