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Characterization of the triadimefon resistant *Puccinia striiformis* f. sp. *tritici* isolates in China

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Abstract
Wheat stripe rust, caused by *Puccinia striiformis* f. sp. *tritici* (*Pst*), is a devastating disease that seriously threatens the production of crops worldwide. Triadimefon is the widely-used fungicide for controlling the disease in China; however, as the fungicide targets a single site (position 401 in the 134th codon of the *Cyp51* gene), the extensive application imposes a strong selection pressure on the pathogens, which may potentially lose the effect over time. In this study, 176 *Pst* field isolates sampled from different regions of Xinjiang were determined for their sensitivity to triadimefon because it is the few frequent *Pst* outbreak and representative area in China. The results showed that the *Pst* isolates collected from Yili, Xinjiang, exhibited a strong resistance to triadimefon with an average EC_{50} of 0.263 μg/mL, despite the rest of the isolates maintaining high sensitivity to triadimefon. The triadimefon-resistant and triadimefon-sensitive isolates did not display significant differences in sporulation, but the triadimefon-resistant isolates exhibited weaker adaptive traits in their latent period and urediniospore germination rate than the triadimefon-sensitive isolates. No cross-resistance was found for the other two fungicides, flubeneteram or pyraclostrobin; however, cross-resistance for the demethylation inhibitor (DMI) fungicides, tebuconazole and hexaconazole, was found. Genome sequencing revealed that the Tyrosine (Y) at 134 residue was mutated to Phenylalanine (F) in the Xinjiang isolates. Our study revealed that a natural mutation in *Pst* led to the efficacy loss of triadimefon to control the disease.

Keywords Wheat stripe rust, *Puccinia striiformis* f. sp. *tritici*, Triadimefon, Fungicide sensitivity

Background

Wheat stripe rust, a serious fungal disease caused by *Puccinia striiformis* f. sp. *tritici*, can lead to severe yield losses of wheat (Chen 2005; Wellings 2011). Located in the northwest of China, the wheat production in Yili accounts for two-thirds of cereals in Xinjiang region. Wheat stripe rust in Xinjiang region is characteristically severe than in other wheat-producing regions of China (Chen et al. 2023). Yili County is one of the severely suffered regions of wheat stripe rust, which is largely due to its mixed planting of winter and spring wheat and the humid climate (Li et al. 2017; Ma 2018). In the last two decades, the disease outbreak area and the resulting wheat yield lost in Yili County have increased each year, and the yield loss of wheat was up to 20–50% in pandemic years (Li et al. 2010). Deployment of rust-resistant wheat varieties has been one of the most effective measures to control wheat stripe rust outbreak, but the fast evolvement of *Pst* often leads to the rapid loss of resistance in cultivated varieties (Ma 2018; Zhao and Kang 2023). Alternatively, fungicides have been extensively used to control the disease (Carmona et al. 2020; Brandt et al. 2021).

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There are over twenty registered fungicides used to control wheat stripe rust in China (http://www.chinapesticide.org.cn/). Of which, triadimefon, thifluzamide, and azoxystrobin are widely-used, which inhibit the fungal protein demethylation, the succinate dehydrogenase activity, and quinone-outside-inhibitors, respectively. Triadimefon, the first demethylation inhibitor (DMI)-type fungicide that was developed and released to market by Bayer Co. in 1976, is the most widely used fungicide against stripe rust (Stammler et al. 2009; Kang et al. 2010). It is the first commercially used triazole fungicide in China, which was registered and applied to control wheat rust disease in 1981 (Zhang et al. 2004). In the past 40 years, triadimefon has successfully controlled wheat stripe rust, leaf rust, and powdery mildew with the merits of low toxicity, broad spectrum, and low price (Line 2002; Kang et al. 2019). However, the selection pressure imposed by persistent fungicide use can lead to the rapid development of pathogens (Chen 2014). It has been reported that many phytopathogens are developing resistance to different types of fungicides (Liu et al. 2022).

The risk level assessment indicated that many biological characteristics of *Pucciniales* spp., such as a short life cycle, rapid reproduction rate, airborne nature of spores, and the sexual and asexual reproductive patterns, are highly similar to the identified fungicide-resistant pathogens, such as *Blumeria* and *Phytophthora* spp., although all rust fungi were classified as low-risk fungi according to Fungicide Resistance Action Committee (FRAC) in 2007 (Brent and Hollomon 2007; Grimmer et al. 2015). Following the report of decreased sensitivity of wheat leaf rust to triazole fungicides (Stammler et al. 2009), resistance to triazole fungicides in soybean rust was also found in South America and Asia (Schmitz et al. 2014). We previously identified a *Pst* parental isolate that displayed resistance to triadimefon with an EC_{50} value of 1.88 μg/mL, and the progeny showed EC_{50} values between 0.06 and 7.89 μg/mL. In the progenies, twenty-six of them were more resistant to triadimefon than the parental isolate (Tian et al. 2019). The resistance of wheat stem rust, stripe rust, and leaf rust to triazole fungicides has been repeatedly reported in China recently (Wu et al. 2020; Zhan et al. 2022; Ji et al. 2023), which should deserve attention and further study.

