Research Article

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Control of wheat powdery mildew using fluopyram seed treatment

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Abstract

BACKGROUND: Wheat powdery mildew is an airborne multi-cycle disease caused by *Blumeria graminis* f. sp. *tritici*. This disease can cause severe yield reduction or total crop loss. Fluopyram is a succinate dehydrogenase inhibitor (SDHI) used for the prevention and control of gray mold, powdery mildew, and downy mildew in fruits and vegetables.

RESULTS: We used fluopyram to treat wheat seeds and demonstrated excellent control of powdery mildew. Fluopyram treatment did not affect wheat seed germination. After seed treatment, the residual amounts of fluopyram in harvested wheat grains and in soil were lower than the maximum residue limit (MRL, 0.07 mg kg^{-1}). We explored the mechanism of action of fluopyram on wheat powdery mildew using eukaryotic reference transcriptome analysis. The differentially expressed genes (DEGs) in wheat plants treated with fluopyram were mostly enriched in the photosynthesis pathway. SPAD (soil–plant analysis development) value measurements showed a significant increase in chlorophyll content after treatment. The enzyme activity of chitinase and the relative expression levels of related genes (*Cht3* and *Cht4*) were significantly up-regulated, indicating that the defense response of wheat was activated.

CONCLUSION: Fluopyram seed treatment is expected to be developed for the control of wheat powdery mildew. The research in this study will provide important theoretical basis for controlling wheat powdery mildew caused by *Blumeria graminis* f. sp. *tritici* in the field.

Supporting information may be found in the online version of this article.

Keywords: wheat powdery mildew; fluopyram; seed treatment; residue and degradation dynamics; mechanism

1 INTRODUCTION

Leaf diseases can significantly reduce wheat yield. Powdery mildew is the main leaf disease of wheat in Jiangsu, China. It is caused by *Blumeria graminis* f. sp. *tritici*. Wheat powdery mildew has rapid outbreaks and can cause serious plant harm.[1] The degree of powdery mildew occurrence is affected by temperature, humidity, and rainfall.[2,3] High temperature and humidity promote disease development.[4,5] After initial infection by wheat powdery mildew, the pathogen can spread from the basal leaves to the flag leaves, gradually forming a white powdery mold layer on the stem and leaves. In severe cases, it can spread to the ears and awns, leading to reduced photosynthesis, increased respiration, increased evaporation intensity, and disrupted metabolism. This results in yellowing and leaf withering, which reduce wheat yield and quality.

Prevention and control of wheat powdery mildew in China mainly rely on agricultural control (selecting disease-resistant wheat varieties, strengthening water and fertilizer management, and eradicating diseased wheat seedlings) and chemical control.[6,7] However, the extensive application of inorganic nitrogen fertilizers and increased wheat plant density promote the occurrence of wheat powdery mildew. Also, *Blumeria graminis* f. sp. *tritici* exhibits a high degree of resistance to chemical controls.[8] Major-gene resistance to powdery mildew in wheat varieties with little quantitative background resistance can decrease or be lost over a short number of planting years.[9] Therefore, in China, the control of powdery mildew depends on stem and leaf sprays and seed treatments with triazole fungicides. However, due to long-term use of conventional triazole fungicides in China for controlling powdery mildew, *Blumeria graminis* f. sp. *tritici* have developed high resistance to them.[10,11] Currently, wheat seeds are treated with tebuconazole, difenoconazole, triadimefon, thiram, and carbendazim, which can effectively kill overwintering *Blumeria graminis* f. sp. *tritici* and have a good inhibitory effect on the early development of wheat powdery mildew.[12] However,

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the inhibition of later disease development is poor, and 1–2 stem and leaf sprays are also needed to effectively control disease development. In addition, because of fungicide damage to plants under low-temperature conditions, triazoles pose a high risk to wheat seedlings during seed treatment.[13] Therefore, it is important to identify new fungicides combined with different application methods to manage wheat powdery mildew.

Seed treatment technology can augment seed resistance.[14] Compared to the control technology of conventional stem and leaf spray treatments, seed treatments reduce both labor costs and pesticide use.[15,16] Triazole fungicides are commonly used as seed treatments to control wheat diseases. At present, seed treatment applications on wheat include using thiamethoxam and imidacloprid to prevent wheat aphids, using fludioxonil to prevent wheat crown rot, using triadimefon to prevent wheat powdery mildew and wheat rust, using difenoconazole to control wheat dwarf bunt, and using tebuconazole to reduce the disease index of wheat smut and wheat root rot and also increase wheat yield.[17–21]

Fluopyram is a succinate dehydrogenase inhibitor (SDHI) developed by Bayer Crop Science (Shanghai, China).[22] It inhibits mitochondrial respiration by hindering electron transfer of succinate dehydrogenase in the respiratory chain, and it also inhibits fungal growth. Fluopyram is used to manage diseases on over 70 crops, especially diseases caused by *Sclerotinia sclerotiorum*, *Botrytis cinerea*, and *Podosphaera xanthii*.[23,24] It is also an excellent nematicide.[25,26] Fluopyram has strong systemic activity. It can be absorbed by treated plant surfaces and translocated upward.[27,28] The favorable qualities of fluopyram suggested that it might be useful as a seed treatment agent to prevent wheat powdery mildew. In this study, we measured the control effect of fluopyram seed treatment on wheat powdery mildew in the field and evaluated the safety of seed treatment on wheat seed germination. We evaluated the residual dynamics of fluopyram seed treatment in wheat and its residues in soil. We also used eukaryotic reference transcriptome analysis to study the mechanism of fluopyram seed treatment in inhibiting wheat powdery mildew.

2 MATERIALS AND METHODS

2.1 Chemical compound, fungal strain, and wheat varieties

Technical-grade fluopyram (98%) was provided by AnaStandard (Yangzhou, China). This compound was dissolved in acetone at 10 000 μg mL^{-1} and stored at 4 °C prior to use. Technical-grade fluopyram (1000 μg mL^{-1} acetonitrile solution) was provided by Sinopharm (Beijing, China) as a standard for the determination of residue and degradation dynamics. A 41.7% fluopyram aqueous suspension concentrate (SC) and a 43% tebuconazole aqueous SC were provided by Bayer (Beijing, China).

The *Blumeria graminis* f. sp. *tritici* strain was isolated from infected wheat ears in a field where chemical fungicides had never been used to control wheat powdery mildew by single spore isolation. The strain was stored at the Central Laboratory, Zhenjiang Academy of Agricultural Sciences (Jurong, China).

