



**Full Length Article**

## Molecular Identification of the *Gibberella fujikuroi* Species Complex Associated with Rice Bakanae and its Management in Jiangsu Province, China

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### Abstract

Rice bakanae disease (RBD) caused by *Gibberella fujikuroi* species complex (GFSC) is a rice (*Oryza sativa* L.) seed-borne disease worldwide. In this report, 122 strains of *Fusarium* spp. isolated from RBD-infected rice plants were identified. Phylogenetic analysis based on *translation elongation factor 1- $\alpha$*  (*TEF1- $\alpha$* ) sequences, as well as two-year field trials against RBD were conducted using eight fungicide products-representative products registered by the Institute for the Control of Agrochemicals, Ministry of Agriculture of China (ICAMA). Among the 122 strains, four species of GFSC were identified: *F. fujikuroi* (representing 74.59%), *F. verticillioides* (18.03%), *F. proliferatum* (5.74%) and *F. andiyazi* (1.64%). Phylogenetic analysis showed broad genetic diversity among the strains, which were clearly grouped into four different clusters. In field trials in 2016 and 2017, the control effect of recommended doses of the eight fungicides were in the following order: penflufen > phenamacril > mixture of fludioxonil and metalaxyl-M > mixture of thiophanate-methyl, metalaxyl, and azoxystrobin > prochloraz > fludioxonil > mixture of carbendazim (MBC) and thiram > hymexazol. Penflufen and phenamacril provided a >97% control effect. Prochloraz, fludioxonil, and mixture of MBC and thiram, which are representative of about 86% of the relevant products registered by ICAMA, provided a weak control effect. This was the first report of the population structure of GFSC associated with RBD in Jiangsu Province, as well as the second record of *F. andiyazi* associated with RBD in China. © 2019 Friends Science Publishers

**Keywords:** Rice bakanae disease; *Gibberella fujikuroi* species complex; Translation elongation factor 1- $\alpha$ ; Disease management

### Introduction

Rice bakanae disease (RBD) caused by the *Gibberella fujikuroi* species complex (GFSC) is one of the most serious and oldest known seed-borne diseases of rice (*Oryza sativa* L.). RBD occurs in rice-producing worldwide (Singh and Sunder, 2012) and was first recognized in Japan in 1828 (Ito and Kimura, 1931). RBD is known to cause significant yield losses from 50% to >70% in various rice-growing countries (Ou, 1985; Rood, 2004). In addition, a variety of mycotoxins produced by the GFSC can cause serious food safety concerns (Kini *et al.*, 2002; Desjardins and Proctor, 2007; Kim *et al.*, 2012; Zainudin and Perumal, 2015; Ali *et al.*, 2018).

Three species of GFSC associated with RBD have been recognized, *i.e.*, *Fusarium fujikuroi*, *F. verticillioides*, *F. proliferatum* (Desjardins *et al.*, 2000; Carter *et al.*, 2008; Amatulli *et al.*, 2010; Hsuan *et al.*, 2011). More recently, *F. andiyazi*, first described in sorghum in Africa (Marasas *et al.*, 2001), has been reported to be associated with RBD (Prà *et al.*, 2010; Wulff *et al.*, 2010; Choi *et al.*, 2018; Rong

*et al.*, 2018). Identification of GFSC has relied on a variety of methods such as morphological comparisons (O'Donnell *et al.*, 1998a, b), sexual crosses to tester strains (Leslie, 1991), application of isozyme technology (Huss *et al.*, 1996), mycotoxin profiling (Rheeder *et al.*, 2002), RAPD (random amplification of polymorphic DNA) analysis (Voigt *et al.*, 1995), AFLP (amplified fragment length polymorphism) analysis (Moretti *et al.*, 2004), RFLP (restriction fragment length polymorphism) and multiple gene sequence data analysis (O'Donnell *et al.*, 2000; Patiño *et al.*, 2006), but most of these methods are technically demanding and laborious. Some simple molecular identification methods have been widely used as alternatives, such as rDNA internal transcribed spacer (ITS) gene sequence analysis (Tan and Niessen, 2003; Quazi *et al.*, 2013),  $\beta$ -tubulin gene sequence analysis (Van-Poucke *et al.*, 2012) and *translation elongation factor 1- $\alpha$*  (*TEF1- $\alpha$* ) gene sequence analysis (Geiser *et al.*, 2004).

