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Metabolomics profiling reveals *p*-aminobenzoic acid enhances resistance to *Fusarium* head blight in wheat

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Abstract

Fusarium head blight (FHB) not only causes severe yield losses but also mycotoxin contamination in wheat, posing a serious threat to food security and public health. The mechanisms of resistance to FHB in wheat are critical for effective prevention and control of the pathogen. In this research, we investigated and analyzed the metabolite changes induced by FHB colonization in the FHB-resistant cultivar Lianmai12 through *Fusarium graminearum* inoculation and mock inoculation. A total of 1001 metabolites were detected, 109 of which were significantly changed due to FHB infection. The majority of these 109 metabolites belonged to alkaloids, flavonoids, phenolic acids, lipids and organic acids. The most enriched KEGG pathways were plant hormone signal transduction and phenylpropanoid biosynthesis, which may constitute the major defence responses to FHB challenge. The metabolite *p*-aminobenzoic acid (PABA) significantly suppressed the growth of mycelia and the production of conidia in vitro. Further studies revealed that spraying PABA at early anthesis on wheat spikes reduced the development of FHB disease. These results provide preliminary insights into the metabolic basis of resistance in Lianmai12 and will be beneficial in the development of potential biocontrol agents against FHB.

Keywords Wheat, *Fusarium* head blight, *p*-aminobenzoic acid, Metabolomics

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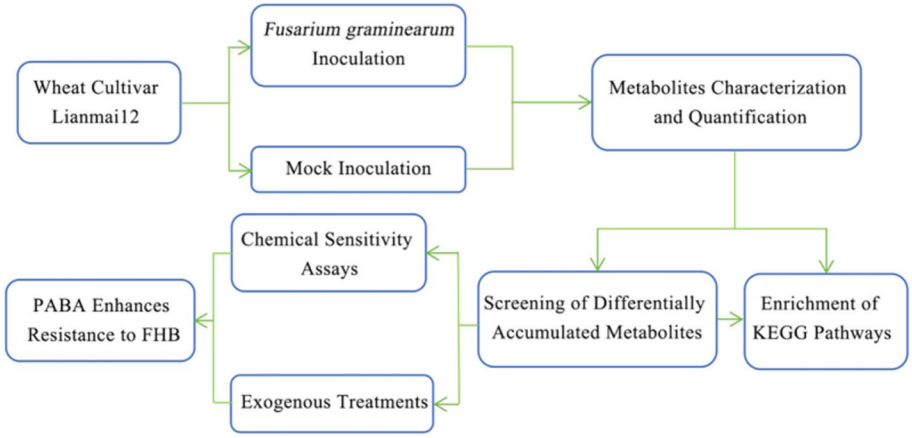
Graphical Abstract



Introduction

Fusarium head blight (FHB) is one of the most destructive wheat diseases affecting grain yield and quality, especially in the humid and subhumid environments of temperate and subtropical wheat production areas (Singh et al., 2023; Steiner et al., 2017; Zheng et al., 2021). The disease is mainly caused by various fungal species from the genus *Fusarium*. Most importantly, mycotoxins such as deoxynivalenol or nivalenol produced by the pathogen accumulate in the grain and pose a serious threat to human and animal health. Due to changes in climate and cropping systems, FHB has become a more widespread disease in recent decades (Alisaac et al., 2023). The middle and lower reaches of the Yangtze River are frequent areas of FHB in China. Nowadays, FHB has become a major disease even in the Huang-Huai wheat production area, where it has seldom occurred. In 2012, more than 10 Mha of wheat production area in China suffered from FHB, with a yield loss of 301.5 ~ 1877.3 kg/ha in some areas (Zhang et al., 2021). In 2018, the incidence area of FHB exceeded 5.67 Mha, accounting for 37.5% of wheat sown area in China (Huang et al., 2019). Chemical fungicides are commonly used to control FHB and mycotoxin contamination. However, the extensive use of chemical fungicides not only causes severe ecological damage, but also promotes the evolution of fungal resistance. Therefore, the development of novel, effective and low-risk compounds or the improvement of host resistance to FHB are effective strategies to reduce FHB damage.

FHB resistance is a complex quantitative trait governed by multiple small to medium effect quantitative trait loci (QTL) and is vulnerable to different environmental conditions. Over 556 QTLs have been identified for FHB resistance, with 8 QTLs designated from common wheat or alien species: *Fhb1* (Lagudah et al., 2019; Li et al., 2019), *Fhb2* (Cuthbert et al., 2007), *Fhb3* (Qi et al., 2008), *Fhb4* (Xue et al., 2010), *Fhb5* (Xue et al., 2011), *Fhb6* (Cainong et al., 2015), *Fhb7* (Wang et al., 2020), and *Fhb8* (Wang et al., 2024). However, the mechanisms of resistance and susceptibility to FHB in wheat are much less understood than the identification of QTLs. Breeding of resistant cultivars has been hampered by a lack of comprehension about the associated genetic mechanisms (Li et al., 2018). Research on the mechanisms of FHB resistance in wheat is essential to explore more genetic resources and provide more options and directions for breeding. In recent years, functional omics has become an effective approach to the identification of potential genes and the revealing of resistance mechanisms. Gene expression profiles, defence mechanisms and predicted resistance genes during *Fusarium* spp. challenge in wheat have been investigated in several transcriptome studies (Biselli et al., 2018; Li et al., 2018; Michel et al., 2021; Pan et al., 2018; Wang et al., 2018). Researchers have widely described the regulatory roles of phytohormones such as abscisic acid, gibberellic acid, salicylic acid, jasmonic acid, and ethylene in wheat defense against FHB (Brauer et al., 2019; Buhrow et al., 2021; Wang et al., 2018), whereas information on metabolite changes in wheat after FHB infection is limited.

Changes in metabolic profiles have provided key insights into the metabolic network in wheat against pathogen invasion, which is valuable for the exploration of metabolites, genetic resources and novel biological control agents. Metabolomic analyses of the resistant wheat cultivar Sumai 3 indicate that phytohormone signalling,

phenolamine and flavonoid metabolic pathways play roles in restricting the expansion of FHB (Zhao et al., 2022). The application of exogenous auxin increased susceptibility to FHB, and the knockdown of *TaTIR1*, the gene encoding the auxin receptor, increased resistance to FHB (Su et al., 2021). Metabolomic research have shown that phenylalanine and malate significantly inhibit the growth of *F. graminearum* (Dong et al., 2023), and spraying exogenous kaempferide, apigenin, proline, and alanine increases the resistance to FHB (Su et al., 2021; Zhao et al., 2021).

Several elite wheat cultivars with FHB resistance have been developed in China through the utilization of resistance genes, such as Fhb1 and Fhb7. However, the majority of FHB-resistant wheat germplasms are spring-like cultivars from the middle and lower reaches of the Yangtze River in China, with fewer FHB-resistant cultivars in the Huang-huai wheat production area. This study compares the metabolic changes in response to FHB colonization by ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) using Lianmai 12, a commercial wheat cultivar that has exhibited FHB resistance for years in the Huang-huai wheat production area. The results of this study will clarify the mechanisms of resistance to FHB infection in wheat from a metabolite perspective. The anti-FHB activity of PABA will be investigated to evaluate its potential as a biocontrol agent and to provide a new strategy for FHB control.

Materials and methods

Plant materials and FHB inoculation

This study utilized Chinese wheat cultivars, namely Lianmai12, Lianmai7, Zhoumai18, Huaimai20, and Huaimai33, which were planted in the greenhouse of Lianyungang Academy of Agricultural Sciences (Lianyungang, China). The *F. graminearum* strain 19JAFG used for inoculation was obtained from Jiangsu Academy of Agricultural Sciences and cultured on potato dextrose agar (PDA) medium at 25℃ for 3 days. Then, 5 fungal plugs at the edge of the mycelium were transferred into 300 mL conical flasks containing 6% mung bean broth medium and cultured with shaking at 200 rpm and 25℃ for 3 days. After filtering, the conidia were counted using a hemocytometer and diluted with sterile water to a concentration of 10^5/mL. Since single floret inoculation is the most commonly applied method for wheat FHB identification with good accuracy and stability, each spike was inoculated with 10 μL conidial suspension into a single middle floret for FHB resistance evaluation (Feng et al., 2018; Su et al., 2021). To ensure that the spikelets were sufficiently pathogenic at 72 h after inoculation and to obtain adequate quantities of pathogenic spikelets for metabolite extraction, each spike was inoculated with 10 μL of conidial suspension into a pair of middle florets for metabolite analysis (Zhao et al., 2021). The control plants received a mock inoculation with 10 μL of sterile water. The infected spikes were sealed in plastic bags for 72 h to maintain moisture. Three groups of biological replicates, each containing 25 spikes, were performed for all the wheat cultivars and the percentage of scabbed spikelets was recorded at 21 days post inoculation to evaluate FHB resistance.

Metabolite extraction and UPLC-MS/MS

Metabolites were extracted from the inoculated spikelets of Lianmai12 at 3 days post inoculation. Three biological replicates of 10 spikelets each were inoculated with FHB conidia as the treatment group. The control groups were inoculated with sterile water as a mock inoculation. The spikelets were harvested simultaneously and designated FHB-1, FHB-2, FHB-3, CK-1, CK-2, and CK-3. The spikelet samples were frozen in liquid nitrogen and stored at -80℃ for further metabolite detection.

All the chemicals and solvents used for metabolite extraction and detection were high-performance liquid chromatography grade. The inoculated spikelets of Lianmai12, FHB-1, FHB-2, FHB-3, CK-1, CK-2, and CK-3 were ground to powder after lyophilization. Metabolite extraction was carried out by infusing 0.1 g of each spikelet powder into 1.2 ml of pre-cooled 70% aqueous methanol. Dissolved samples were placed at 4℃ for 12 h, during which time samples were vortexed six times to enhance the extraction of metabolites. After centrifugation at 12,000 rpm for 10 min, the supernatant was collected and filtered through a microporous membrane filter (0.22 μm pore size) for subsequent UPLC-MS/MS analysis.

Ultra-performance liquid chromatography was performed on a SHIMADZU Nexera X2 system using an Agilent SB-C18 column. The column temperature was set to 40℃, and the flow rate was 0.35 mL/min. The mobile phase consisted of phase A (0.1% formic acid in ultrapure water) and phase B (0.1% formic acid in acetonitrile). The elution gradient was B-phase ratio of 5% at 0 min, which was linearly increased to 95% within 9 min and maintained at 95% for 1 min. The B-phase ratio decreased to 5% at 10.00–11.10 min and equilibrated at 5% for 14 min. An applied Biosystems 4500 QTRAP was used for tandem mass spectrometry. The electrospray ionization temperature was 550℃, the ion spray voltage was 5500 V (positive ion mode)/-4500 V (negative ion mode). The ion source gas I, gas II, and curtain gas were set to 50, 60, and 25 psi, respectively. The collision-activated dissociation parameter was set to high. The metabolites were quantified using the multiple reaction monitoring mode of a triple quadrupole mass spectrometer.