Zhenmai 168 and Sumai 3 wheat varieties were provided by Zhenjiang Academy of Agricultural Sciences. Zhenmai 168 is susceptible to powdery mildew, and it was used for indoor and field experiments. When conducting the glasshouse induced disease experiment, Sumai 3 was planted around Zhenmai 168. During the jointing stage (Z31, Zadoks growth stage) of Sumai 3, the isolated *Blumeria graminis* f. sp. *tritici* strain was artificially inoculated.[29] After the outbreak of wheat powdery mildew in Sumai 3, it induced the occurrence of wheat powdery mildew on Zhenmai 168 in the field.

2.2 Potted plant test for fungicide control of wheat powdery mildew

Zhenmai 168 was used for the indoor potted plant test. Its seedlings were prepared for use during the third-leaf stage (Z13). The 10 000 μg mL^{-1} of fluopyram methanol solution was diluted with 0.1% Tween 80 aqueous solution to obtain concentrations of 0.1, 0.5, 1, 2, and 10 μg mL^{-1} for stem leaf spray treatments. At 24 h after the stem leaf spray, the fresh spores of powdery mildew produced within 24 h on the diseased wheat leaves were shaken off evenly onto potted wheat seedlings treated with fluopyram. The cultivation conditions for wheat powdery mildew were 19 °C and a 12 :12 h (light/dark) photoperiod.

The inoculation and culture method of powdery mildew on wheat with a fluopyram seed treatment were consistent with the method of fluopyram stem leaf spray treatment. The fluopyram seed treatment method was seed dressing, which involved mixing 41.7% fluopyram SC and wheat seeds with a small amount of water to ensure even coverage of the fungicide on the surface of the wheat seeds. The concentrations of fluopyram in the seed treatment were 0%, 0.1%, 0.2%, 0.5%, 1%, and 2% (The ratio of the mass of active ingredients in 41.7% fluopyram SC to the mass of wheat seeds.) At 7 days after inoculation, the disease index between blank controls and treatments was calculated to evaluate the indoor toxicity of fluopyram against wheat powdery mildew.[30] Each concentration was repeated three times within each experiment, and the experiment was repeated three times. Blank control meant that neither wheat seeds nor leaves have been treated with fungicides.

$$\text{Disease index} = \sum(\text{number of diseased leaves in each level} \times \text{relative level value}) / (\text{the total number of leaves investigated} \times 9) \times 100$$

Level 0: The wheat leaves do not develop wheat powdery mildew.

Level 1: The lesion area accounts for less than 6% of the entire leaf area.

Level 3: The lesion area accounts for 6% to 15% of the entire leaf area.

Level 5: The lesion area accounts for 16% to 25% of the entire leaf area.

Level 7: The lesion area accounts for 26% to 50% of the entire leaf area.

Level 9: The lesion area accounts for more than 50% of the entire leaf area.

2.3 RNA extraction, reverse transcription PCR, qPCR, and eukaryotic reference transcriptome analysis

The RNA Easy Fast Plant Tissue Kit (DP452; TIANGEN, Beijing, China) was used to extract the total RNA from wheat leaves. The HiScript II qRT SuperMix for quantitative real-time polymerase chain reaction (qPCR + gDNA wiper) (Vazyme, Nanjing, China) was used for reverse transcription polymerase chain reaction (PCR).[31] The ChamQ SYBR qPCR Master Mix (without ROX) (Vazyme) was used for qPCR.

During the jointing stage (Z31) of wheat, the flag leaves of five wheat plants for each treatment were conducted for total RNA

extraction. The collected fresh leaves were immediately manually ground with liquid nitrogen in a mortar. After grinding, the ground leaf tissues were immediately added to the lysis buffer in the RNA Easy Fast Plant Tissue Kit and quickly mixed (add 1 mL lysate buffer per 50–100 mg tissue). The experimental equipment for collecting and grinding leaves were needed to be disinfected in advance, and RNase free plastic products and pipette tips should be used to prevent RNA degradation and cross-contamination. Afterwards, total RNA extraction was performed according to the experimental steps in the RNA Easy Fast Plant Tissue Kit.

Then, reverse transcription PCR and qPCR were performed to determine the relative expression of the *Chi-3*, *Cht1*, *Cht2*, *Cht3*, and *Cht4* genes. *β-Actin* gene was used as reference gene. The $^{\Delta\Delta}$Ct method was used for the analysis of qPCR. The collected flag leaves were subjected to eukaryotic reference transcriptome analysis by Shanghai Majorbio Bio-pharm Technology Co., Ltd (Shanghai, China).

The primers used for qPCR to determine the relative expression of *Chi-3*, *Cht1*, *Cht2*, *Cht3*, and *Cht4* genes are listed in Table 1. The experiment was conducted in two plots, and each plot had three biological replicates. Here, a plot represented an independent experimental unit in our experimental design. Each plot also served as a biological replicate in this study and was incorporated as a random effect in the mixed-effects model approach.

2.4 Determination of chitinase activity

The blank control flag leaves and fluopyram-treated flag leaves were both collected during the jointing stage (Z31, early stage of wheat powdery mildew infection) of wheat. The flag leaves were used to determine the enzyme activity of chitinase with the Plant Chitinase ELISA kit (Ruifan, Shanghai, China).

2.5 Residue and degradation dynamics of fluopyram

Briefly, 0.04 mL of technical-grade fluopyram (1000 μg mL^{-1} acetonitrile solution) was dissolved in 3.92 mL of 80% acetonitrile to prepare a stock solution with a concentration of 10 000 μg mL^{-1} and stored at 4 °C prior to use. The stock solution was added to 80% acetonitrile to prepare standard samples with concentrations of 0.625, 1.25, 2.5, 5, 10, and 20 μg mL^{-1}. The residue and degradation dynamics of fluopyram were determined by liquid chromatography–mass spectrometry (LC–MS) with an Absciex 4500 mass spectrometer detector and an Agilent 1290 UPLC system (AB Sciex, Framingham, MA, USA).