Resistance risk assessment is important to prevent and defer the development of pathogens for fungicide resistance (Grimmer et al. 2014). It is important to make a resistance risk assessment for the prevalent *Pst* isolates in fields (Miao et al. 2020). Cross-resistance investigation is also necessary to determine whether a given fungicide-resistant pathogen is resistant to other fungicides, which is also important for deferring the resistance development of fungal pathogens. Zhan et al. (2022) conducted a triadimefon resistance assessment on 446 *Pst* isolates which were nation widely collected. The result showed that the fungicide turned out from low-risk use to a moderate risk, which provided guidance for the use of triadimefon and similar fungicides in the wheat-producing regions in China.

This study aimed to determine the resistance risk levels of *Pst* isolates sampled from Yili County by the sensitivity test to triadimefon and other widely used fungicides in Yili County, in order to guide the fungicide use scientifically.

Results

Effect of triadimefon on *Pst* pisolates from Yili County

In this study, the sensitivity to triadimefon in 176 *Pst* isolates from six counties in Yili region was determined. The results are shown in Table 1 (the sensitivity is shown in Additional file 1: Table S2). The EC_{50} values range from 0.007 to 2.000 μg/mL with a mean EC_{50} value of 0.263 μg/mL in the isolates. The sensitivity of *Pst* isolates to triadimefon varied in the samples from different counties. Among them, the average EC_{50} value in Chaxian (CX) and Xinyuan (XY) were significantly higher than other four counties. The average EC_{50} value of *Pst* was highest in CX (0.370 μg/mL), followed by that in XY (0.251 μg/mL). The average EC_{50} values for the isolates collected from TKS (Tekesi), ZS (Zhaosu), NLK (Nileke), and GL (Gongliu) counties were 0.222, 0.181, 0.178, and 0.140 μg/mL, respectively.

Resistance levels of *Pst* population

The EC_{50} value for triadimefon on *Pst* was reported to be the sensitive baseline of 0.19 μg/mL (Zhan et al. 2022). Based on this criteria, we showed that there were nine low-resistance isolates and two medium-resistance isolates in Yili County (Table 2), which account for 5.114% and 1.136% of the total sampled population. The remaining 93.75% isolates were all sensitive to triadimefon,

Table 1 EC_{50} value of *Puccinia striiformis* f. sp. *tritici* in Yili County

Location (county)	Range of EC_{50} (μg/mL)	Mean EC_{50} (μg/mL)[a]
Cha (CX)	0.025–0.271	0.128bc
Nileke (NLK)	0.054–0.173	0.128bc
Tekesi (TKS)	0.064–0.329	0.131bc
Zhaosu (ZS)	0.059–0.176	0.123d
Xinyuan (XY)	0.058-1.100	0.157a
Gongliu (GL)	0.007–0.650	0.140b

[a] EC_{50}, half-maximal effective concentration, means 50% inhibition of urediniospore germination. Different letters after the data indicate statistically significant differences ($P < 0.05$), which tested by Duncan's multiple range test

Table 2 Level and frequency of triadimefon resistance in *Puccinia striiformis* f. sp. *tritici*

Location (county)	Frequency of different RI[a] values			Mean RI[b]	Highest RI	Frequency of resistance
	$5 < R \leq 10$	$10 < R \leq 40$	$RI > 40$			
Cha (CX)	8.696	0.000	0.000	0.673 cd	1.428	8.696
Nileke (NLK)	0.000	0.000	0.000	0.676 cd	0.912	0.000
Tekesi (TKS)	6.667	0.000	0.000	0.689c	1.733	6.667
Zhaosu (ZS)	0.000	0.000	0.000	0.650d	0.925	0.000
Xinyuan (XY)	2.857	2.857	0.000	0.828a	5.788	5.714
Gongliu (GL)	9.756	2.439	0.000	0.736b	3.421	12.195

[a] Resistance index, the resistance index (RI) = EC_{50} of the tested isolate/EC_{50} of the sensitive isolate

[b] Different letters after the data indicate statistically significant differences ($P < 0.05$), which tested by Duncan's multiple range test

and no isolates exhibited high resistance to triadimefon (RI > 40). According to the race identification results (Additional file 1: Table S3), the medium-resistant isolate was CYR34 and low-resistance isolates were CYR33 and G22-083. Notably, CYR34 and CYR33 are the dominant races in Yili.