Chemical sensitivity assays for *Fusarium*

The *F. asiaticum* strains 1312 and 0301 were obtained from Xuzhou Academy of Agricultural Sciences. Both the *F. asiaticum* and *F. graminearum* were used in the experiment. The effects of PABA were evaluated on solid PDA media supplemented with 0.1 mg/mL, 0.2 mg/mL, 0.4 mg/mL, or 0.8 mg/mL PABA dissolved in 4‰ DMSO. PDA media and media supplemented with 4‰ DMSO alone were used as controls. FHB strain discs were obtained from the growing edge of one colony plate using a 5 mm diameter circular punch. The discs were then inoculated separately into the centers of 90 mm petri dishes containing solid PDA media, 4‰ DMSO-amended PDA media, or PABA-amended PDA media. There were three biological replicates in the experiment. The plates were incubated for 3 days in the dark at 25℃. The diameter of the colonies was measured daily, and a circle was drawn along the growth edge of mycelium on the plate for 3 days. The average colony diameter, mycelial growth rate, and inhibition rate (Duan et al., 2022) were calculated.

To study the effect of PABA on *Fusarium* spp. conidia production, 5 fungal discs were inoculated into 200 mL of 6% mung bean broth medium supplemented with 0.1 mg/mL, 0.2 mg/mL, 0.4 mg/mL, or 0.8 mg/mL PABA with shaking at 200 rpm and 25℃ for 2 days. The conidia concentrations were counted by using the hemocytometer. Three sets of replicates were set up in the test.

Exogenous PABA treatments on *F.* graminearum-infected spikes

Ten spikes of similar size were selected at early anthesis and inoculated with 10 μL of 1×10^5/mL conidial suspension per spike in one middle floret. A total of 3 groups of biological replicates were performed for the wheat cultivars Lianmai12, Lianmai7, Zhoumai18, Huaimai20 and Huaimai33. The inoculated spikes were moisturized in plastic bags for three days. A first spray of 2 mg/mL PABA with 4‰ DMSO as solvent was then applied. A second spray of the same concentration of PABA solution was applied the following day. The plants in the control group were sprayed with sterile water and 4‰ DMSO. The percentage of scabbed spikelets of inoculated spikes was recorded at 21 days post inoculation.

Data preprocessing and analysis

Compound analysis was based on the Metware database (Metware Biotechnology Co., Ltd, Wuhan, Hubei, China). In the characterization of substances, the isotopic signals, the repetitive signals containing K^+, Na^+, NH_4^+ and the repetitive signals of fragment ions of higher molecular weight are removed. Chromatographic peaks were integrated and corrected using MultiaQuant based on the retention time and peak shape of each metabolite detected in different samples, to ensure accurate qualitative and quantitative analysis.

Principal component analysis (PCA) was performed with R package prcomp. Set the argument scale = TRUE in prcomp to standardize the data. After Log_2 transformation and mean centering of the data, orthogonal partial least squares discriminant analysis (OPLS-DA) was performed using the OPLSR. Anal of the R package. We used the variable importance for projection (VIP) value (with threshold > 1) in the OPLS-DA model and the fold change value (with threshold ≥ 2 or ≤ 0.5) to determine differentially accumulated metabolites (DAMs) between groups. The heatmap was plotted using the complex heatmap of the R package. In KEGG pathway analysis, two pathways with integrative properties, the metabolic pathway (ko01100) and biosynthesis of secondary metabolites (ko01110), were not involved in the analysis. Significance analysis of experimental data for each group was performed using one-way ANOVA.

Results

Wheat cultivar Lianmai12 exhibits FHB resistance

Disease symptoms caused by FHB were investigated in the wheat cultivars Huaimai33, Lianmai7, Zhoumai18, Huaimai20 and Lianmai12. At 21 days post inoculation, the spikes inoculated with *F. graminearum* showed varying degrees of bleaching (Fig. 1A). Symptoms of FHB infection on Huaimai33, Lianmai7, Zhoumai18, and Huaimai20 were clearly observed not only on the inoculated spikelet but also above and below it, while Lianmai12 bleached only on the spikelets close to the inoculation point. Notably, the rachises of Lianmai12 remained green, whereas the rachises of Huaimai33, Lianmai7, Zhoumai18 and Huaimai20 almost bleached because of disease development (Fig. 1B). The percentage of scabbed spikelets of Lianmai12 was significantly lower than that of Huaimai33, Lianmai7, Zhoumai18, Huaimai20 ($P < 0.001$)(Fig. 1C). These results indicated that Lianmai12 had better FHB resistance than the other tested cultivars in the Huang-Huai wheat production area.

Metabolic responses to pathogen infection

To investigate the metabolic characteristics of Lianmai12 after FHB infection, wide-targeted metabolomic analysis based on UPLC-MS/MS was used. A total of 1001 metabolites were identified, including 183 flavonoids, 152 lipids, 129 phenolic acids, 107 organic acids, 104 alkaloids, 98 amino acids and derivatives, 71 saccharides and alcohols, 60 nucleotides and derivatives, 41 lignans and coumarins, 18 vitamins, 15 terpenoids, 6 quinones, 1 tannin, and 16 others. The identified metabolites were

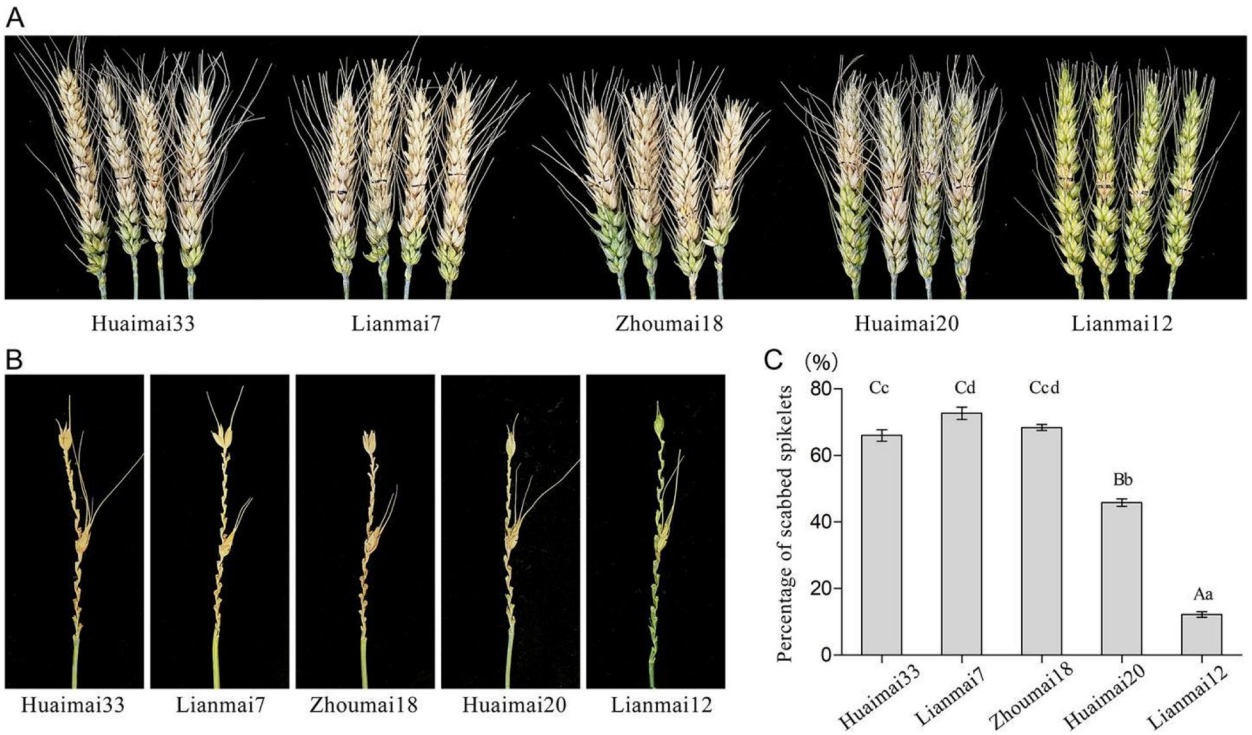


Fig. 1 Phenotypes of spikes inoculated with *F. graminearum* at 21 days post inoculation. **A** Blight symptoms on the spikes of different cultivars. **B** Blight symptoms on the rachises of different cultivars. **C** The percentage of scabbed spikelets of different cultivars. The data shown are the means ± SD. Different letters represent significantly different values (uppercase letters indicate $P < 0.001$, lowercase letters indicate $P < 0.05$)

analyzed by PCA to evaluate the overall magnitude of variability between the samples. Despite slight separation observed in one replicate of the CK group, PCA analysis clearly distinguished between samples from the CK and FHB groups (Fig. 2A). The two principal components explained 60.32% of the total variance. The first principal component (PC1) and the second principal component (PC2) explained 42.14% and 18.18%, respectively, of the variance. The differences in metabolite responses between the CK group and the *F. graminearum*-inoculated group were significant.

In order to filter out the information unrelated to the classification and to maximize the distinction between the samples, supervised multivariate statistical analysis, OPLS-DA, was performed. As shown in the OPLS-DA score plots, the contribution of the T score was 42% and

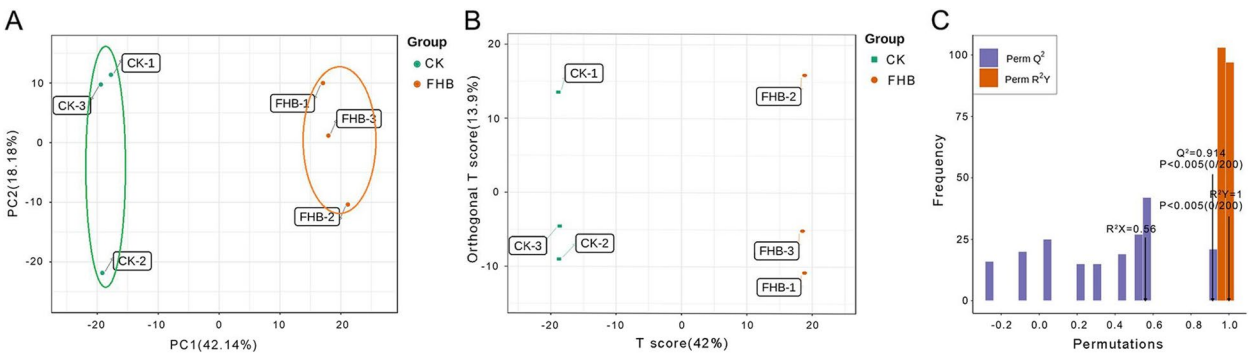


Fig. 2 Principal component analysis (PCA) and orthogonal partial least squares discriminant analysis (OPLS-DA) for the mock inoculated groups (CK) and the *F. graminearum* inoculated groups (FHB) at 3 days post inoculation. **A** PCA score plots for the metabolites in the spikelets between the CK and FHB groups. PC1 means the first principal component, PC2 means the second principal component. **B** OPLS-DA scatter plots for all samples. **C** Permutation tests of OPLS-DA modeled on CK versus FHB groups

the contribution of the orthogonal T score was 13.9%. The CK group was on the left side while the FHB group was on the right side based on the abscissa of the score plots, which further indicated the impact of differentiation on FHB infection in the wheat cultivar Lianmai12 (Fig. 2B). The model along with 200 permutation tests showed that $R^2X = 0.56$, $R^2Y = 1$, and $Q^2 = 0.914$, with $P < 0.005$ for both Q^2 and R^2, confirming that the model was reliable without overfitting for subsequent DAMs screening and analysis (Fig. 2C).