Residue and degradation dynamics experiments were conducted in a glasshouse. Whole wheat plants were collected on 23 November 2022, 7 December 2022, 23 December 2022, 16 January 2023, 15 March 2023, 5 May 2023, and 23 May 2023 after seed treatment to determine the degradation dynamics of fluopyram in wheat. The soil from wheat roots was collected to determine the soil residues of fluopyram. Soil and wheat samples were dried and crushed at 50 °C. Then, 10 mL of 80% acetonitrile was added to 0.5 g of the sample and rotated for extraction at 26 °C for 40 min. The sample solution was centrifuged at 10 000 rpm and 26 °C for 5 min to obtain 2 mL of the supernatant. Then, 0.1 g of PSA purification agent was added to the supernatant, rotated, and purified for 10 min before centrifugation. Finally, after centrifugation, the supernatant was collected for analysis by LC–MS. The detection conditions of LC–MS are listed in Table 2. The experiment was conducted in two plots, and each plot had three biological replicates. Here, a plot represented an independent experimental unit in our experimental design. Each plot also served as a biological replicate in this study and was incorporated as a random effect in the mixed-effects model approach.

Table 1. Primers used in this study

Gene	Primer sequence	GeneBank number
Cht1	Cht1-F: GGCACCGACCTGCTCAAC Cht1-R: ATGATGTTGGTGATCACA	KR049247
Cht2	Cht2-F: GGGACCGACCTGCTCAAC Cht2-R: CGAAGGTTTAGGTGACTGC	KR049248
Cht3	Cht3-F: ACAGTCACCCAAACCTTCG Cht3-R: GGCCACCGTTGATGATGTTAG	KR049249
Cht4	Cht4-F: AGCACCGACCTGCTCAAT Cht4-R: TGTGATCACGTCGTGGCTC	KR049250
Chi-3	Chi-3-F: CTACTTAACAACCCGGAC Chi-3-R: TCATGGCTTGAGGGTTTC	KJ507387
β-Actin	ACTIN-F: CTCCCTCACAACAACCGC ACTIN-R: TACCAGGAACTTCCATACCAAC	AB181991.1

2.6 Determination of SPAD in wheat leaves

SPAD (soil–plant analysis development) determination on wheat flag leaves was accomplished by using a chlorophyll meter (SPAD-502 Plus; Konica Minolta, Tokyo, Japan). The experiment was conducted in two plots, and each plot had three biological replicates. Here, a plot represented an independent experimental unit in our experimental design. Each plot also served as a biological replicate in this study and was incorporated as a random effect in the mixed-effects model approach.

2.7 Field experiments and safety test for wheat sprouting

The test site was located in the test field of the Agricultural Science and Technology Innovation Center Park of Zhenjiang Academy of Agricultural Sciences. The test soil was 'Magan,' with medium fertility and an organic matter content of about 1.85%. The wheat varieties tested were Zhenmai 168 and Sumai 3. The disease conditions for field experiments were set as outdoor natural disease and glasshouse induced disease. When conducting the glasshouse induced disease experiment, Sumai 3 was used to induce the occurrence of wheat powdery mildew on Zhenmai 168. There were four treatments: seed treatment with 0.5% fluopyram, seed treatment with 1% fluopyram, stem leaf spray with 43% tebuconazole SC 225 mL hm^{-2}, and blank control. Each treatment had three repeats, yielding a total of 24 plots. The area of each plot was 20 m^2, and the plots were arranged in random blocks. The fluopyram seed treatment was performed on 15 November 2022, the sowing date was 16 November 2022, and the sowing method was strip sowing. The 43% tebuconazole SC stem leaf spray was made on the early stage of wheat powdery mildew (the outdoor natural disease experiment was conducted on 18 April 2023, and the glasshouse induced disease experiment was conducted on 5 March 2023). A 1.5 L handheld manual pneumatic sprayer was used for spraying, and the spray volume was 650 L hm^{-2} (1300 mL per plot). A single application was made. The blank control area was sprayed with an equivalent amount of water. The investigation on the occurrence of wheat powdery

Table 2. Detection conditions of liquid chromatography–mass spectrometry (LC–MS).

Detection conditions	
Chromatographic column	Agilent C18 column 100 mm × 2.1 mm, 1.7 μm)
Mobile phase A	0.1% formic acid–water
Mobile phase B	Acetonitrile
Column temperature	40 °C
Injection volume	1 μL
Velocity of flow	0.2 mL min^{-1}
Gradient elution procedure	50% A (0–0.7 min), 50–10% A (0.7–3 min), 10% A (3–4.5 min), 10–50% A (4.5–4.51 min), 50% A (4.51–7 min)
Ion source	Electrospray ion source
Scanning mode	Positive ion switching scanning
Detection method	Multi-response monitoring (MRM)
Curtain gas	35 kPa
Ionspray voltage	5500 kPa
Temperature	500 °C
Ion source gas1	40 °C
Ion source gas2	40 °C

mildew in the outdoor natural disease experiments was conducted on 28 April 2023. The investigation on the occurrence of wheat powdery mildew in the glasshouse induced disease experiment was conducted on 15 March 2023 and 3 April 2023. The five-point sampling method was used for field investigation. In each plot, we randomly selected five points and investigated 100 ears at each point, with a total of 500 ears. The number and severity of the disease were recorded, and the disease index and disease control effect (control effect of disease index) were calculated.

$$\text{Disease control effect} = (\text{disease index of blank control} - \text{disease index of the treatment}) / \text{disease index of blank control} \times 100\%.$$

Table 3. Effects of fluopyram seed treatment on emergence of wheat

Treatments	15 °C Emergence rate (%)	25 °C Emergence rate (%)
Blank control	95.00 ± 0.11 ^{a}	97.17 ± 0.14 ^{a}
0.1% Fluopyram seed treatment	94.67 ± 0.18 ^{abc}	96.74 ± 0.27 ^{ab}
0.2% Fluopyram seed treatment	94.00 ± 0.14 ^{bc}	96.87 ± 0.19 ^{ab}
0.5% Fluopyram seed treatment	94.13 ± 0.27 ^{bcd}	96.00 ± 0.24 ^{bc}
1% Fluopyram seed treatment	93.33 ± 0.24 ^{d}	94.98 ± 0.29 ^{c}
2% Fluopyram seed treatment	90.33 ± 0.38 ^{e}	91.30 ± 0.34 ^{d}

Note: Values are means and standard errors of six replicates. Means with different letters are significantly different ($P < 0.05$, analysis of variance, Tukey's honest significant difference, see Supporting Information Tables S1–S5).

Following treatment with 0%, 0.1%, 0.2%, 0.5%, 1%, and 2% fluopyram, the wheat seeds were placed on absorbent paper that was kept moist. The cultivation conditions for wheat seeds were 15 °C and 25 °C, with a 12 h:12 h (light/dark) photoperiod. After 5 days, the germination of wheat seeds with different treatments was scored.