At present, no rice varieties have been observed to be completely resistant to RBD. In China, RBD management depends on benzimidazole fungicides, particularly

carbendazim (MBC). These fungicides have been widely and extensively used since the 1970s but MBC resistance has generally removed their efficacy (Chen *et al.*, 2014). In the 1980s, imidazole fungicides, particularly prochloraz, applied as rice seed disinfectants were observed to be effective against MBC-resistant strains. However, prochloraz-resistant strains have been frequently isolated concomitantly with reduced field control effects of prochloraz, and the frequency of field-resistant strains (minimum inhibitory concentration > 3.125  $\mu\text{g}/\text{mL}$ ) was greater than 80% (Chen *et al.*, 2012). Up to 20 Jul 2018, 137 fungicide products (including single and compounded agents) used for RBD management have been registered by the Institute for the Control of Agrochemicals, Ministry of Agriculture of China (ICAMA); however, the effective component of about 66% (90 out of 137 registered products) of these products is prochloraz or MBC, and the effective component of about 20% (28/137) of these products is fludioxonil. Thus, the situation of resistance management remains very serious.

Despite many studies worldwide, there is little information on the population structure of the GFSC associated with RBD and the field control effects of fungicides against RBD in Jiangsu Province, China. The aims of the research were to: (i) identify the population structure of the GFSC associated with RBD in Jiangsu Province; (ii) identify the GFSC based on *TEF1- $\alpha$*  sequences; and (iii) field test the effect of eight fungicide products (representative fungicides registered by ICAMA) in the control of RBD.

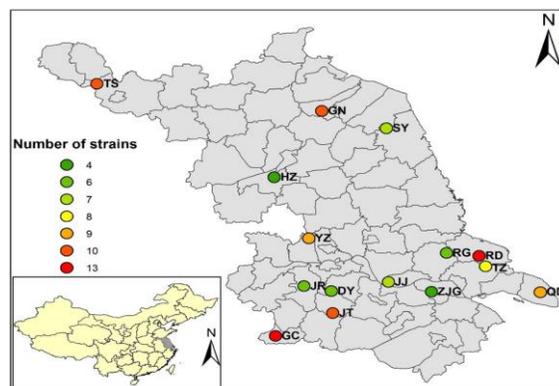
## Materials and Methods

### Fungal Cultures and DNA Extraction

RBD-infected rice plant samples were collected from different fields (one sample in each field) in 15 counties (cities) in Jiangsu Province, China, in 2016. Strains of *Fusarium* spp. were isolated according to Tateishi and Chida (2000) and initially selected based on morphological characters of *Fusarium* spp. (Mathur and Kongsdal, 2003). Finally, a total of 122 single-spore strains of *Fusarium* spp. were obtained (Fig. 1). The pure cultures of the 122 single-spore strains were multiplied in potato dextrose broth at 26°C on a shaking incubator at 120 rpm for 3 days. Harvested mycelial mats were used for total genomic DNA extraction following a modified CTAB method (Dubey and Singh, 2008).

### Species Identification and Phylogenetic Analysis

The PCR amplification assays using *TEF1- $\alpha$*  primers: ef1 (5'-ATGGGTAAGGARGACAAGAC-3') and ef2 (5'-GGARGTACCAGTSATCATGTT-3') (O'Donnell *et al.*, 1998b). PCR amplifications were performed in a 50- $\mu\text{L}$  volume containing 5  $\mu\text{L}$  10 $\times$ Taq DNA polymerase PCR