In the six counties, the most triadimefon-resistant isolates were found in GL, where 12.195% of the isolates are resistant. In CX, the resistance frequency was 8.696%, slightly lower than those in GL. Among the resistant isolates, two medium resistance isolates were detected in GL and XY, with a resistance index of 10.026 and 10.525, respectively. However, low resistance isolates accounted for 8.696, 3.333, 2.857, and 12.195% in CX, TKS, XY, and GL, respectively. All isolates from NLK and ZS regions were sensitive to triadimefon.

The parasitic fitness of the isolates

To determine whether parasitic fitness was compromised in triadimefon-resistant isolates, eight triadimefon-resistant and twelve triadimefon-sensitive isolates were selected to determine spore germination rate, latent period, sporulation intensity, and spot expansion rate. Table 3 showed that there were no significant differences in triadimefon-resistant and -sensitive isolates in regarding of sporulation intensity and lesion expansion rate. However, the resistant isolates showed weaker latent period and lower spore germination rate than the sensitive isolates, where the latent period in resistant isolates was significantly longer than that of the sensitive isolates; in addition, the spore germination rate in resistant isolates was significantly lower than that of the sensitive isolates.

Parasitic fitness assays revealed that the relative parasitic fitness of the resistant isolates was lower than that of the sensitive isolates. However, three of the eight resistant isolates displayed relatively high parasitic fitness levels, indicating that their viability was not low compared to the sensitive isolates, which may facilitate them to be the dominant races in field.

Cross-resistance on four other fungicides

Eight isolates with triadimefon-resistant and twelve sensitive isolates were selected to test EC_{50} values for *Pst* against the four fungicides using the detached leaf method (Table 4). The correlation results showed that there was no cross-resistance between triadimefon and the two other fungicides, flubeneteram, and pyraclostrobin ($\rho < 0.3$) (Fig. 1). However, there was a cross-resistance to DMI fungicides, with a low correlation between triadimefon and tebuconazole ($0.3 \leq \rho < 0.5$) and a moderate correlation between triadimefon and hexaconazole ($0.5 \leq \rho < 0.8$). Thus, isolates that were resistant to triadimefon also became resistant to tebuconazole and hexaconazole.

Control efficacy on triadimefon-resistant and -sensitive isolates

Medium resistance isolate XY8-6, low resistance isolate CX4-4, and sensitive isolate TKS2-4 were selected for protective and therapeutic tests, respectively. As shown in Fig. 2, the medium resistance isolates still produced spores after the 260 μg/mL fungicide application, but the low resistance isolate produced few spores and even no spores under a therapeutic treatment. Interestingly, the sensitive isolate treated with 260 μg/mL triadimefon displayed inhibited spore growth. These results indicated that triadimefon was more effective in the sensitive isolates.

Determination of the *CYP51* gene mutation sites by KASP

SNP genotyping, using primers from Zhan et al. (2022) on 176 *Pst* isolates, results were consistent with the detached leaf method (Table 5). Of the isolates, some showed resistance to triadimefon in bioassay, and the mutations were detected at the 134th amino acid residues

Table 3 Comparison of parasitic fitness indices between triadimefon-sensitive and triadimefon-resistant isolates of *Puccinia striiformis* f. sp. *tritici*