2.8 Statistical analysis

For the experiment that involved only three treatments, we utilized the DPS software and performed the analysis using the least significant difference (LSD) method.

To evaluate the effects of fluopyram seed treatment on emergence rate of wheat seedlings, we initially used a mixed-effects model approach [lmer function from the lme4 package using R (version 4.3.1)] to account for the potential random effects of plots and experimental replicates, with treatment as the fixed effect. *Post hoc* comparisons were performed using Tukey's honest significant difference (HSD) test (emmeans package) to control for multiple comparisons.

The half maximal effective concentration (EC_{50}) values were calculated with the probit regression of the percentage of inhibition against the logarithmic value of fungicides concentrations.[32]

To estimate the half-life of the substance, we employed a nonlinear least squares (NLS) method to account for the fact that the initial measurement was taken on the seventh day. Using this method, we estimated the initial quantity N0, which were then used to calculate the decay constant (λ) and the half-life using the formula $T1/2 = \ln(2)/\lambda$. A confidence interval for the half-life T1/2 was also calculated to assess the precision of the estimate.

3 RESULTS

3.1 Sensitivity of *Blumeria graminis* f. sp. *tritici* to fluopyram

The sensitivity of the *Blumeria graminis* f. sp. *tritici* to fluopyram was tested by the potted plant method. In the fluopyram stem leaf spray treatment, the EC_{50} value of *Blumeria graminis* f. sp. *tritici* strain to fluopyram was 3.06 μg mL^{-1} ($y = 4.3937 + 1.2483x$, $r^2 = 0.9837$, y: probability value; x: logarithmic concentration of fungicide). For the fluopyram seed treatment, wheat seedlings treated with fluopyram did not develop wheat powdery mildew at the onset of the blank control disease. Continued observations for 7 days showed that the wheat seedlings treated with fluopyram did not develop wheat powdery mildew. These results suggested that the preventive effect of fluopyram seed treatment against wheat powdery mildew was better than that of the fluopyram stem leaf spray under the condition of artificial inoculation of wheat powdery mildew during the third-leaf stage (Z13) of wheat indoors.

3.2 Evaluation of the safety of fluopyram seed treatment on wheat

The effects of fluopyram seed treatment on the emergence rate of wheat seedlings were determined. We used multiple concentrations of fluopyram for seed treatment under conditions of 15 °C and 25 °C. At concentrations of 0.1%, 0.2%, 0.5% and 1% fluopyram seed treatment, the emergence rate of wheat seeds was greater than 90%. The germination of wheat seeds in the fluopyram seed treatment at 25 °C was slightly greater than the emergence rate at 15 °C (Table 3).

3.3 Residue and degradation dynamics of fluopyram seed treatment

The degradation of pesticides in the soil and plants is related to their environmental safety. Therefore, we measured the residue and degradation dynamics of fluopyram seed treatment in wheat and its soil residues. The half-life values of 0.5% and 1% fluopyram seed treatments in the field were 28.34 and 23.03 days, respectively (Table 4). The initial concentrations of fluopyram in 0.5% and 1% fluopyram seed treatments were 27.24 and 52.24 mg kg^{-1}, respectively. Following wheat harvest, the residual levels of fluopyram in wheat grains and soil were detected. The residual amounts in the seeds and soil for the 0.5% fluopyram seed treatment were 7.21 and 1.04 μg kg^{-1}, respectively. The residual amounts in the seeds and soil for the 1% fluopyram seed treatment were 4.41 and 11.39 μg kg^{-1}, respectively (Fig. 1).

3.4 Control effects of fluopyram seed treatment on wheat powdery mildew in the field

The earlier results indicated that fluopyram seed treatment has the potential for use in the control of wheat powdery mildew. We therefore set up two field conditions (outdoor natural disease experiment and glasshouse induced disease experiment) to explore the control effect of fluopyram seed treatment on wheat powdery mildew. We used 43% tebuconazole SC 225 mL hm^{-2} stem leaf spray as the standard control agent. Under natural or induced disease conditions, the 1% fluopyram seed treatment provided the best control of wheat powdery mildew, followed by 43% tebuconazole SC stem and leaf spray. The least effective treatment was the 0.5% fluopyram seed treatment. Under the condition of induced disease in a glasshouse, with the duration of powdery mildew, the control effect of either seed treatment or stem leaf spray treatment gradually diminished (Table 5).

3.5 Eukaryotic reference transcriptome analysis of wheat after seed treatment with fluopyram

We conducted eukaryotic reference transcriptome analysis on wheat leaves from plants treated with 1% fluopyram seed treatment and a blank control. Compared to the blank control group, the treatment group had a total of 3176 differentially expressed genes (DEGs), of which 700 genes were up-regulated and 2476 genes were down-regulated (Fig. 2(A)). Cluster analysis showed significant differences in gene expression profiles between the treatment group and the control group (Fig. 2(B)). Gene Ontology (GO) functional annotation analysis was conducted on DEGs. The results showed that in the molecular functions, DEGs were mainly enriched in GO terms such as catalytic activity and binding. In the cellular components, DEGs were mainly enriched in GO terms as membrane, membrane part, organelle, organelle part, and cell part. In biological processes, DEGs were mainly enriched in GO terms as cellular process and metabolic process (Fig. 2(C)). GO enrichment analysis was conducted on DEGs, and it showed that DEGs were mainly enriched in photosynthesis (light harvesting in photosystem I), green leaf volatile biosynthetic process, and lipoxygenase pathway (Fig. 3(A)). Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis was conducted on DEGs, and it showed that DEGs were mainly enriched in photosynthesis – antenna proteins, ribosome, carbon fixation in photosynthetic organism, glyoxylate and dicarboxylate metabolism, and photosynthesis pathways (Fig. 3(B)).

3.6 Effects of fluopyram seed treatment on enzyme activity and transcription level of chitinase in wheat

Chitinase plays an important role in wheat disease resistance. We determined if fluopyram seed treatment increased the activity of chitinase and enhanced the resistance to wheat powdery mildew. We studied the effects of fluopyram seed treatment on chitinase activity and the relative expression of related genes (*Chi-3*, *Cht1*, *Cht2*, *Cht3*, and *Cht4*) during the early stage of wheat powdery mildew infection (wheat jointing stage, Z31). The results showed that 1% fluopyram seed treatment effectively enhanced the activity of chitinase (Fig. 4) and the relative expression of *Chi-3* and *Cht4* genes in wheat (Fig. 5).