Screening and analysis of DAMs

The VIP value of the OPLS-DA model (threshold > 1) and the fold change value (threshold ≥ 2 or ≤ 0.5) were used to screen for DAMs between the CK and FHB groups. A total of 109 DAMs were obtained from the screening. A heatmap of the classification and changes in DAMs in response to FHB infection in Lianmai12 is shown in Fig. 3. The differential metabolites in the heatmap show opposite expression characteristics between the CK and FHB groups, suggesting that the invasion of FHB activates the corresponding metabolic pathways in Lianmai12 and stimulates the synthesis of metabolites. Alkaloids (26 compounds), flavonoids (18 compounds), phenolic acids (17 compounds), lipids (13 compounds), organic acids (11 compounds) were the most representative DAMs among the compound classes.

Table 1 shows the 50 DAMs with $|\text{Log}_2$ fold change$| > 2$. The order of the DAMs is sorted by fold change value. The plant hormone signal transduction (ko04075) pathway annotated with N6-isopentenyladenine and jasmonic acid, the flavonoid biosynthesis (ko00941) pathway annotated with sakuranetin, naringenin and neohesperidin, the isoflavonoid biosynthesis (ko00943) pathway annotated with naringenin and prunetin generally involved in plant defence and stress responses. The *p*-coumaryl alcohol and coniferyl alcohol were annotated to phenylpropanoid biosynthesis (ko00940), which is responsible for cell wall formation in plants. Figure 4A shows the top 15 upregulated DAMs and top 5 downregulated DAMs according to the fold change value. Among the 109 DAMs, L-prolyl-L-phenylalanine (18,321.48-fold), 3,4-*O*-dicaffeoylquinic acid methyl ester (15,773.70-fold), 3,24-dihydroxy-17,21-semiacetal-12(13)oleanolic fruit (11,361.04-fold), heptadecanoic acid (5247.19-fold), and 3,4-dimethoxyphenyl acetic acid (4868.00-fold) were significantly upregulated. The VIP score plot (Fig. 4B) shows the top 20 DAMs according to the VIP value. Volcano plot analysis was further applied to visualize the differential metabolites between the CK and FHB groups. The volcano plot shows that 97 metabolites were upregulated and 12 metabolites were downregulated in Lianmai12 after FHB invasion (Fig. 4C). The metabolites labeled 'a' to 'g' in Fig. 4C are L-prolyl-L-phenylalanine (a), 3,4-*O*-dicaffeoylquinic acid methyl ester (b), 3,24-dihydroxy-17,21-semiacetal-12(13)oleanolic fruit (c), heptadecanoic acid (d), 3,4-dimethoxyphenyl acetic acid (e), *p*-aminobenzoic acid (f) and *N*-formylmethionine (g). These compounds are the top 15 upregulated DAMs by the fold change value and the top 20 by the VIP value. It is hypothesized that these compounds may be associated with resistance to FHB in Lianmai12.

Enrichment of KEGG pathways

To identify potential pathways involved in resistance responses following FHB attack in Lianmai12, differential metabolites were mapped to the KEGG database and 332 of them could be annotated, of which 46 were DAMs. As shown in Fig. 5, the two pathways enriched with the greatest number of DAMs were phenylpropanoid biosynthesis and flavonoid biosynthesis. The phenylpropanoid biosynthesis pathway was the most abundant class detected in this study. A total of 24 metabolites were mapped to KEGG pathways, and 8 of them were DAMs: *p*-coumaraldehyde, cinnamic acid, *p*-coumaryl alcohol, caffeic acid, coniferyl alcohol, ferulic acid, *trans*-5-*O*-(*p*-coumaroyl)shikimate, and coniferin. The most significant pathways of enrichment at 72 h postinfection were the plant hormone signal transduction pathway and phenylpropanoid biosynthesis pathway. In addition, the plant hormone signal transduction, riboflavin metabolism, folate biosynthesis, betalain biosynthesis and isoflavonoid biosynthesis were the pathways with the highest value of rich factor. Three DAMs, namely, N6-isopentenyladenine, jasmonic acid and (-)-jasmonoyl-L-isoleucine, were mapped to plant hormone signal transduction pathways. Ribitol, lumichrome and riboflavin (vitamin B2) were mapped to riboflavin metabolism pathways. Only one DAM was mapped to the folate biosynthesis pathway (*p*-aminobenzoic acid) or the betalain biosynthesis pathway (3,4-dihydroxy-L-phenylalanine). Two DAMs, namely, naringenin and prunetin were assigned to isoflavonoid biosynthesis pathways. With the exception of the betalain biosynthesis pathway, most of the DAMs mapped to the above pathways were significantly upregulated after inoculation with FHB, implying that these metabolic pathways or metabolites may be associated with the FHB defense response mechanism in Lianmai12.

The anti-FHB activity of PABA on wheat

An in vitro assay of partial DAMs revealed the antifungal activity of PABA. FHB pathogenic strains, 1 *F. graminearum* strain and 2 *F. asiaticum* strains, discs (5 mm) were inoculated on solid PDA media, DMSO-amended PDA media, and PABA-amended PDA media for three days (Fig. 6A). The mycelial growth inhibition rate did

Fig. 3 The heat map of the 109 differentially accumulated metabolites. CK: mock inoculated groups; FHB: *F. graminearum* inoculated groups

Table 1 Partial differentially accumulated metabolites with $|Log_2$ fold change$|> 2$

Compound names	CAS	VIP	Fold change (Log_2)	KEGG pathways
L-prolyl-L-phenylalanine	13,589–02-1	1.54	14.16	/
3,4-*O*-dicaffeoylquinic acid methyl ester	/	1.54	13.95	/
3,24-dihydroxy-17,21-semiacetal-12(13)oleanolic fruit	/	1.54	13.47	/
heptadecanoic acid	506–12-7	1.54	12.36	/
3,4-dimethoxyphenyl acetic acid	93–40-3	1.54	12.25	/
tryptamine	61–54-1	1.52	4.88	ko00380,ko00901,ko01100,ko01110
p-coumaroylmalic acid	/	1.29	4.88	/
lysoPE 17:1	/	1.53	4.79	/
lysoPE 17:1(2n isomer)	/	1.53	4.79	/
lysoPE 16:1(2n isomer)	/	1.53	4.67	/
p-aminobenzoic acid	150–13-0	1.54	4.37	ko00790,ko01100
N-feruloylserotonin	68,573–23-9	1.52	4.31	/
N-feruloyltryptamine	53,905–13-8	1.53	4.14	/
N-formylmethionine	4289–98-9	1.54	3.96	ko00270
serotonin	50–67-9	1.54	3.87	ko00380,ko01100
ribosyladenosine	/	1.54	3.86	/
lysoPE 16:1	/	1.54	3.83	/
sakuranetin	2957–21-3	1.52	3.72	ko00941,ko01110
7-*O*-methylnaringenin	/	1.52	3.69	/
N6-isopentenyladenine	2365–40-4	1.22	3.52	ko00908,ko01110,ko04075
lysoPE 14:0(2n isomer)	/	1.51	3.26	/
diosmetin-7-*O*-glucoside	20,126–59-4	1.49	3.05	/
1-*O*-cinnamoyl-*β*-*D*-glucose	40,004–96-4	1.50	2.79	/
jasmonic acid	77,026–92-7	1.54	2.79	ko00592,ko01100,ko01110,ko04075
p-coumaryl alcohol	3690–05-9	1.53	2.73	ko00940,ko01100,ko01110
N-*trans*-*p*-coumaroylputrescine	34,136–53-3	1.46	2.69	ko00330,ko01100
N-*p*-coumaroyl-*N*′-feruloylputrescine	380,302–96-5	1.53	2.64	/
p-coumaroylcadaverine	/	1.46	2.63	/
4-methoxyphenylpropionic acid	/	1.53	2.61	/
indole-5-carboxylic acid	1670–81-1	1.54	2.57	/
coniferyl alcohol	458–35-5	1.53	2.57	ko00940,ko00998,ko01100,ko01110
apigenin-7,4′-dimethyl ether	5128–44-9	1.53	2.55	/
N-*cis*-*p*-coumaroylhydroxyputrescine	/	1.51	2.48	/
10-heptadecenoic acid	29,743–97-3	1.42	2.42	/
N-(4′-*O*-glycosyl)-*p*-coumaroyl agmatine	/	1.43	2.42	/
naringenin	480–41-1	1.54	2.42	ko00941,ko00943,ko01100,ko01110
N-*trans*-*p*-coumaroylhydroxyputrescine	/	1.50	2.41	/
5,2′-dihydroxy-7,8-dimethoxyflavone glycosides	/	1.48	2.36	/
N-*cis*-*p*-coumaroylputrescine	/	1.51	2.36	/
L-tartaric acid	87–69-4	1.14	2.28	ko00630,ko01100
acetryptine	3551–18-6	1.48	2.25	/
1-*O*-caffeoyl-(6-*O*-glucosyl)-*β*-*D*-glucose	/	1.50	2.20	/
prunetin	552–59-0	1.54	2.18	ko00943
methyl linolenate	301–00-8	1.52	2.11	/
kaempferide-3-*O*-(6″-malonyl)glucoside	/	1.52	2.11	/
rubusic acid	23,984–26-1	1.52	2.10	/
N-trans-*p*-coumaroylagmatine	7295–86-5	1.52	2.06	ko00330,ko01100
2′-*O*-methyladenosine	2140–79-6	1.51	-2.09	/
feruloylhistamine	94,848–18-7	1.53	-2.39	/
neohesperidin	13,241–33-3	1.12	-3.44	ko00941