Isolates/ sensitivity[a]	Urediniospore germination rate (%)[b]	Latent period (h)[b]	Sporulation intensity (spore×10^4/mm^2)[b]	Lesion expansion speed (mm)[b]	Relative parasitic fitness[b]
GL 3-2/LR	76.33c	233.20a	5.60a	6.90a	1.83ab
TKS1-1/LR	79.33c	232.13a	7.46a	8.72a	2.55a
GL 1-2/LR	75.33c	214.40b	5.79a	7.58a	2.03ab
CX 4-4/LR	81.00c	226.80a	5.39a	6.58a	1.93ab
CX 5-3/LR	74.33c	233.33a	5.01a	7.41a	1.60b
XY8-6/MR	71.33c	228.47a	5.54a	8.33a	1.73ab
XY 1-4/LR	76.00c	235.80a	7.29a	8.47a	2.35ab
GL 8-3/LR	81.33c	217.47b	6.04a	7.38a	2.26ab
Mean	76.87B	227.70 A	6.02 A	7.67 A	2.03
NLK 5-4/S	90.67a	218.80a	5.23a	6.26a	2.17ab
ZS 1-3/S	82.67b	213.67b	4.63a	7.15a	1.79ab
TKS 2-5/S	83.67b	220.07a	5.78a	7.18a	2.20ab
TKS 2-1/S	83.6 b	214.93b	6.32a	8.68a	2.46a
ZS 5-1/S	87.67a	218.27b	5.69a	7.18a	2.29ab
TKS 6-3/S	85.00b	214.93b	5.13a	7.24a	2.03ab
TKS 3-1/S	82.33b	232.20a	5.11a	6.99a	1.81ab
GL 3-3/S	86.33a	224.93a	5.71a	7.91a	2.19ab
NLK 4-3/S	82.00b	225.53a	5.54a	7.25a	2.01ab
CX 2-2/S	85.33b	224.00a	6.02a	7.31a	2.29ab
NLK 2-2/S	74.67c	210.07b	5.69a	7.10a	2.02ab
XY 5-3/S	79.67c	212.13b	6.40a	8.62a	2.40ab
Mean	83.64 A	219.13B	5.60 A	7.41 A	2.14

[a] S, MR, and LR represent sensitive, medium, and low resistant to triadimefon, respectively

[b] Different letters after the data indicate statistically significant differences ($P < 0.05$), which tested by Duncan's multiple range test

of the *CYP51* gene. Of these, the low resistance isolate CX4-4 showed homozygous mutation, while the rest 10 resistant isolates were heterozygous (Fig. 3a). Analysis of EC_{50} values and genotypes confirmed that Y134F point mutation in the *CYP51* gene led to the resistance to triadimefon in *Pst* isolates from Yili (Fig. 3b), which is consistent with the results of Zhan et al. (2022).

Discussion

Triadimefon is the major fungicide widely used for the control of wheat stripe rust in China. Our previous study showed that triadimefon-resistant isolates have appeared in several provinces in China, but no resistant isolates had yet been found in Xinjiang (Zhan et al. 2022). The emergence of fungicide-resistant isolates dramatically reduced the effectiveness of the fungicide. Therefore, continuously monitoring the resistance level and frequency to DMI fungicides in *Pst* isolates and evaluating the resistance risk will help guide the precise selection and application of fungicides and provide a theoretical basis to defer the occurrence and development of pesticide resistance for wheat stripe rust. In this study, we examined the *Pst* isolates collected in Yili County, Xinjiang in 2022, and determined their sensitivity to triadimefon, where the higher resistance levels were found in GL and CX isolates than the other four counties (Table 2). The reasons could be: (1) The selection pressure imposed by extensive use of fungicides. Located in the center of six counties in Yili region, Gongliu County is the key area of wheat stripe rust prevention and control. The special geographic location requires that Gongliu County must pay much attention to preventing *Pst* outbreak. In the wheat growing season, triadimefon has been frequently applied. (2) Sexual reproduction drives genetic evolution. Tian et al. (2019) found that sexually reproducing *Pst* offspring demonstrated higher levels of resistance to triadimefon than their parental lines. Barberry is widely distributed in Xinjiang (Zhao et al. 2018), which may support the sexual reproduction of *Pst* in Yili region. Of the 176 tested isolates, 11 resistant isolates were detected with a resistance frequency of 6.25%. This is the first report that *Pst* isolates collected in Xinjiang Province are resistant to triadimefon.

Table 4 EC_{50} of *Puccinia striiformis* f. sp. *tritici* to four other fungicides

Isolates	Triadimefon	Pyraclostrobin	Flubeneteram	Tebuconazole	Hexaconazole
GL 8-3/LR	1.0644	0.2045	0.1881	0.3729	0.8403
CX 4-4/LR	1.6714	0.2596	0.1502	0.2439	0.1426
TKS 1-1/LR	1.5692	0.215	0.1317	0.2776	0.1791
GL 1-2/LR	1.0597	0.4212	0.0742	0.3665	0.686
XY 1-4/LR	1.0236	0.1125	0.4891	0.2772	0.3841
CX 5-3/LR	1.4393	0.101	0.0877	0.193	0.2144
GL 3-2/LR	1.3022	0.0812	0.101	0.0848	0.7181
XY 8-6/MR	1.9997	0.0899	0.3347	0.2191	0.4574
ZS 1-3/S	0.1692	0.0647	0.092	0.0842	0.4574
ZS 5-1/S	0.1244	0.1411	0.0736	0.0752	0.0722
TKS 2-1/S	0.1693	0.1151	0.0706	0.1005	0.0935
NLK 2-2/S	0.1395	0.1298	0.4586	0.1827	0.1276
TKS 6-3/S	0.149	0.0647	0.1022	0.2397	0.0617
XY 5-3/S	0.0582	0.1068	0.106	0.1207	0.0867
GL 3-3/S	0.1403	0.0947	0.8016	0.0982	0.1684
NLK 5-4/S	0.1643	0.1326	0.1872	0.1255	0.1819
NLK 4-3/S	0.1848	0.4629	0.144	0.1301	0.1754
TKS 3-1/S	0.1198	0.0966	0.0893	0.1579	0.1768
CX 2-2/S	0.2502	0.0533	0.1069	0.0627	0.051
TKS 2-5/S	0.1661	0.1079	0.4183	0.0867	0.1067