3.7 Fluopyram seed treatment can enhance the leaf color of wheat

Wheat powdery mildew infected wheat leaves have reduced photosynthesis that causes yield losses. In this study, we determined the effect of fluopyram seed treatment on the SPAD value of wheat. The SPAD value represents the relative content of chlorophyll and reflects plant greenness. The results showed that 1% fluopyram seed treatment significantly enhanced the SPAD value of wheat plant leaves (Table 6).

4 DISCUSSION

Wheat powdery mildew, caused by obligate parasitic fungus, can occur in various growth stages of wheat.[33] Wheat powdery mildew consumes wheat nutrients, leading to increased wheat respiration and transpiration and the reduction of carbohydrate accumulation and transportation. These effects weaken the stress resistance of wheat and make it more susceptible to disease.[34] Wheat powdery mildew infection in the early growth stage of wheat can affect the development of wheat roots and reduce wheat yield and quality.[35] Due to the variability of *Blumeria graminis* f. sp. *tritici* sensitivity and long-term fungicide use, *Blumeria graminis* f. sp. *tritici* has developed resistance to many triazole fungicides.[36,37] In 2009, 99.09% of *Blumeria graminis* f. sp. *tritici* strains in major wheat areas in China were resistant to triadimefon.[38] Previous study found that quinone outside inhibitors (QoIs), SDHIs, and some biogenic fungicides provide good inhibitory effects on wheat powdery mildew.[39,40]

With the implementation of straw returning to the field in China, the occurrence of wheat diseases has increased and the corresponding pesticide consumption has also increased. This trend is not compatible with green and sustainable development.[41,42] Seed treatment technology not only helps to prevent and control diseases but also enhances plant stress resistance. It is a technology that helps achieve a reduction in pesticide applications.

Table 4. Degradation dynamic parameters of fluopyram in wheat

Treatments	Dissipation equation	Half-life (days)	95% Confidence interval
0.5% Fluopyram seed treatment	$C_t = 27.24e^{-0.024t}$	28.34	(24.48, 33.17)
1% Fluopyram seed treatment	$C_t = 52.24e^{-0.030t}$	23.03	(19.95, 26.92)

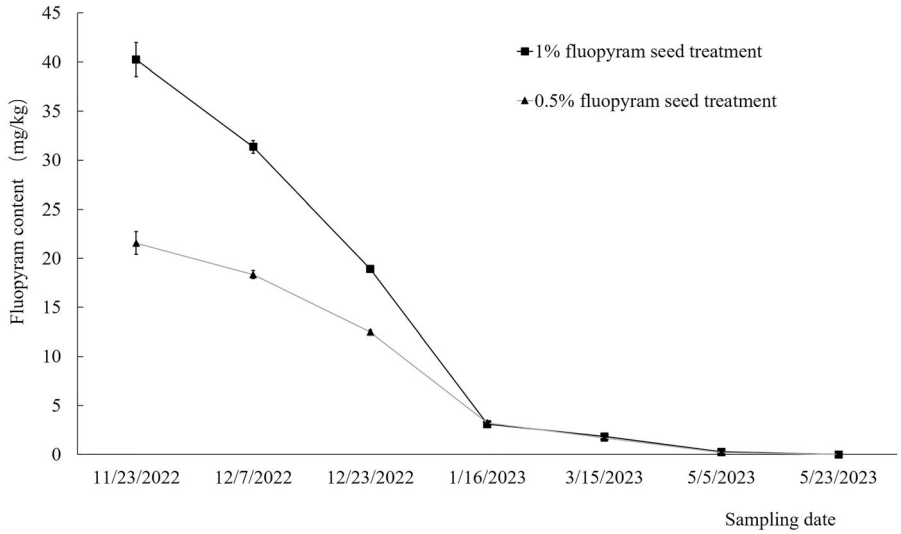

Figure 1. Degradation curve of fluopyram in wheat. Values are means and standard errors of six replicates. The degradation curve was based on actual monitoring data obtained from glasshouse conditions (Jurong, Jiangsu, China). Different regions and glasshouse conditions may lead to differences in degradation curves.

Table 5. Control effects of fluopyram seed treatment on Zhenmai 168 in field

	Disease control effect (%)		
	Outdoor natural disease experiment	Glasshouse induced disease experiment	
Treatments	28 April 2023	15 March 2023	3 April 2023
0.5% Fluopyram seed treatment	81.90 ± 0.38 [a]	68.32 ± 1.37 [a]	42.07 ± 0.86 [a]
1% Fluopyram seed treatment	92.64 ± 0.35 [c]	94.01 ± 0.55 [c]	78.05 ± 0.69 [c]
43% Tebuconazole SC 225 mL hm^{-2}	85.64 ± 0.58 [b]	89.16 ± 0.81 [b]	67.23 ± 0.54 [b]

Note: The tested fungicides were 41.7% fluopyram suspension concentrate (SC) and 43% tebuconazole SC. Values are means and standard errors of six replicates. Means with different letters are significantly different ($P < 0.05$, analysis of variance, LSD, see Supporting Information, Tables S6–S12).

Fluopyram has good control effects on cucumber powdery mildew and strawberry powdery mildew through stem and leaf spray, and root-knot nematode through irrigation root, dipping root and soil treatment.[43–45] At present, there is no registered seed treatment agent of fluopyram in China, but in Canada, a seed treatment agent (Velum® Rise) composed of fluopyram and penflufen has been registered for the prevention and control of soil borne diseases caused by *Rhizoctonia solani* and nematodes. Furthermore, 57.1% fluopyram seed treatment suspension (ILeVO) was registered in Australia for the prevention and control of rape black stem rot and soybean sudden death syndrome caused by Fusarium.[46] Currently, there is no research on the control of wheat powdery mildew with fluopyram, especially the control methods of seed treatment. In this study, we found that fluopyram seed treatment provided good control of wheat powdery mildew. Meanwhile, the emergence rate of wheat seeds remained above 90% after 1% fluopyram seed treatment.