/ indicates no annotation to CAS or KEGG pathways

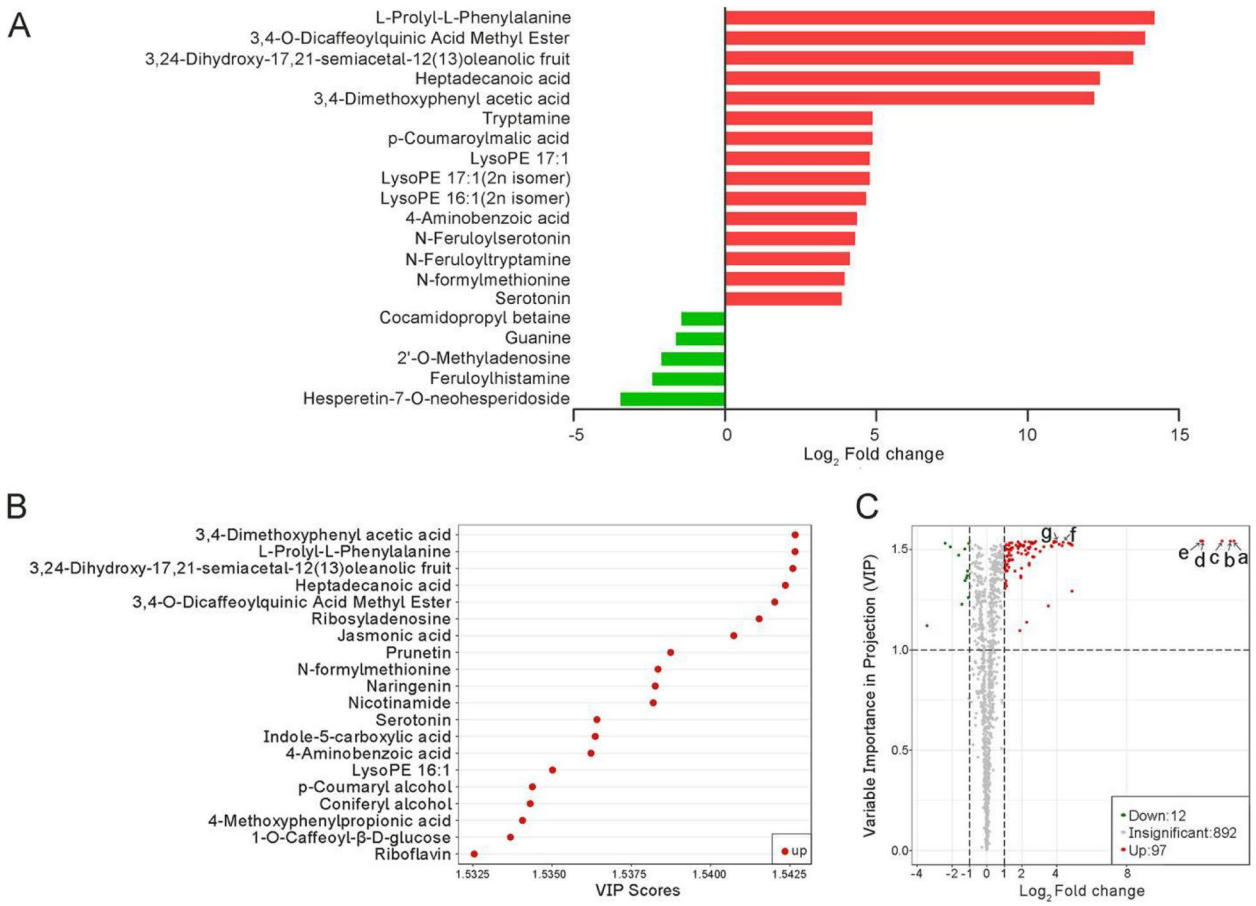


Fig. 4 Screening of the DAMs. **A** The top 15 upregulated DAMs and the top 5 downregulated DAMs according to the fold change value. **B** The top 20 DAMs by the VIP value. **C** Volcano plot of differential metabolites. L-prolyl-L-phenylalanine (a), 3,4-*O*-dicaffeoylquinic acid methyl ester (b), 3,24-dihydroxy-17,21-semiacetal-12(13)oleanolic fruit (c), heptadecanoic acid (d), 3,4-dimethoxyphenyl acetic acid (e), *p*-aminobenzoic acid (f), and *N*-formylmethionine (g) are labeled in the volcano plot

not significantly differ between mycelia incubated on DMSO-amended PDA media and those incubated on normal PDA media, suggesting that 4‰ DMSO as a solvent had no significant effect on the mycelial growth rate ($P > 0.05$) (Fig. 6B). In contrast, compared with the DMSO-amended PDA media and PDA media, the PABA-amended PDA media significantly suppressed the growth of the mycelia ($P < 0.001$) (Fig. 6A, B). Furthermore, the concentration of PABA was negatively correlated with the growth rate of the pathogen mycelium but positively correlated with the inhibition rate of mycelial growth, with inhibition rates of 25.4% ~ 31.4% and 42.87% ~ 52.48% at 0.4 mg/mL and 0.8 mg/mL between different FHB strains, respectively (Fig. 6B, Table 2). The effect of PABA on the ability of FHB strains to produce conidia was investigated by inoculating equivalent quantities of discs into conidia-producing media supplemented with 0.1 mg/ml, 0.2 mg/ml, 0.4 mg/ml, or 0.8 mg/ml PABA. Figure 6C shows that the concentration of conidia decreased significantly with increasing concentration of PABA, indicating that PABA was able to inhibit the production of *Fusarium* spp. conidia. The antifungal activity of PABA was further investigated in wheat by comparing the percentage of scabbed spikelets between wheat cultivars sprayed with H_2O, DMSO or PABA after inoculation (Fig. 6D and E). Spraying 2 mg/mL PABA did not inhibit the infection, as it was clearly observed that all wheat spikelets successfully infected and invaded neighboring spikelets (Fig. 6E). The percentage of scabbed spikelets of wheat sprayed with PABA was significantly lower than that sprayed with H_2O or DMSO ($P < 0.05$), averaging 30.67% ~ 57.99% and 29.54% ~ 55.89% lower, respectively, across wheat cultivars. These results demonstrated that PABA exhibited anti-FHB activity during pathogen challenge.

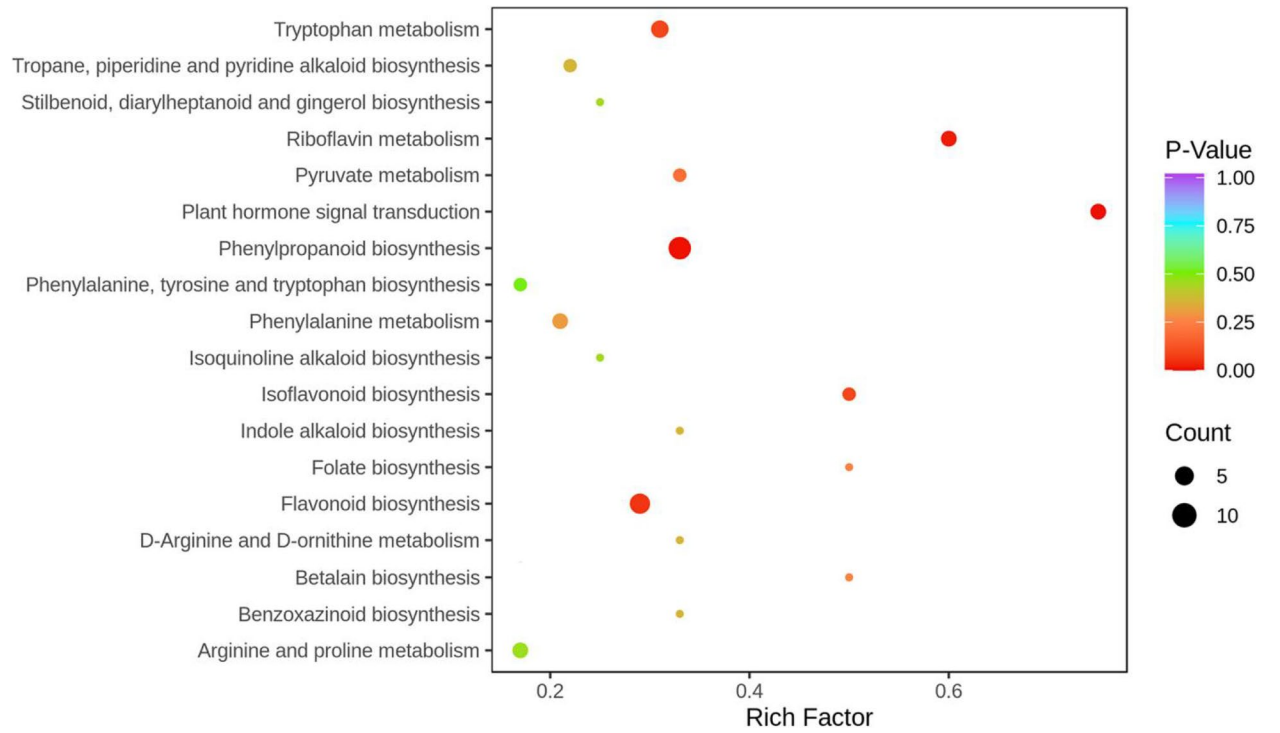


Fig. 5 Top 18 KEGG enrichment pathways of the annotated DAMs

Discussion

F. graminearum is one of the most widespread fungal diseases of commercially essential crops, such as wheat, barley and maize, and is capable of causing FHB in wheat and barley and *Giberella* ear rot in maize (Balcerzak et al., 2012). Given the limited understanding of resistance mechanisms to combat FHB in wheat, the identification of host resistance factors could assist in the control of FHB. Metabolomics is a nonbiased, comprehensive and high-throughput method for analyzing complex metabolic compounds that theoretically allows the identification and quantification of every metabolite (Gauthier et al., 2015). The number of identified metabolites related to FHB resistance depends on the genetic background of the material and the analytical strategy, for example, 340 metabolites based on wheat near isogenic lines in an LC-LTQ-Orbitrap study and 45 metabolites based on six wheat cultivars/lines in a GC–MS study (Gunnaiah et al., 2012; Hamzehzarghani et al., 2008). In this study, metabolome profiling between *F. graminearum* inoculation and mock inoculation of Lianmai12, a Chinese commercial wheat cultivar resistant to FHB, was conducted by using UPLC-MS/MS technology. A total of 1001 compounds were detected following the inoculation and mock inoculation. These metabolites were quantified and analyzed to investigate the potential defense mechanisms of Lianmai12 in response to FHB disease. Our study showed that the plant hormone signal transduction, the phenylpropanoid biosynthesis, and the flavonoid biosynthesis pathways were significantly enriched in Lianmai12 at 3 days post inoculation. Profiling of the recombinant inbred lines carrying resistant and susceptible alleles of QTL-*Fhb2* revealed a higher abundance of metabolites belonging to phenylpropanoid and flavonoid biosynthetic pathways in the resistant lines (Dhokane et al., 2016), which is consistent with the results of our analysis. Metabolomic analysis of the resistant wheat cultivar Sumai 3 also revealed the important role of plant hormone signal transduction pathway and flavonoid metabolic pathways in limiting the spread of the pathogen (Zhao et al., 2022).