The resistant isolates from natural populations can become dominant due to parasitic fitness (Miao et al. 2020). However, it may lead to a 'fitness cost' and the evolutionary trade-off, which, to some extent, explains the absence of resistant isolates in natural populations for certain pathogens (Oliver 2014; Hawkins and Fraaije 2018). The cost of the fungicide-resistant pathogens may be observed with compromised spore germination, lesion expansion, sporulation intensity, infection efficiency, and overwintering capacity, which decreased the competition to sensitive isolates in field (Mikaberidze and Mcdonald 2015). Our study showed that resistant isolates displayed longer latent periods and lower spore germination rates compared to the sensitive isolates, which is consistent with the observation of Klosowski et al. (2016). The resistant isolates were less virulent than the sensitive counterparts in fields generally, but there were some tested resistant isolates showing high parasite fitness.

Cross-resistance tests indicated that the triadimefon-resistant isolates showed certain degrees of resistance to tebuconazole and hexaconazole but not to flubeneteram and pyraclostrobin (Fig. 1). Therefore, the field use should avoid DMI-type fungicides combining with triadimefon; instead, applying with flubeneteram and pyraclostrobin, may prolong the warranty of triadimefon.

Under both protective and therapeutic treatments, triadimefon had a more significant effect on the sensitive isolates (Fig. 2), but the resistant isolates could still sporulate even at the highest recommended dose, indicating the high risk of resistance in fields. Indeed, the resistant isolates CYR33 and CYR34 become the dominant races in Yili.

This study confirmed that *Pst* isolates are resistant to triadimefon emerging in Yili County. We must be aware that the extensive use of triadimefon may lead to widespread resistant isolates. It is urgent to carry out comprehensive fungicide resistance monitoring in all wheat-producing areas of Xinjiang for *Pst*, and determine their sensitivity to triadimefon, in order to scientifically control the wheat stripe rust in this area.

Conclusions

The sensitivity to the fungicide triadimefon for 176 *Pst* isolates collected from Yili County was determined in this study. The widely-used triadimefon could impose selection pressure among *Pst* population and reduce the efficacy of the fungicide. Although triadimefon exhibited control efficacy against wheat stripe rust, we found the triadimefon-resistant *Pst* isolates in Xinjiang. The point mutation of Y134F in the genome of resistant isolates conferred resistance to triadimefon. The finding urges us for better fungicide resistance risk management and to develop new fungicides.