The long-term application of chemical agents can also cause some problems, such as excessive residue, plant phytotoxicity, and impact on the ecological environment. Due to its strong plant absorption, fluopyram can remain in the edible parts of fruits and vegetables. Research has been conducted on the residual behavior of fluopyram in apples, watermelons, pomegranates, and mangoes.[47–50] Nematicides are usually applied through soil treatment to prevent and control nematode disease, which can cause plant phytotoxicity, such as dazomet, metham-sodium, fluensulfone and fluopyram.[51] In view of this, the residue and degradation dynamics of fluopyram in wheat and the residues in soil after seed treatment were studied. We found that the residual amounts of fluopyram on harvested wheat grains and in soil were lower than the maximum residue limit (MRL, 0.07 mg kg^{-1}). The types and changes of soil microorganisms can have complex impacts on crop growth, such as *Aspergillus flavus*, which can harm peanuts and corn.[52] But there are also beneficial microorganisms, such as actinomycetes, which can produce antibiotics to inhibit pathogens and secrete cytokinin to promote crop growth.[53] Further research is needed to study whether the use of fluopyram seed treatment will affect beneficial microorganisms or have a control effect on soil borne diseases such as wheat stem rot and wheat sharp eyespot.

In addition, to explore the mode of action of fluopyram seed treatment for managing wheat powdery mildew, we used a eukaryotic reference transcriptome to analyze the expression of DEGs in wheat plants after treatment. GO enrichment analysis and KEGG enrichment analysis showed that DEGs have the highest enrichment in photosynthesis-related pathways. And after treatment, the chlorophyll content of wheat leaves also significantly increased. Photosynthesis not only provides energy for

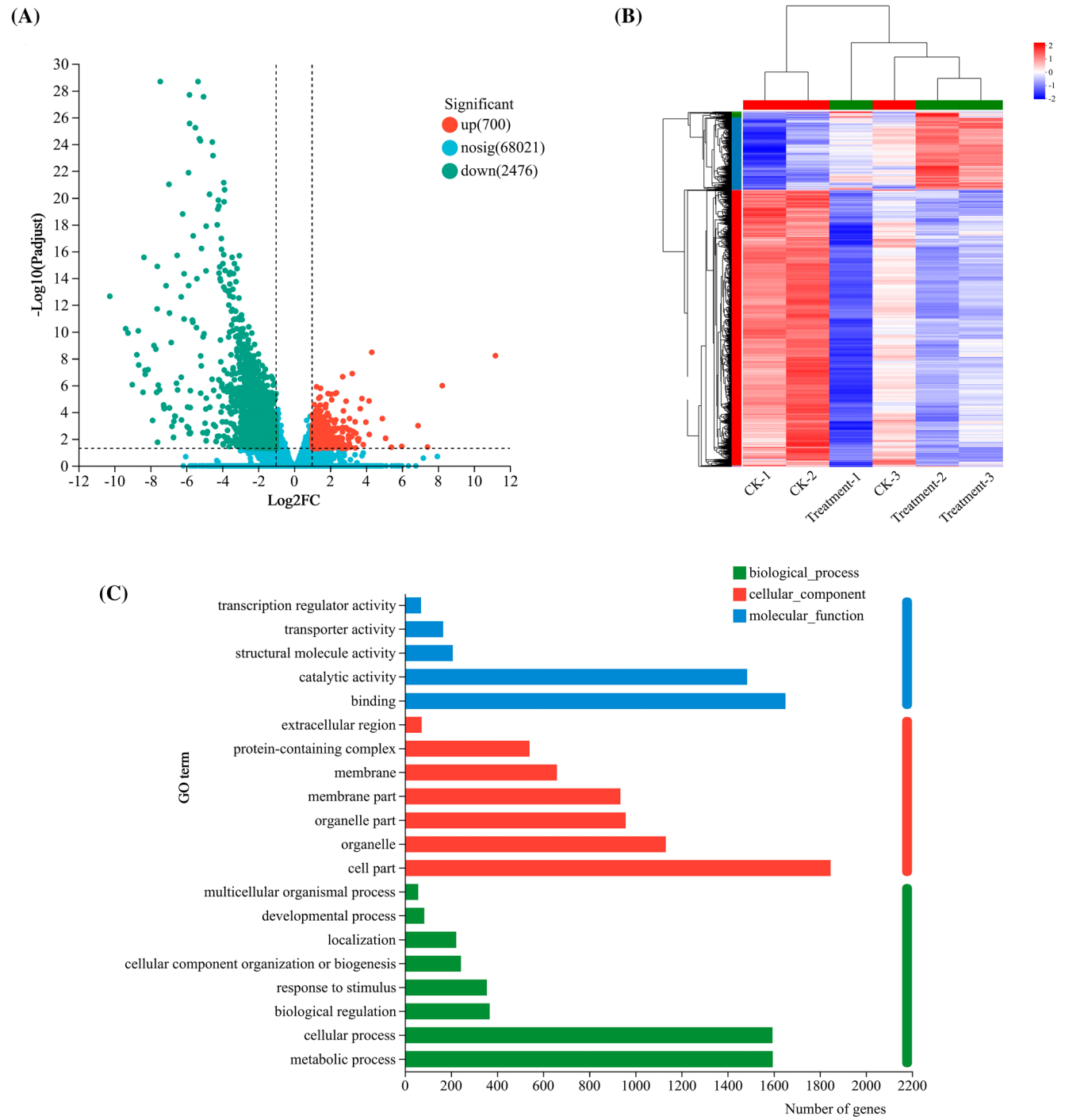

Figure 2. Volcano map, cluster analysis heatmap, and GO annotation analysis of differentially expressed genes (DEGs) of Zhenmai 168 treated with 1% fluopyram. Volcano map (A), cluster analysis heatmap (B), and GO annotation analysis (C) of DEGs of Zhenmai 168 treated with 1% fluopyram. Fold change (FC) in $\log_2$FC represents the expression level of the treatment group and control group (A and B). The horizontal axis represents the number of DEGs annotated to GO terms, and the vertical axis represents the GO terms (C).

plants but also generates various antioxidant substances (peroxidase and superoxide dismutase), which can effectively reduce harmful oxygen free radicals in cells. This reduces the oxidative stress level in plant cells and enhances their resistance to stress.[54] In addition, photosynthesis is closely related to plant disease resistance. Plants can synthesize and accumulate many disease-resistance substances (antimicrobial peptides and substance phosphorus) through the energy and organic matter generated by photosynthesis. This process enhances plant resistance to pathogenic microorganisms.[55]

Chitinase plays an important role in plant defense and is closely related to plant disease resistance. It can hydrolyze chitin in the cell wall during the early stage of mycelial growth, resulting in the rupture of pathogenic bacterial cells.[56] Chitinase activity has been detected in over 100 plant species, including rice, wheat, and cotton.[57] When induced by pathogen infection, virus