Phytohormones are known to participate in plant defense during biotic stress and to influence the severity of FHB in wheat (Alazem et al., 2015; Qi et al., 2012; Shigenaga et al., 2016). The phytohormone jasmonic acid reportedly plays a positive role in the early infection of *F. graminearum*, whereas abscisic acid may be associated with susceptibility to FHB (Qi et al., 2016; Su et al., 2021; Sunic et al., 2023; Wang et al., 2018). *TaAOC*, *TaAOS*, and *TaOPR3*, genes involved in the jasmonic acid pathway, have been shown to positively regulate FHB resistance (Fan et al., 2019). Exogenous jasmonic acid treatment of wheat heads reduced the growth of *F. graminearum* and FHB symptoms, in contrast to abscisic acid treatment,

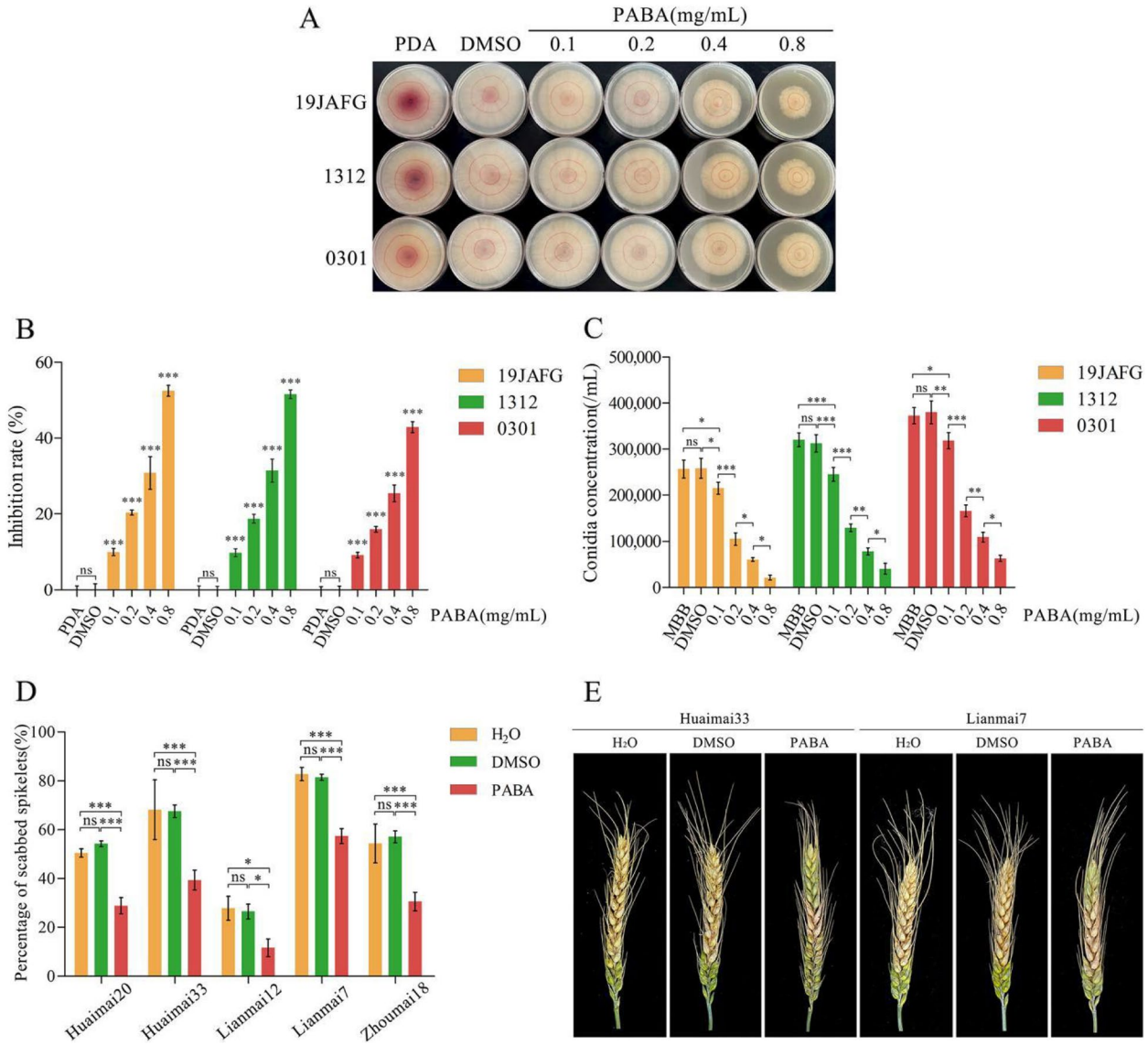


Fig. 6 The effects of PABA on the growth of FHB strains. **A** Suppression of the mycelia in vitro by PABA at 3 days. The rings marked on the plates indicate the edge of hyphal growth every 24 h. PDA and DMSO mean that the strain was cultured on the potato dextrose agar medium and the DMSO-amended potato dextrose agar medium, respectively. **B** Inhibition rate of the mycelial growth by PABA at 3 days. PDA and DMSO mean that the strain was cultured on the potato dextrose agar medium and the DMSO-amended potato dextrose agar medium, respectively. **C** Effect of PABA on conidia production at 48 h. MBB and DMSO mean that the strain was cultured on the mung bean broth medium and the DMSO-amended mung bean broth medium, respectively. **D** The percentage of scabbed spikelets of different cultivars after the application of exogenous PABA at 21 days post inoculation. H_2O, DMSO and PABA represent the control groups sprayed with H_2O or DMSO, and the experimental groups sprayed with PABA, respectively. **E** Exogenous spraying of PABA reduces FHB disease phenotype in wheat cultivars Huaimai33 and Lianmai7. H_2O, DMSO and PABA represent the control groups sprayed with H_2O or DMSO, and the experimental groups sprayed with PABA, respectively. The results are presented as the means ± SD. Asterisks indicate statistical significance. *** indicates $P < 0.001$, ** indicates $P < 0.01$, * indicates $P < 0.05$, ns indicates no significance ($P > 0.05$)

which was found to increase FHB symptoms (Qi et al., 2016). Additionally, *Fusarium* spp. can hijack phytohormones, such as abscisic acid, to establish or promote infection (Buhrow et al., 2021). These studies support our experimental results. In our metabolomic study, the plant hormone signal transduction pathway was the most enriched KEGG pathway. Jasmonic acid was upregulated 6.92-fold, and its bioactive conjugate (-)-jasmonoyl-L-isoleucine was upregulated 2.77-fold at 3 days post inoculation. Moreover, abscisic acid did not change significantly, which may be one of the bases of resistance in wheat cultivar Lianmai12.

Table 2 Correlation analysis of the anti-FHB activity of PABA in vitro

FHB Strains	Correlation	PABA concentration	Average colony diameter			Average growth rate of mycelium	Average inhibition rate
			24 h	48 h	72 h		
19JAFG	PABA concentration	1.000	-0.995**	-0.996**	-0.989**	-0.988**	0.989**
	Average colony diameter at 24 h		1.000	0.998**	0.997**	0.996**	-0.997**
	Average colony diameter at 48 h			1.000	0.998**	0.998**	-0.998**
	Average colony diameter at 72 h				1.000	1.000**	-1.000**
	Average growth rate of mycelium					1.000	-1.000**
	Average inhibition rate						1.000
1312	PABA concentration	1.000	-0.974**	-0.985**	-0.989**	-0.991**	0.989**
	Average colony diameter at 24 h		1.000	0.994**	0.995**	0.994**	-0.994**
	Average colony diameter at 48 h			1.000	0.998**	0.998**	-0.998**
	Average colony diameter at 72 h				1.000	1.000**	-1.000**
	Average growth rate of mycelium					1.000	-1.000**
	Average inhibition rate						1.000
0301	PABA concentration	1.000	-0.953**	-0.981**	-0.989**	-0.989**	0.989**
	Average colony diameter at 24 h		1.000	0.993**	0.986**	0.985**	-0.986**
	Average colony diameter at 48 h			1.000	0.997**	0.996**	-0.997**
	Average colony diameter at 72 h				1.000	1.000**	-1.000**
	Average growth rate of mycelium					1.000	-1.000**
	Average inhibition rate						1.000

** means $P < 0.01$, * means $P < 0.05$

Plant metabolites provide chemical defense not only by inducing specific defense signaling pathways but also by exerting antimicrobial activity and reinforcing cell walls. The phenylpropanoid biosynthesis pathway, which was enriched in the highest number of DAMs in Lianmai12 after infection, activates the synthesis of phenolic phytoalexin in crops (Cho et al., 2015). Phytogenous phenolic acids (such as caffeic acid (Lima et al., 2016; Yuvamoto et al., 2007) and ferulic acid (Yan et al., 2023), flavonoids (such as prunetin (Kuete et al., 2011; Wabo et al., 2007), sakuranetin (Grecco et al., 2014; Hasegawa et al., 2014; Liu et al., 2023) and prunin (Salas et al., 2012), and alkaloids (such as putrescine (Song et al., 2023), tryptamine (Gardiner et al., 2013) and serotonin (Du Fall et al., 2013) have been reported to exhibit antimicrobial activity directly. Yan et al. (2023) reported that ferulic acid treatment inhibited the synthesis of ergosterol in *F. graminearum* and impaired the cell membrane of hyphae. Patzke et al. (2018) found that ferulic acid was very effective in inhibiting the growth of *Botrytis cinerea* and that suppression of infection in wounded and contaminated grapes was achieved by adjusting the concentration of ferulic acid in the phenolic emulsion. Li et al. (2021) suggest that caffeic acid has a crucial role in defense against *Ralstonia solanacearum* infection in tobacco and is a potential and effective antibacterial agent for the control of bacterial wilt. According to Sidiq et al. (2021), nicotinamide is an effective candidate for controlling FHB disease. It suppressed fungal cell growth and mycotoxin production in wheat and barley. After *F. graminearum* infection, metabolites with antimicrobial properties also increased in spikelets of Lianmai12. We identified 18 antimicrobial compounds among the DAMs, 16 of which were upregulated. The evidence suggests that these metabolites may have antifungal effects on resistance to FHB in the wheat cultivar Lianmai12. However, further studies on the process of wheat-fungal interactions and the mechanisms by which pathogens escape from the wheat defense system are needed because several host-generated defense compounds, such as ferulic acid and putrescine, may induce the hypertranscription of deoxynivalenol biosynthetic genes and subsequently lead to deoxynivalenol accumulation (Gardiner et al., 2010; Ma et al., 2021; Ponts et al., 2011). The application of natural products and plant extracts as safer antimicrobials against plant pathogens has become an increasingly popular topic in agriculture. Feng et al. (2023) developed cinnamaldehyde nanoemulsions, which overcame the negative characteristics of cinnamaldehyde such as high volatility, poor water solubility and easy oxidative degradation, and showed good efficacy against rice sheath blight, wheat sheath blight, and wheat take-all diseases with good biosafety. Plant Tonic9 (EOX-SOV; Sovereign Innovations Sdn Bhd, Malaysia in collaboration with EOX International b.v. Netherlands),

contains palm oil and palm kernel oil with lauric acid, has good antifungal activity and protective properties against rice blast disease (Abed-Ashtiania et al., 2018; Walters et al., 2003).