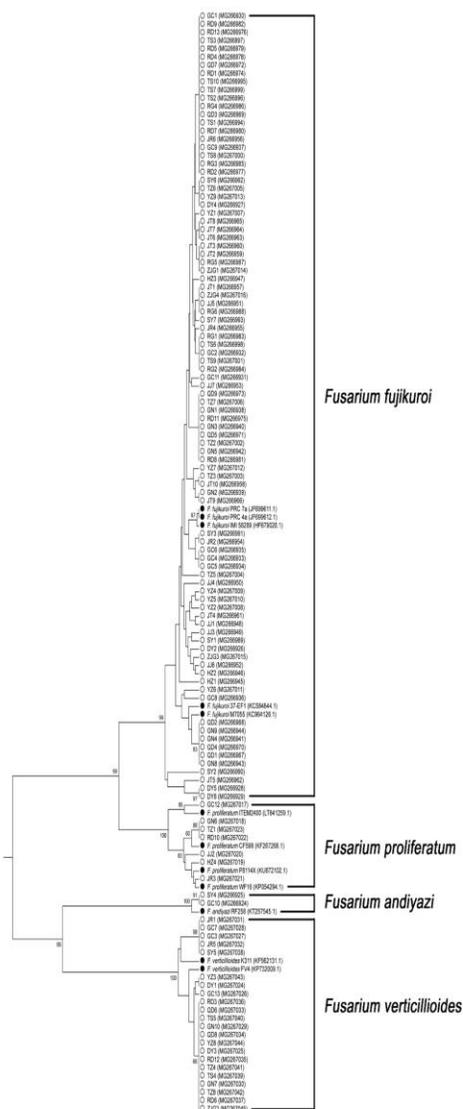
**Fig. 1:** Map showing locations in Jiangsu Province, China, where RBD-infected rice plants were collected for GFSC identification. The lettering indicates the code of the different counties (cities) of collection, and the color of circles indicates the number of strains collected

buffer (Sangon Biotech Co., Ltd., Shanghai, China), 1  $\mu\text{L}$  dNTP (10 mM), 3  $\mu\text{L}$   $\text{MgCl}_2$  (25 mM), 1  $\mu\text{L}$  of each primer (10  $\mu\text{M}$ ), 0.5  $\mu\text{L}$  Taq polymerase (5U/ $\mu\text{L}$ ) (Sangon Biotech Co., Ltd., Shanghai, China), 1  $\mu\text{L}$  fungal genomic DNA (20–50 ng DNA), and 37.5  $\mu\text{L}$  ddH<sub>2</sub>O. The PCR amplifications were performed in a T100 thermal cycler (Bio-Rad Laboratories Inc., Hercules, CA, USA) set to parameters described by Amatulli *et al.* (2010). The PCR products were separated on 1.7% agarose gels in 1  $\times$  Tris-acetate buffer and photographed after staining with gelred. The PCR product from each strain was purified and sequenced in both directions with primers ef1 and ef2 by Sangon Biotech Co., Ltd. (Shanghai, China).

All nucleotide sequences for each strain were submitted to GenBank (accession numbers: MG266924–MG267045). These sequences were subjected to BLAST analysis (<http://www.ncbi.nih.gov/index.html>) in GenBank database of NCBI. The non-redundant best-hits of these sequences corresponded to 12 *TEF1- $\alpha$*  gene sequences from four species of *Fusarium*, *i.e.*, *F. andiyazi*, *F. fujikuroi*, *F. proliferatum*, and *F. verticillioides* (Table S1). The 12 sequences were used as reference sequences for phylogenetic analysis. Phylogenetic analyses were conducted using MEGA version 4 (Tamura *et al.*, 2007). The dendrogram was obtained by conducting bootstrap analyses (1,000 replicates, removing gaps) with the unweighted pair group method with arithmetic mean (UPGMA) (Fig. 2).