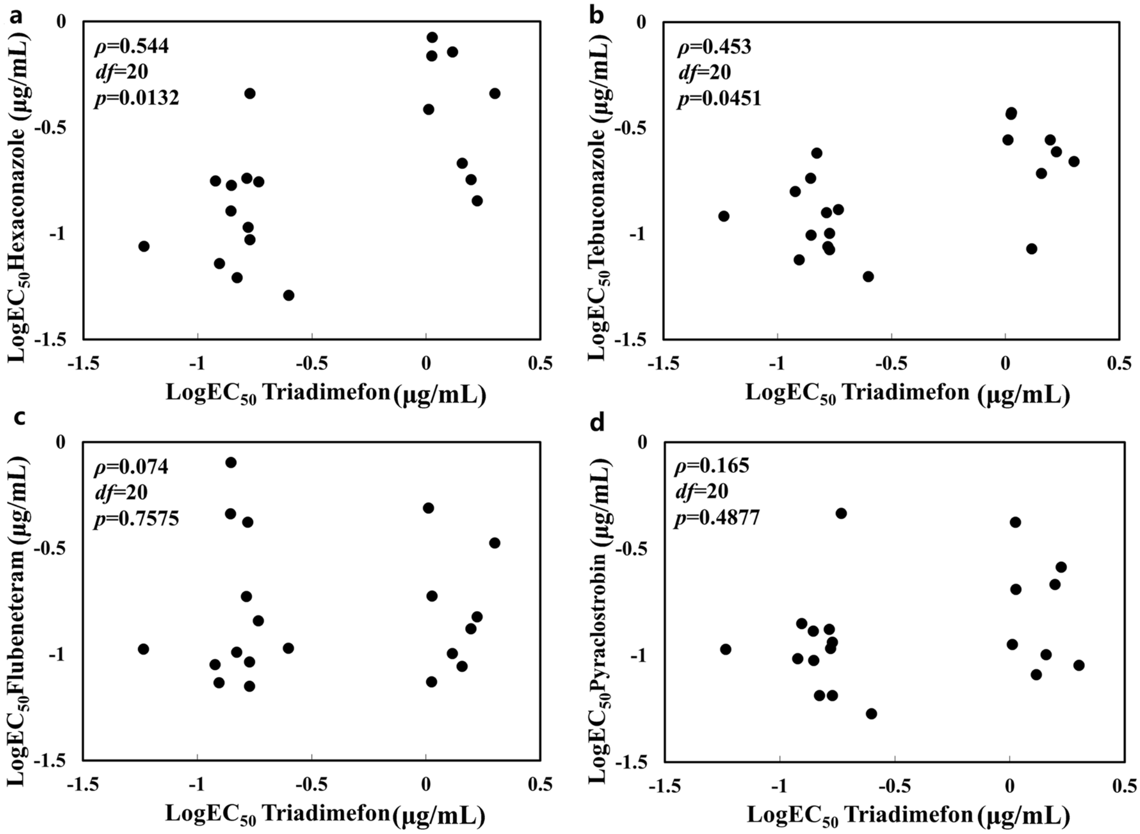

Fig. 1 Correlation analysis of resistance to triadimefon and other four fungicides. **a** Hexaconazole. **b** Tebuconazole. **c** Flubeneteram. **d** Pyraclostrobin. $\rho < 0.3$ means poor correlation; $0.3 \leq \rho < 0.5$ means low level correlation; $0.5 \leq \rho < 0.8$ means medium correlation; $\rho > 0.8$ means high correlation

Methods

The *Pst* isolates and fungicides

One hundred and seventy-six *Pst* isolates from six counties in Yili region of Xinjiang were collected in 2022 (Additional file 1: Table S1). Susceptible wheat cultivar mingxian 169 (MX169), 97.5% triadimefon, 97.5% tebuconazole, 97% pyraclostrobin, and 97.5% hexaconazole were all purchased from Xi'an Hytech Agrochemicals Co., Ltd., Shaanxi, China. The 97% flubeneteram was provided by Professor Guangfu Yang from Central China Normal University. All the above fungicides were resuspended in acetone. 6-Benzylaminopurine (6-BA) (Duly BioInc), agar powder (Solarbio Company), EDTA, Tris (Xi'an Walson Company), and Taq DNA polymerase (Dalian TaKaRa Company) were purchased from the indicated companies.

Measurement of *Pst* sensitivity to triadimefon

The triadimefon sensitivity of 176 isolates sampled from Yili County was determined and the EC_{50} value was calculated according to the method described by Xia et al. (2022).

Parasite fitness determination

Eight triadimefon-resistant isolates and twelve sensitive isolates were selected to determine parasitic fitness on MX169, including spore germination rate, sporulation intensity, lesion expansion rate, and latency. The relative parasitic fitness is indicated by (sporulation intensity × summer spore germination rate)/latent period (Bai et al. 2018; Zhan et al. 2022).

The spore germination rate determination: Fresh urediniospores were collected after propagation, and 0.01 g spores were placed in a centrifuge tube with 2 mL of Novec7100. 200 μL of spore suspension was applied evenly on 1.5% agar plate and incubated in a dark dew chamber for 24 h. The spore germination was recorded under a light microscopy (Bai et al. 2018).