Lignin biosynthesis is an off-shoot process of the phenylpropanoid pathway in plants (Ninkuu et al., 2023). The precursor compounds related to lignin biosynthesis, including *p*-coumaraldehyde, *p*-coumaryl alcohol, caffeic acid, coniferyl alcohol, ferulic acid, and coniferin (Boerjan et al., 2003; Kumar et al., 2020), all increased after FHB infection. Studies have demonstrated that cultivar resistance to FHB in wheat depends on biochemical factors that limit the spread of the pathogen in spikes and that the combination of cell wall components and lignification are critical for the mechanism of FHB type II resistance (Lahlali et al., 2016; Lionetti et al., 2015). Based on these findings, it is hypothesized that the limited bleaching symptoms observed on the Lianmai12 rachis after infection may be due to the strengthening of cell wall defenses that prevent or slow the spread of the pathogen.

During the metabolic profiling of Lianmai12, we paid particular attention to the elevated trend of PABA after infection, which is an environmentally friendly bioactive metabolite that has shown antifungal activity based on the ability to inhibit cytokinesis and antibacterial activity by altering outer membrane integrity (Jiang et al., 2023). Studies have proven that PABA, found in the secretion of *Lysobacter antibioticus*, has stable broad-spectrum antifungal activity in pears, which significantly reduces the symptoms of bitter rot disease (Laborda et al., 2019; Zhu et al., 2022). Goodwin et al. (2018) found that PABA affected salicylic acid-related gene expression and induced resistance against *Pseudomonas syringae* in tomato. Jiang et al. (2023) reported that PABA exhibited antibacterial activity against the soybean pathogen *Xanthomonas axonopodis* pv. *glycines* by reducing the expression of several membrane integrity-related genes and the content of membrane lipopolysaccharides. Nevertheless, studies on the resistance of PABA to *Fusarium* spp. in wheat are still to be conducted. To investigate whether PABA has a protective effect against FHB in wheat, we first verified its direct antifungal activity in vitro. Consistent with the above findings, our results showed that PABA inhibited mycelial growth and conidial production of *Fusarium* spp. in vitro. Further field trials showed that PABA protected wheat from FHB disease and significantly reduced the percentage of scabbed spikelets in resistant and susceptible cultivars. As PABA and its derivatives are widely used in the pharmaceutical industry, for example in sunscreens, PABA and its metabolites are almost exclusively excreted in the urine and are generally considered to be non-toxic and well-tolerated (Krátký et al., 2020; Sowinska et al., 2019). This offers the potential for research into the development of novel PABA-based biocontrol agents, although the environmental safety of PABA needs to be further investigated.

Conclusions

In summary, we conducted a comprehensive analysis of the metabolic profiles of Lianmai12, an FHB-resistant commercial wheat cultivar in China, during early infection. Our study revealed the key roles of the plant hormone signal transduction pathway and the phenylpropanoid biosynthesis pathway in the FHB resistance process. In particular, we demonstrated not only the anti-FHB activity of PABA, but also the protective effect of PABA against FHB disease in wheat for the first time. In the future, we will further investigate the mechanisms of PABA against FHB and explore the possibility of PABA-based bioagents in agriculture. We hope to provide a theoretical basis for the application of PABA and contribute to the development of new biopesticides with high efficacy, safety and environmental friendliness against FHB.

Abbreviations

FHB	*Fusarium* head blight
PABA	*p*-Aminobenzoic acid
QTL	Quantitative trait loci
UPLC-MS/MS	Ultra-performance liquid chromatography-tandem mass spectrometry
PCA	Principal component analysis
OPLS-DA	Orthogonal partial least squares discriminant analysis
DAMs	Differentially accumulated metabolites
VIP	Variable importance for the projection

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s43014-024-00291-z.

Supplementary Material 1

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Authors' contributions

YT and JF contributed to the generation of the idea for the experiment, funding acquisition, experimental design, manuscript writing and submission. KW, MG, DX and FC assisted in acquiring the resources and writing the first draft. GZ and MH performed the experiments. XL and YS participated in the investigation and analysis of the data. All authors proofread the manuscript before submission.

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Data availability
The metabolomic datasets used in this study are available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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References

Abed-Ashtiania, F., Arzanloua, M., Nasehib, A., Kadirb, J., Vadamalaib, G., & Azadmard-Damirchi, S. (2018). Plant tonic, a plant-derived bioactive natural product, exhibits antifungal activity against rice blast disease. *Industrial Crops and Products,112*, 105–112. https://doi.org/10.1016/j.indcrop.2017.11.013

Alazem, M., & Lin, N. S. (2015). Roles of plant hormones in the regulation of host-virus interactions. *Molecular Plant Pathology,16*(5), 529–540. https://doi.org/10.1111/mpp.12204

Alisaac, E., & Mahlein, A. K. (2023). Fusarium head blight on wheat: Biology, modern detection and diagnosis and integrated disease management. *Toxins,15*(3), Article 192. https://doi.org/10.3390/toxins15030192

Balcerzak, M., Harris, L. J., Subramaniam, R., & Ouellet, T. (2012). The feruloyl esterase gene family of *Fusarium graminearum* is differentially regulated by aromatic compounds and hosts. *Fungal Biology,116*(4), 478–488. https://doi.org/10.1016/j.funbio.2012.01.007

Biselli, C., Bagnaresi, P., Faccioli, P., Hu, X. K., Balcerzak, M., Mattera, M. G., Yan, Z., Ouellet, T., Cattivelli, L., & Valé, G. (2018). Comparative transcriptome profiles of near-isogenic hexaploid wheat lines differing for effective alleles at the 2DL FHB resistance QTL. *Frontiers in Plant Science., 9*, 37. https://doi.org/10.3389/fpls.2018.00037

Boerjan, W., Ralph, J., & Baucher, M. (2003). Lignin biosynthesis. *Annual Review of Plant Biology,54*, 519–546. https://doi.org/10.1146/annurev.arplant.54.031902.134938

Brauer, E. K., Rocheleau, H., Balcerzak, M., Pan, Y. L., Fauteux, F., Liu, Z. Y., Wang, L., Zheng, W. J., & Ouellet, T. (2019). Transcriptional and hormonal profiling of *Fusarium graminearum*-infected wheat reveals an association between auxin and susceptibility. *Physiological and Molecular Plant Pathology,107*, 33–39. https://doi.org/10.1016/j.pmpp.2019.04.006

Buhrow, L. M., Liu, Z. Y., Cram, D., Sharma, T., Foroud, N. A., Pan, Y. L., & Loewen, M. C. (2021). Wheat transcriptome profiling reveals abscisic and gibberellic acid treatments regulate early-stage phytohormone defense signaling, cell wall fortification, and metabolic switches following Fusarium graminearum challenge. *Bmc Genomics,22*(1), Article 798. https://doi.org/10.1186/s12864-021-08069-0

Cainong, J. C., Bockus, W. W., Feng, Y. G., Chen, P. D., Qi, L. L., Sehgal, S. K., Danilova, T. V., Koo, D. H., Friebe, B., & Gill, B. S. (2015). Chromosome engineering, mapping, and transferring of resistance to *Fusarium* head blight disease from *Elymus tsukushiensis* into wheat. *Theoretical and Applied Genetics,128*(6), 1019–1027. https://doi.org/10.1007/s00122-015-2485-1

Cho, M. H., & Lee, S. W. (2015). Phenolic phytoalexins in rice: Biological functions and biosynthesis. *International Journal of Molecular Sciences,16*(12), 29120–29133. https://doi.org/10.3390/ijms161226152

Cuthbert, P. A., Somers, D. J., & Brulé-Babel, A. (2007). Mapping of Fhb2 on chromosome 6BS: A gene controlling Fusarium head blight field resistance in bread wheat (Triticum aestivum L.). *Theoretical and Applied Genetics,114*(3), 429–437. https://doi.org/10.1007/s00122-006-0439-3

Dhokane, D., Karre, S., Kushalappa, A. C., & McCartney, C. (2016). Integrated metabolo-transcriptomics reveals Fusarium head blight candidate resistance genes in wheat QTL-Fhb2. *Plos one,11*(5), Article e0155851. https://doi.org/10.1371/journal.pone.0155851

Dong, Y. F., Xia, X. B., Ahmad, D., Wang, Y. H., Zhang, X., Wu, L., Jiang, P., Zhang, P., Yang, X. J., Li, G., & He, Y. (2023). Investigating the resistance mechanism of wheat varieties to Fusarium head blight using comparative metabolomics. *International Journal of Molecular Sciences,24*(4), Article 3214. https://doi.org/10.3390/ijms24043214

Du Fall, L. A., & Solomon, P. S. (2013). The necrotrophic effector SnToxA induces the synthesis of a novel phytoalexin in wheat. *New Phytologist,200*(1), 185–200. https://doi.org/10.1111/nph.12356

Duan, S. N., Jin, J. J., Gao, Y. T., Jin, C. L., Mu, J. Y., Zhen, W. C., Sun, Q. X., Xie, C. J., & Ma, J. (2022). Integrated transcriptome and metabolite profiling highlights the role of benzoxazinoids in wheat resistance against *Fusarium* crown rot. *Crop Journal,10*(2), 407–417. https://doi.org/10.1016/j.cj.2021.06.004

Fan, Y. H., Hou, B. Q., Su, P. S., Wu, H. Y., Wang, G. P., Kong, L. R., Ma, X., & Wang, H. W. (2019). Application of virus-induced gene silencing for identification of FHB resistant genes. *Journal of Integrative Agriculture,18*(10), 2183–2192. https://doi.org/10.1016/s2095-3119(18)62118-5