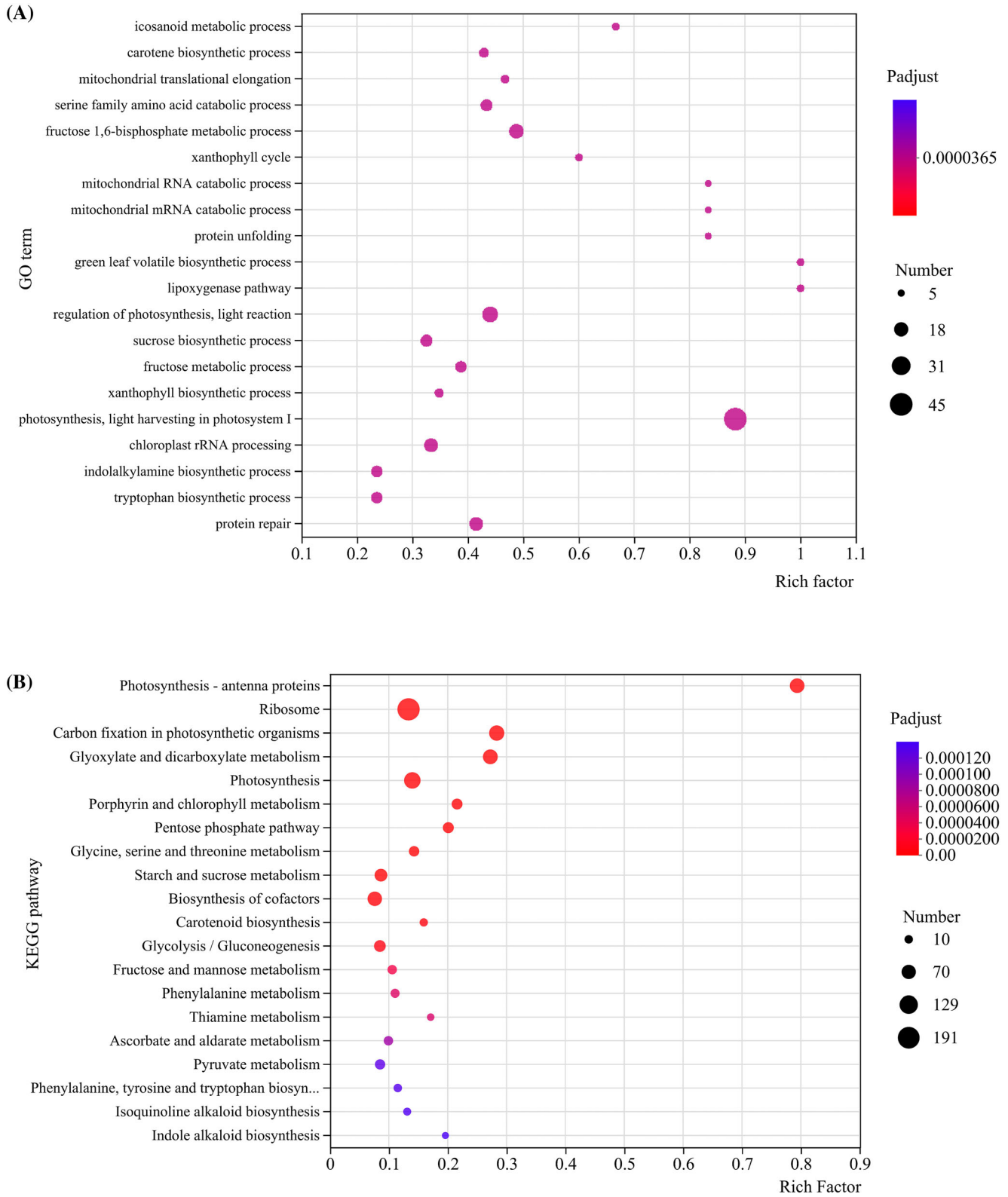

Figure 3. GO enrichment analysis and KEGG enrichment analysis of differentially expressed genes (DEGs) of Zhenmai 168 treated with 1% fluopyram. GO enrichment analysis (A) and KEGG enrichment analysis (B) of DEGs of Zhenmai 168 treated with 1% fluopyram. The horizontal axis represents the rich factor of DEGs annotated to GO terms (A) and KEGG pathways (B). The vertical axis represents the enriched GO terms (A) and KEGG pathways (B). The dots from purple to red indicate the *P*-adjusted value from large to small, and the size of the dots indicates the number of DEGs annotated to GO terms (A) and KEGG pathways (B).

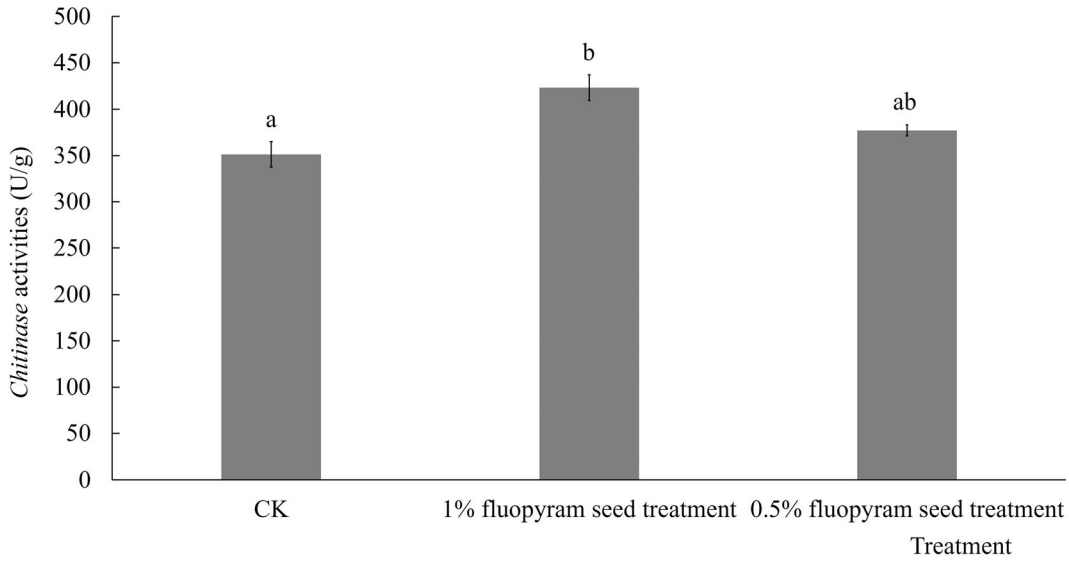

Figure 4. Effects of fluopyram seed treatment on the activity of *chitinase* in wheat. Values are means and standard errors of six replicates. Means with different letters are significantly different ($P < 0.05$, analysis of variance, LSD, see Supporting Information Tables S13–S15).