Latent period: The plant leaves were inoculated with five microliters of spore suspension (0.002 g/mL). The newly developed uredinia ruptures (Urediniospores break through the epidermis of wheat leaves) were observed every 6 h at 7 days post-inoculation. The latent period (h) was determined from the time of initial inoculation to the rupture of uredinia. Sporulation

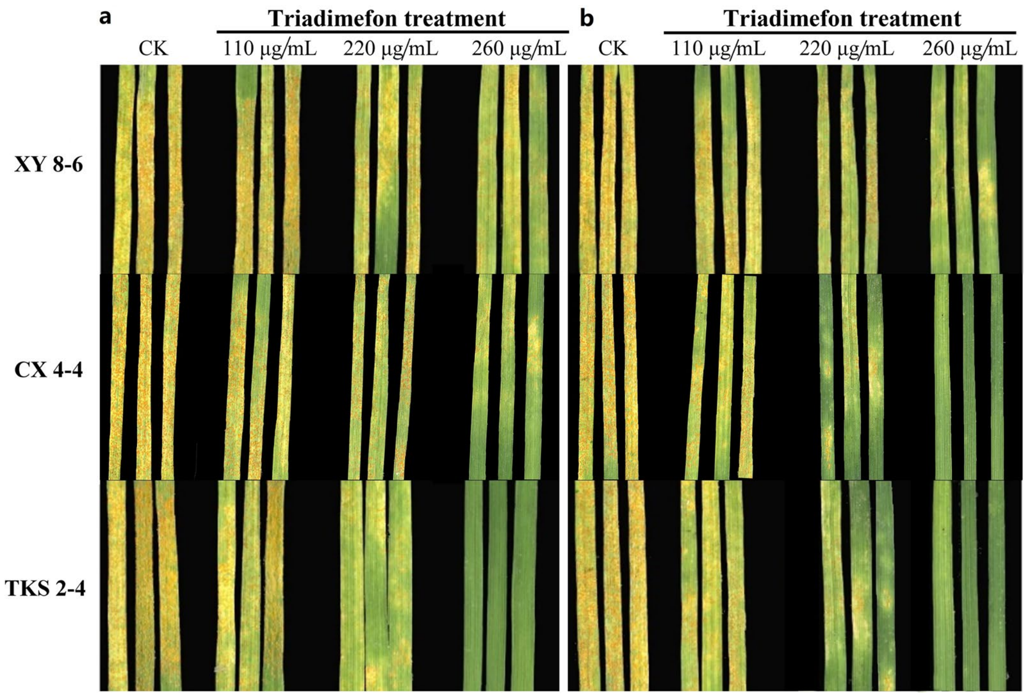

Fig. 2 Control effect of triadimefon on resistant and sensitive *Pst* isolates. **a** Protective effect of triadimefon. **b** Therapeutic effect of triadimefon. XY 8-6: medium resistance isolate; CX 4–4: low resistance isolate; TKS 2-4: sensitive isolate

Table 5 Results of KASP typing of resistant isolates

Isolate	Toxicity regression equation	R^2	EC_{50} (μg/mL)	Resistance level	Result of KASP
XY 8-6	Y = 0.607X + 4.817	0.994	1.999	Medium-resistant	X:Y
GL 1-1	Y = 0.813X + 4.773	0.991	1.905	Medium-resistant	X:Y
CX 4-4	Y = 0.756X + 4.831	0.995	1.671	Low-resistant	Y:Y
TKS 1-1	Y = 0.820X + 4.840	0.994	1.569	Low-resistant	X:Y
GL 9-6	Y = 0.765X + 4.866	0.985	1.497	Low-resistant	X:Y
CX 5-3	Y = 0.729X + 4.885	0.985	1.439	Low-resistant	X:Y
GL 3-2	Y = 0.496X + 4.943	0.977	1.302	Low-resistant	X:Y
GL 8-2	Y = 0.747X + 4.928	0.960	1.250	Low-resistant	X:Y
GL 8-3	Y = 0.776X + 4.980	0.991	1.064	Low-resistant	X:Y
GL 1-2	Y = 0.637X + 4.984	0.996	1.060	Low-resistant	X:Y
XY 1-4	Y = 0.751X + 4.992	0.989	1.024	Low-resistant	X:Y
CX 1-1	Y = 1.035X + 5.431	0.999	0.383	Sensitive	X:X

intensity: After the latent period, urediniospores were collected continuously for five days. A certain amount of Novec7100 was added to prepare the urediniospore suspension. The number of urediniospores was counted under a light microscope, and sporulation intensity was determined as spore production amount/spore production area (unit: spore/mm^2).

Lesion expansion rate: The length of onset was measured for five continuous days after the end of the latent period. The average rate of spot expansion was calculated. Ten leaves were measured for the tests and the experiments were repeated three times.