Feng, C., Liu, H., & Tang, Z. (2018). Fusarium graminearum inoculation on wheat head. *Bio-protocol,8*(15), e2964. https://doi.org/10.21769/BioProtoc.2964

Feng, J., Sun, L., Zhai, T., Liang, Q., Jiang, T., & Chen, Z. (2023). Preparation of cinnamaldehyde nanoemulsions: Formula optimization, antifungal activity, leaf adhesion, and safety assessment. *Industrial Crops and Products,200*, 116825. https://doi.org/10.1016/j.indcrop.2023.116825

Gardiner, D. M., Kazan, K., Praud, S., Torney, F. J., Rusu, A., & Manners, J. M. (2010). Early activation of wheat polyamine biosynthesis during Fusarium head blight implicates putrescine as an inducer of trichothecene mycotoxin production. *Bmc Plant Biology,10*, Article 289. https://doi.org/10.1186/1471-2229-10-289

Gardiner, D. M., Stephens, A. E., Munn, A. L., & Manners, J. M. (2013). An ABC pleiotropic drug resistance transporter of *Fusarium graminearum* with a role in crown and root diseases of wheat. *Fems Microbiology Letters,348*(1), 36–45. https://doi.org/10.1111/1574-6968.12240

Gauthier, L., Atanasova-Penichon, V., Chéreau, S., & Richard-Forget, F. (2015). Metabolomics to decipher the chemical defense of cereals against *Fusarium graminearum* and deoxynivalenol accumulation. *International Journal of Molecular Sciences,16*(10), 24839–24872. https://doi.org/10.3390/ijms161024839

Goodwin, P. H., Trueman, C., Loewen, S. A., & Tazhoor, R. (2018). Variation in the responsiveness of induced resistance against *Pseudomonas syringae* pv. *tomato* by *Solanum lycopersicum* treated with para-aminobenzoic acid. *Physiological and Molecular Plant Pathology,104*, 31–39. https://doi.org/10.1016/j.pmpp.2018.08.007

Grecco, S. D., Dorigueto, A. C., Landre, I. M., Soares, M. G., Martho, K., Lima, R., Pascon, R. C., Vallim, M. A., Capello, T. M., Romoff, P., Sartorelli, P., & Lago, J. H. G. (2014). Structural crystalline characterization of sakuranetin - an antimicrobial flavanone from twigs of *Baccharis retusa* (Asteraceae). *Molecules,19*(6), 7528–7542. https://doi.org/10.3390/molecules19067528

Gunnaiah, R., Kushalappa, A. C., Duggavathi, R., Fox, S., & Somers, D. J. (2012). Integrated metabolo-proteomic approach to decipher the mechanisms by which wheat QTL (Fhb1) contributes to resistance against Fusarium graminearum. *Plos One,7*(7), Article e40695. https://doi.org/10.1371/journal.pone.0040695

Hamzehzarghani, H., Paranidharan, V., Abu-Nada, Y., Kushalappa, A. C., Dion, Y., Rioux, S., Comeau, A., Yaylayan, V., & Marshall, W. D. (2008). Metabolite profiling coupled with statistical analyses for potential high-throughput screening of quantitative resistance to *Fusarium* head blight in wheat. *Canadian Journal of Plant Pathology,30*(1), 24–36. https://doi.org/10.1080/07060660809507493

Hasegawa, M., Mitsuhara, I., Seo, S., Okada, K., Yamane, H., Iwai, T., & Ohashi, Y. (2014). Analysis on blast fungus-responsive characters of a flavonoid phytoalexin sakuranetin; accumulation in infected rice leaves, antifungal activity and detoxification by fungus. *Molecules,19*(8), 11404–11418. https://doi.org/10.3390/molecules190811404

Huang, C., Jiang, Y., Wu, J., Qu, K., & Yang, J. (2019). Occurrence characteristics and reason analysis of wheathead blight in 2018 in China. *Plant Protection, 45*(2), 160–163. https://doi.org/10.16688/j.zwbh.2018414

Jiang, Y. H., Liu, T., Shi, X. C., Herrera-Balandrano, D. D., Xu, M. T., Wang, S. Y., and Laborda, P. (2023). *p*-Aminobenzoic acid inhibits the growth of soybean pathogen *Xanthomonas axonopodis* pv. *glycines* by altering outer membrane integrity. *Pest Management Science*, *79*(10), 4083-4093. https://doi.rog/10.1002/ps.7608.

Krátký, M., Konečná, K., Janoušek, J., Brablíková, M., Janďourek, O., Trejtnar, F., Stolaříková, J., & Vinšová, J. (2020). 4-Aminobenzoic acid derivatives: Onverting folate precursor to antimicrobial and cytotoxic agents. *Biomolecules,10*(1), Article 9. https://doi.org/10.3390/biom10010009

Kuete, V., Nono, E. C. N., Mkounga, P., Marat, K., Hultin, P. G., & Nkengfack, A. E. (2011). Antimicrobial activities of the CH_2Cl_2-CH_3OH (1: 1) extracts and compounds from the roots and fruits of *Pycnanthus angolensis* (Myristicaceae). *Natural Product Research,25*(4), 432–443. https://doi.org/10.1080/14786419.2010.522577

Kumar, V., Hatan, E., Bar, E., Davidovich-Rikanati, R., Doron-Faigenboim, A., Spitzer-Rimon, B., Elad, Y., Alkan, N., Lewinsohn, E., & Oren-Shamir, M. (2020). Phenylalanine increases chrysanthemum flower immunity against *Botrytis cinerea* attack. *Plant Journal,104*(1), 226–240. https://doi.org/10.1111/tpj.14919

Laborda, P., Li, C. H., Zhao, Y. Y., Tang, B., Ling, J., He, F., & Liu, F. Q. (2019). Antifungal metabolite *p*-aminobenzoic acid (pABA): Mechanism of action and efficacy for the biocontrol of pear bitter rot disease. *Journal of Agricultural and Food Chemistry,67*(8), 2157–2165. https://doi.org/10.1021/acs.jafc.8b05618

Lagudah, E. S., & Krattinger, S. G. (2019). A new player contributing to durable *Fusarium* resistance. *Nature Genetics,51*(7), 1070–1071. https://doi.org/10.1038/s41588-019-0454-3

Lahlali, R., Kumar, S., Wang, L. P., Forseille, L., Sylvain, N., Korbas, M., Muir, D., Swerhone, G., Lawrence, J. R., Fobert, P. R., Peng, G., & Karunakaran, C. (2016). Cell wall biomolecular composition plays a potential role in the host type II resistance to Fusarium head blight in wheat. *Frontiers in Microbiology,7*, Article 910. https://doi.org/10.3389/fmicb.2016.00910

Li, G. Q., Zhou, J. Y., Jia, H. Y., Gao, Z. X., Fan, M., Luo, Y. J., Zhao, P. T., Xue, S. L., Li, N., Yuan, Y., Ma, S. W., Kong, Z. X., Jia, L., An, X., Jiang, G., Liu, W. X., Cao, W. J., Zhang, R. R., Fan, J. C., … Ma, Z. Q. (2019). Mutation of a histidine-rich calcium-binding-protein gene in wheat confers resistance to Fusarium head blight. *Nature Genetics,51*(7), 1106-+. https://doi.org/10.1038/s41588-019-0426-7

Li, S., Pi, J., Zhu, H., Yang, L., Zhang, X., & Ding, W. (2021). Caffeic acid in tobacco root exudate defends tobacco plants from infection by Ralstonia solanacearum. *Frontiers in Plant Science,12*, 690586. https://doi.org/10.3389/fpls.2021.690586

Li, X., Zhong, S. F., Chen, W. Q., Fatima, S. A., Huang, Q. L., Li, Q., Tan, F. Q., & Luo, P. G. (2018). Transcriptome analysis identifies a 140 kb region of chromosome 3B containing genes specific to *Fusarium* head blight resistance in wheat. *International Journal of Molecular Sciences, 19*(3), 852. https://doi.org/10.3390/ijms19030852

Lima, V. N., Oliveira-Tintino, C. D. M., Santos, E. S., Morais, L. P., Tintino, S. R., Freitas, T. S., Geraldo, Y. S., Pereira, R. L. S., Cruz, R. P., Menezes, I. R. A., & Coutinho, H. D. M. (2016). Antimicrobial and enhancement of the antibiotic activity by phenolic compounds: Allic acid, caffeic acid and pyrogallol. *Microbial Pathogenesis,99*, 56–61. https://doi.org/10.1016/j.micpath.2016.08.004

Lionetti, V., Giancaspro, A., Fabri, E., Giove, S. L., Reem, N., Zabotina, O. A., Blanco, A., Gadaleta, A., and Bellincampi, D. (2015). Cell wall traits as potential resources to improve resistance of durum wheat against Fusarium graminearum. Bmc Plant Biology, 15, Article 6. doi: 10.1186/s12870-014-0369-1.

Liu, M. Y., Hong, G. J., Li, H. J., Bing, X. L., Chen, Y. M., Jing, X. F., Gershenzon, J., Lou, Y. G., Baldwin, I. T., & Li, R. (2023). Sakuranetin protects rice from brown planthopper attack by depleting its beneficial endosymbionts. *Proceedings of the National Academy of Sciences of the United States of America, 120*(23), e2305007120. https://doi.org/10.1073/pnas.2305007120

Ma, T. L., Zhang, L. X., Wang, M. H., Li, Y. Q., Jian, Y. Q., Wu, L., Kistler, H. C., Ma, Z. H., & Yin, Y. N. (2021). Plant defense compound triggers mycotoxin synthesis by regulating H2B ub1 and H3K4 me2/3 deposition. *New Phytologist,232*(5), 2106–2123. https://doi.org/10.1111/nph.17718

Michel, S., Wagner, C., Nosenko, T., Steiner, B., Samad-Zamini, M., Buerstmayr, M., Mayer, K., & Buerstmayr, H. (2021). Merging genomics and transcriptomics for predicting Fusarium head blight resistance in wheat. *Genes,12*(1), Article 114. https://doi.org/10.3390/genes12010114

Ninkuu, V., Yan, J. P., Fu, Z. C., Yang, T. F., Ziemah, J., Ullrich, M. S., Kuhnert, N., & Zeng, H. M. (2023). Lignin and its pathway-associated phytoalexins modulate plant defense against fungi. *Journal of Fungi,9*(1), Article 52. https://doi.org/10.3390/jof9010052