### Field Control Effect of Fungicides against RBD

Eight representative fungicide products registered by ICAMA were selected for the RBD field control experiments, *i.e.*, Jinglei (25% prochloraz, EC) was provided by Nanjing Redsun Co. Ltd., Liangdi (25% phenamacril, SC) was provided by Jiangsu Pesticide Research Institute Co. Ltd., Celest (2.5% fludioxonil, FS)



**Fig. 2:** UPGMA analysis inferred from *TEF1-a* gene sequences. Bootstrap values are indicated as percentages above the nodes for maximum parsimony analysis (1,000 replications). GenBank accession number given next to strain code

was provided by Syngenta (China) Investment Co. Ltd., Amashi (22% penflufen, FS) was provided by Bayer Co. Ltd., Huanong (70% hymexazol, WP) was provided by Shenyang Research Institute of Chemical Industry (Nantong) Chemical Science and Technology Co. Ltd., THB (5% MBC+10% thiram, FS) was provided by Jiangsu Tianhebao Agrochemical Co. Ltd., Liangdun (2.5% fludioxonil+3.75% metalaxyl-M, FS) was provided by Syngenta (China) Investment Co. Ltd., and Hemu (6% thiophanate-methyl+3% metalaxyl+3% azoxystrobin, FS) was provided by Sipcarn Agro USA Inc.. Prochloraz, phenamacril, fludioxonil, penflufen, hymexazol, mixture of MBC and thiram, mixture of fludioxonil and metalaxyl-M,

and mixture of thiophanate-methyl, metalaxyl and azoxystrobin were designed for the recommended doses of 0.125, 0.125, 0.075, 1.000, 0.7000, 7.500, 0.125, 0.900 g ai/L, respectively, as the soaking dose (Table 1).

The experiment was conducted in experimental fields of the Zhenjiang Institute of Agricultural Science in Hilly Area of Jiangsu Province (31°58'5.27"N, 119°17'47.23"E in Jiangsu Province, China), during the rice-growing seasons of 2016 and 2017. Infected grains (500 g) of susceptible rice variety "Huaidao 11" were treated by separately soaking in 1 L of solutions of eight different fungicides for 48 h (Table 1). Control treatment was provided by treating seeds using sterilized distilled water (SDW). Each treatment had three replicates. Then the suspension of fungicide products and water was drained out and treated seeds were incubated at 28°C for 48 h to germination under dark conditions. The germinated seeds of each treatment were sown in a separate seed-bed of 1 m × 5 m. At 20 d after sowing, RBD-infected seedlings were counted. Thirty-day-old seedlings were uprooted and individually transplanted into a randomized block design with three replications in 2 m × 20 m plots with 20 cm × 15 cm spacings. The plants were raised following recommended agronomic practices during the rice growing season. In addition, RBD-infected rice plants of each treatment were counted at the jointing and heading stages, respectively.

Collected data were converted into percent seedling, jointing and heading infection, respectively. The field control effect of different fungicides on RBD was determined by the following formula: [(percent infection in SDW control – percent infection in the treatment)/percent infection in SDW control] × 100. Finally, data were analyzed using SPSS 14.0 software (SPSS Inc. Chicago, IL, USA). Fisher's least significant difference (LSD) test at P = 0.05 was used for comparison of treatment means.

## Results

### Isolation of *Fusarium* spp. from RBD-infected Rice Plants

*Fusarium* spp. were isolated from 100% of rice plants exhibiting RBD symptoms, collected from different fields in 15 counties in Jiangsu Province in 2016. There were 122 strains initially confirmed as *Fusarium* spp. based on morphological characters, including orange to salmon-colored pionnotes on the RBD-infected rice plants, initially white then turned to red or violet colonies on potato dextrose agar, initially whitish then turned to light gray or light pink aerial mycelium, microconidia generally appear in chains on monophialides or polyphialides.

### Molecular Identification of *Fusarium* spp.

Amplification of *TEF1-a* yielded an approximately 700-bp fragment and its sequence was used for species