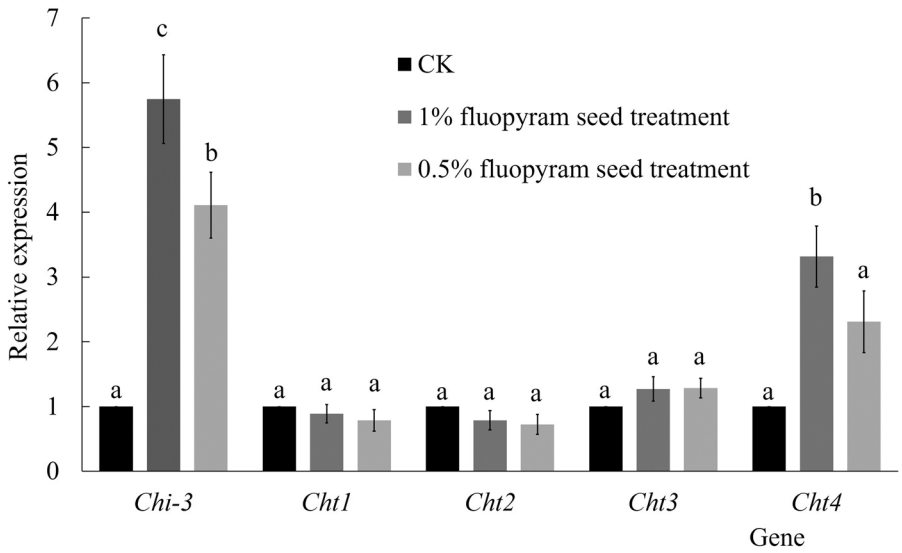

Figure 5. Effects of fluopyram seed treatment on the expression of *Chi-3*, *Cht1*, *Cht2*, *Cht3*, and *Cht4* genes in wheat. Values are means and standard errors of six replicates. Means with different letters are significantly different ($P < 0.05$, analysis of variance, LSD, see Supporting Information Tables S16–S26). Multiple comparisons for the different treatments are restricted to within each gene, and no multiple comparisons were conducted between different genes. The expression of *Chi-3*, *Cht1*, *Cht2*, *Cht3*, and *Cht4* genes in wheat were relative to that of *β-actin*.

Table 6. Effects of fluopyram seed treatment on SPAD (soil–plant analysis development) value in wheat

	SPAD value		
	Outdoor natural disease experiment		Glasshouse induced disease experiment
Treatments	24 March 2023	11 May 2023	24 March 2023
0.5% Fluopyram seed treatment	33.29 ± 0.26 [a]	33.46 ± 0.20 [b]	27.63 ± 0.21 [a]
1% Fluopyram seed treatment	33.92 ± 0.27 [a]	33.83 ± 0.14 [b]	28.88 ± 0.30 [b]
Blank control	33.09 ± 0.30 [a]	32.79 ± 0.20 [a]	27.08 ± 0.26 [a]

Note: Values are means and standard errors of six replicates. Means with different letters are significantly different ($P < 0.05$, analysis of variance, LSD, see Supporting Information, Tables S27–S33).

infection, or other abiotic factors, the activity of chitinase in plants rapidly increases. *In vitro* antibacterial experiments have demonstrated that different chitinases have inhibitory effects on the spore germination and mycelial growth of more than 20 pathogenic fungi, including *R. solani*, *Botrytis cinerea*, *Fusarium oxysporum*, *Fusarium graminearum*, and *Verticillium dahlia*.[58,59] Chitinase is distributed in the roots, stems, leaves, flowers, seeds, and callus of plants, but the content of chitinase varies in different tissues.[60] The five chitinase genes (*Chi-3*, *Cht1*, *Cht2*, *Cht3*, and *Cht4*) in this study had the highest expression in wheat leaves. We found that in the early stage of wheat powdery mildew infection, the activity of chitinase and the relative expression of related genes (*Chi-3* and *Cht4*) were significantly up-regulated in wheat leaves after fluopyram seed treatment. This indicated that fluopyram seed treatment can effectively activate the defense response of wheat to resist powdery mildew infection.

As is well known, fluopyram achieves the goal of preventing and controlling diseases by inhibiting the respiratory function of pathogenic bacteria. This study found that fluopyram seed treatment could enhance wheat disease resistance by improving photosynthesis and chitinase activity. In plants, photosynthesis and respiration are interdependent. Further research is needed to study whether fluopyram, as respiratory inhibitors, can enhance photosynthesis by affecting respiration in wheat. Endophytic fungi play a significant role in plant disease resistance. Both carbendazim and fludioxonil have inhibitory effect on endophytic fungi isolated from wheat ears, but the effect of phenamacril, which have specific activity against Fusarium, on endophytic fungi isolated from wheat ears is weak.[61] Therefore, further research is needed to study whether fluopyram will affect the quantity or activity of beneficial endophytic fungi in wheat, leading to a decrease in plant resistance to other diseases.

5 CONCLUSION

Fluopyram seed treatment provided good control of wheat powdery mildew in the field. It improved the photosynthesis and chitinase activity of wheat, which enhanced stress resistance and resistance to powdery mildew infection. Fluopyram seed treatment did not affect wheat germination, and fluopyram residues in soil and wheat grains were lower than the safety residue standards. Based on the results, fluopyram is expected to be developed as a new agent for controlling wheat powdery mildew. Seed treatment results using fluopyram were better than the spray treatment. Fluopyram improves the disease resistance of wheat, and it may also generate secondary metabolites in wheat that provide a superior control effect on wheat powdery mildew.

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CONFLICT OF INTEREST STATEMENT

No conflict of interest exits in the submission of this manuscript, and the manuscript is approved by all authors for publication.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

AUTHOR CONTRIBUTIONS

Hongfu Yang and Chao Xu conceived the ideas and designed the methodology. Chao Xu, Hong Zhang, Qinyan Wu and Xuebiao Zhang performed the experiments. Chao Xu analyzed the data and wrote the first draft of the manuscript. Xiaomeng Guo, Xiaoya Tian and Hongzhou Chen edited the manuscript.

SUPPORTING INFORMATION

Supporting information may be found in the online version of this article.

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