Pan, Y. L., Liu, Z. Y., Rocheleau, H., Fauteux, F., Wang, Y. L., McCartney, C., & Ouellet, T. (2018). Transcriptome dynamics associated with resistance and susceptibility against Fusarium head blight in four wheat genotypes. *Bmc Genomics,19*, Article 642. https://doi.org/10.1186/s12864-018-5012-3

Patzke, H., & Schieber, A. (2018). Growth-inhibitory activity of phenolic compounds applied in an emulsifiable concentrate - ferulic acid as a natural pesticide against *Botrytis cinerea*. *Food Research International,113*, 18–23. https://doi.org/10.1016/j.foodres.2018.06.062

Ponts, N., Pinson-Gadais, L., Boutigny, A. L., Barreau, C., & Richard-Forget, F. (2011). Cinnamic-derived acids significantly affect *Fusarium graminearum* growth and in vitro synthesis of type B trichothecenes. *Phytopathology,101*(8), 929–934. https://doi.org/10.1094/phyto-09-10-0230

Qi, L. L., Pumphrey, M. O., Friebe, B., Chen, P. D., & Gill, B. S. (2008). Molecular cytogenetic characterization of alien introgressions with gene *Fhb3* for resistance to *Fusarium* head blight disease of wheat. *Theoretical and Applied Genetics,117*(7), 1155–1166. https://doi.org/10.1007/s00122-008-0853-9

Qi, P. F., Balcerzak, M., Rocheleau, H., Leung, W., Wei, Y. M., Zheng, Y. L., & Ouellet, T. (2016). Jasmonic acid and abscisic acid play important roles in host-pathogen interaction between *Fusarium graminearum* and wheat during the early stages of *Fusarium* head blight. *Physiological and Molecular Plant Pathology,93*, 39–48. https://doi.org/10.1016/j.pmpp.2015.12.004

Qi, P. F., Johnston, A., Balcerzak, M., Rocheleau, H., Harris, L. J., Long, X. Y., Wei, Y. M., Zheng, Y. L., & Ouellet, T. (2012). Effect of salicylic acid on *Fusarium graminearum*, the major causal agent of *Fusarium* head blight in wheat. *Fungal Biology,116*(3), 413–426. https://doi.org/10.1016/j.funbio.2012.01.001

Salas, M. P., Reynoso, C. M., Céliz, G., Daz, M., & Resnik, S. L. (2012). Efficacy of flavanones obtained from citrus residues to prevent patulin contamination. *Food Research International,48*(2), 930–934. https://doi.org/10.1016/j.foodres.2012.02.003

Shigenaga, A. M., & Argueso, C. T. (2016). No hormone to rule them all: Interactions of plant hormones during the responses of plants to pathogens. *Seminars in Cell & Developmental Biology,56*, 174–189. https://doi.org/10.1016/j.semcdb.2016.06.005

Sidiq, Y., Nakano, M., Mori, Y., Yaeno, T., Kimura, M., & Nishiuchi, T. (2021). Nicotinamide effectively suppresses *Fusarium* head blight in wheat plants. *International Journal of Molecular Sciences, 22*(6), 2968. https://doi.org/10.3390/ijms22062968

Singh, J., Chhabra, B., Raza, A., Yang, S. H., & Sandhu, K. S. (2023). Important wheat diseases in the US and their management in the 21st century. *Frontiers in Plant Science,13*, 1010191. https://doi.org/10.3389/fpls.2022.1010191

Song, Y. P., Ren, Y. F., Xue, Y. H., Lu, D. D., Yan, T. Y., & He, J. Y. (2023). Putrescine (1,4-Diaminobutane) enhances antifungal activity in postharvest mango fruit against Colletotrichum gloeosporioides through direct fungicidal and induced resistance mechanisms. *Pesticide Biochemistry and Physiology,195*, Article 105581. https://doi.org/10.1016/j.pestbp.2023.105581

Sowinska, M., Morawiak, M., Bochyńska-Czyż M., Lipkowski, A., Ziemińska, E., Zabłocka, B. (2019). Molecular antioxidant properties and in vitro cell toxicity of the p-aminobenzoic acid (PABA) functionalized peptide dendrimers. Biomolecules, 9(3), Article 89. doi: 10.3390/biom9030089.

Steiner, B., Buerstmayr, M., Michel, S., Schweiger, W., Lemmens, M., & Buerstmayr, H. (2017). Breeding strategies and advances in line selection for *Fusarium* head blight resistance in wheat. *Tropical Plant Pathology,42*(3), 165–174. https://doi.org/10.1007/s40858-017-0127-7

Su, P. S., Zhao, L. F., Li, W., Zhao, J. X., Yan, J., Ma, X., Li, A. F., Wang, H. W., & Kong, L. R. (2021). Integrated metabolo-transcriptomics and functional characterization reveals that the wheat auxin receptor TIR1 negatively regulates defense against *Fusarium graminearum*. *Journal of Integrative Plant Biology,63*(2), 340–352. https://doi.org/10.1111/jipb.12992

Sunic, K., Brkljacic, L., Vukovic, R., Katanic, Z., Salopek-Sondi, B., and Spanic, V. (2023). Fusarium head blight infection induced responses of six winter

wheat varieties in ascorbate-glutathione pathway, photosynthetic efficiency and stress hormones. Plants-Basel, 12(21), Article 3720. https://doi.org/10.3390/plants12213720.

Wabo, H. K., Tatsimo, S. N., Tane, P., & Connolly, J. D. (2007). Pycnanthuquinone C: A new terpenoid-quinone from *Pycnanthus angolensis*. *Planta Medica,73*(2), 187–189. https://doi.org/10.1055/s-2007-967103

Walters, D. R., Walker, R. L., & Walker, K. C. (2003). Lauric acid exhibits antifungal activity against plant pathogenic Fungi. *Phytopathol,151*, 228–230. https://doi.org/10.1046/j.1439-0434.2003.00713.x

Wang, H. W., Sun, S. L., Ge, W. Y., Zhao, L. F., Hou, B. Q., Wang, K., Lyu, Z. F., Chen, L. Y., Xu, S. S., Guo, J., Li, M., Su, P. S., Li, X. F., Wang, G. P., Bo, C. Y., Fang, X. J., Zhuang, W. W., Cheng, X. X., Wu, J. W., Kong, L. R. (2020). Horizontal gene transfer of *Fhb7* from fungus underlies *Fusarium* head blight resistance in wheat. *Science, 368*(6493), eaba5435. https://doi.org/10.1126/science.aba5435. 844-+.

Wang, L. P., Li, Q., Liu, Z. Y., Surendra, A., Pan, Y. L., Li, Y. F., Zaharia, L. I., Ouellet, T., & Fobert, P. R. (2018). Integrated transcriptome and hormone profiling highlight the role of multiple phytohormone pathways in wheat resistance against Fusarium head blight. *Plos One,13*(11), Article e0207036. https://doi.org/10.1371/journal.pone.0207036

Wang, X., Li, G. Q., Jia, H. Y., Cheng, R., Zhong, J. K., Shi, J. X., Chen, R. T., Wen, Y. X., & Ma, Z. Q. (2024). Breeding evaluation and precise mapping of *Fhb8* for *Fusarium* head blight resistance in wheat (*Triticum aestivum*). *Plant Breeding,143*(1), 26–33. https://doi.org/10.1111/pbr.13113

Xue, S., Li, G. Q., Jia, H. Y., Xu, F., Lin, F., Tang, M. Z., Wang, Y., An, X., Xu, H. B., Zhang, L. X., Kong, Z. X., & Ma, Z. Q. (2010). Fine mapping Fhb4, a major QTL conditioning resistance to Fusarium infection in bread wheat (Triticum aestivum L.). *Theoretical and Applied Genetics,121*(1), 147–156. https://doi.org/10.1007/s00122-010-1298-5

Xue, S. L., Xu, F., Tang, M. Z., Zhou, Y., Li, G. Q., An, X., Lin, F., Xu, H. B., Jia, H. Y., Zhang, L. X., Kong, Z. X., & Ma, Z. Q. (2011). Precise mapping Fhb5, a major QTL conditioning resistance to Fusarium infection in bread wheat (Triticum aestivum L.). *Theoretical and Applied Genetics,123*(6), 1055–1063. https://doi.org/10.1007/s00122-011-1647-z

Yan, H., Meng, X. Y., Lin, X. F., Duan, N., Wang, Z. P., & Wu, S. J. (2023). Antifungal activity and inhibitory mechanisms of ferulic acid against the growth of Fusarium graminearum. *Food Bioscience,52*, 102414. https://doi.org/10.1016/j.fbio.2023.102414

Yuvamoto, P. D., & Said, S. (2007). Germination, duplication cycle and septum formation are altered by caffeine, caffeic acid and cinnamic acid in *Aspergillus nidulans*. *Microbiology,76*(6), 735–738. https://doi.org/10.1134/s0026261707060136

Zhang, P. P., Guo, C. J., Liu, Z., Bernardo, A., Ma, H. X., Jiang, P., Song, G. C., & Bai, G. H. (2021). Quantitative trait loci for *Fusarium* head blight resistance in wheat cultivars Yangmai 158 and Zhengmai 9023. *Crop Journal,9*(1), 143–153. https://doi.org/10.1016/j.cj.2020.05.007

Zhao, L., Su, P., Hou, B., Wu, H., Fan, Y., Li, W., Zhao, J., Ge, W., Xu, S., Wu, S., Ma, X., Li, A., Bai, G., Wang, H., & Kong, L. (2022). The black necrotic lesion enhanced fusarium graminearum resistance in wheat. *Frontiers Plant Science,13*, 926621. https://doi.org/10.3389/fpls.2022.926621

Zhao, P. Y., Gu, S. B., Han, C., Lu, Y. R., Ma, C. Y., Tian, J. C., Bi, J. J., Deng, Z. Y., Wang, Q. Q., & Xu, Q. (2021). Targeted and untargeted metabolomics profiling of wheat reveals amino acids increase resistance to Fusarium head blight. *Frontiers in Plant Science,12*, Article 762605. https://doi.org/10.3389/fpls.2021.762605

Zheng, T., Hua, C., Li, L., Sun, Z. X., Yuan, M. M., Bai, G. H., Humphreys, G., & Li, T. (2021). Integration of meta-QTL discovery with omics: Owards a molecular breeding platform for improving wheat resistance to *Fusarium* head blight. *Crop Journal,9*(4), 739–749. https://doi.org/10.1016/j.cj.2020.10.006

Zhu, G. Y., Chen, Y., Wang, S. Y., Shi, X. C., Herrera-Balandrano, D. D., Polo, V., & Laborda, P. (2022). Peel diffusion and antifungal efficacy of different fungicides in pear fruit: Structure-diffusion-activity relationships. *Journal of Fungi,8*(5), Article 547. https://doi.org/10.3390/jof8050547

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