Cross-resistance assay

Eight isolates exhibiting triadimefon resistance and twelve triadimefon-sensitive *Pst* isolates were used to determine the cross-resistance against four fungicides, tebuconazole, hexaconazole, pyraclostrobin, and

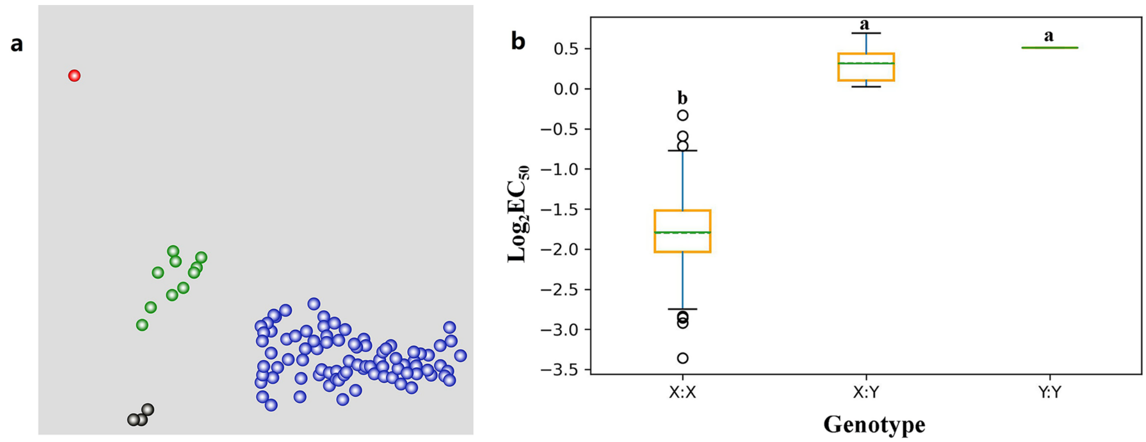

Fig. 3 Single Nucleotide Polymorphism (SNP) allelic configuration of point mutation Y134F in 14α-demethylase enzyme (Cyp51) by the KASP assay. **a** Blue color represents homozygous wild type (AA), green color represents heterozygous mutant (AT), and red color represents homozygous mutant (TT) genotypes. **b** Responses of 176 isolates to triadimefon (estimated as EC_{50}) and Duncan analysis of different genotypes ($P<0.01$)

flubeneteram, using the detached leaf method (Xia et al. 2022). EC_{50} values were calculated for Spearman correlation analysis.

Control efficacy on the resistant isolates

To study the control efficacy of triadimefon on triadimefon-resistant isolates, the growth of the isolates was measured at three concentrations of the fungicide (110, 220, and 260 μg/mL) recommended by the China Pesticide Information Network.

In the triadimefon-protective assays, 10 mL of triadimefon solution was sprayed on MX169 seedlings at the concentrations of 110, 220, and 260 μg/mL. Pathogens were inoculated 24 h later, with 3 μL spore suspension (0.002 g/mL) on each leaf according to methods described by Xia et al. (2022). For triadimefon therapeutic assay, the MX169 seedlings were inoculated with 3 μL spore suspension at a concentration of 0.002 g/mL on each leaf. Seedlings were transferred to a growth chamber after 24 h of moisturizing. Ten milliliters per pot of triadimefon solution at the concentrations described above were sprayed at 3 days after inoculation. The disease symptom was observed at 14 days. Each treatment was repeated three times.

Kompetitive allele specific PCR (KASP)

SNP genotyping of 176 isolates of *Pst* was conducted using the KASP-SNP genotyping method to determine the presence of the Y134F point mutation in *CYP51* gene. Primer sequence and PCR amplification methods were according to the procedures described by Zhan et al. (2022).

Abbreviations

6-BA	6-Benzylaminopurine
CX	Cha County
DMI	demethylation inhibitor
FRAC	Fungicide Resistance Action Committee
GL	Gongliu County
KASP	Kompetitive Allele Specific PCR
MX169	susceptible wheat cultivar mingxian 169
NLK	Nileke County
Pst	*Puccinia striiformis* f. sp. *tritici*
SDHI	succinate dehydrogenase inhibitor
TKS	Tekesi County
XY	Xinyuan County
ZS	Zhaosu County

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s42483-023-00205-w.

Additional file 1: Table S1. Sampling information in the Yili County in 2022. **Table S2.** Determination of sensitivity of 176 *Puccinia striiformis* f. sp. *tritici* isolates. **Table S3.** 176 isolates of *Puccinia striiformis* f. sp. *tritici* and their virulence binary formulae.

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Author's contributions

AZ, YF, XG, and YL analyzed the data and wrote the manuscript. AZ, GZ, YF, XG, and YL carried out the experiments. AZ analyzed the data. GZ, ZK, and LH designed the study. All authors read and approved the final manuscript.

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Availability of data and materials

Not applicable.

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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