**Table 1:** Fungicide products used for seed treatment

No.	Fungicide products	Chemical group	Manufacturer	Registration no. <sup>a</sup>	Trade name <sup>b</sup>	Dosage (g ai/L) <sup>c</sup>
T1	25% Prochloraz, EC <sup>d</sup>	Imidazole	Nanjing Redsun Co. Ltd.	PD20080162	Jinglei	0.125
T2	25% Phenamacril, SC	Cyanoacrylate	Jiangsu Pesticide Research Institute Co. Ltd.	PD20121670	Liangdi	0.125
T3	2.5% Fludioxonil, FS	Pyrrrole	Syngenta (China) Investment Co. Ltd.	PD20050196-F01-11	Celest	0.075
T4	22% Penflufen, FS	Pyrazole amide	Bayer Co. Ltd.	LS20150048	Amashi	1.000
T5	70% Hymexazol, WP	Isoxazole	Shenyang Research Institute of Chemical Industry (Nantong) Chemical Science and Technology Co. Ltd.	PD20130704	Huanong	0.700
T6	5% Carbendazim + 10% Thiram, FS	Mixed	Jiangsu Tianhebao Agrochemical Co. Ltd.	PD20091290	THB	7.500
T7	2.5% Fludioxonil + 3.75% Metalaxyl-M, FS	Mixed	Syngenta (China) Investment Co. Ltd.	PD20096644	Liangdun	0.125
T8	6% Thiophanate-methyl + 3% Metalaxyl + 3% Azoxystrobin, FS	Mixed	Sipcam Agro USA Inc.	PD20142522	Hemu	0.900
T9	CK <sup>e</sup>	-	-	-	-	-

<sup>a</sup> Indicates the number of the fungicide products registered by ICAMA

<sup>b</sup> Trade name of fungicide product in China

<sup>c</sup> The recommended dose for seed soaking treatment

<sup>d</sup> EC, SC, FS and WP indicate emulsifiable concentrates, suspension concentrates, flowable concentrate for seed treatment and wettable powder, respectively

<sup>e</sup> CK means treating seeds using sterilized distilled water

**Table 2:** Field control effect of fungicide products against RBD in Jiangsu Province, China, in 2016 and 2017

No.	2016 <sup>a</sup>						2017 <sup>a</sup>					
	Seedling stage		Jointing stage		Heading stage		Seedling stage		Jointing stage		Heading stage	
	PI (%) <sup>b</sup>	CE (%) <sup>c</sup>	PI (%)	CE (%)	PI (%)	CE (%)	PI (%)	CE (%)	PI (%)	CE (%)	PI (%)	CE (%)
T4	0.00 ± 0.00 f	100.00 ± 0.00 a	0.00 ± 0.00 f	100.00 ± 0.00 a	0.25 ± 0.03 e	98.42 ± 0.11 a	0.00 ± 0.00 f	100.00 ± 0.00 a	0.00 ± 0.00 f	100.00 ± 0.00 a	0.33 ± 0.03 g	98.26 ± 0.11 a
T2	0.00 ± 0.00 f	100.00 ± 0.00 a	0.00 ± 0.00 f	100.00 ± 0.00 a	0.40 ± 0.04 e	97.48 ± 0.21 a	0.00 ± 0.00 f	100.00 ± 0.00 a	0.00 ± 0.00 f	100.00 ± 0.00 a	0.45 ± 0.04 g	97.63 ± 0.23 a
T7	0.34 ± 0.02 e	87.64 ± 0.51 b	0.85 ± 0.07 e	91.54 ± 0.34 b	1.15 ± 0.13 d	92.74 ± 0.90 b	0.28 ± 0.03 e	87.56 ± 1.52 b	0.98 ± 0.06 e	91.85 ± 0.81 b	2.95 ± 0.33 f	84.43 ± 1.76 b
T8	0.40 ± 0.04 e	85.45 ± 0.92 b	0.90 ± 0.02 e	91.04 ± 0.61 b	1.28 ± 0.14 d	91.92 ± 0.89 b	0.34 ± 0.02 e	84.89 ± 1.45 b	1.05 ± 0.07 e	91.26 ± 1.47 b	3.04 ± 0.09 f	83.96 ± 0.58 b
T1	0.84 ± 0.05 d	69.45 ± 1.76 c	1.85 ± 0.04 d	81.59 ± 1.32 c	5.05 ± 0.13 c	68.14 ± 1.88 c	0.85 ± 0.05 d	62.22 ± 3.92 c	2.20 ± 0.15 d	81.70 ± 1.03 c	5.05 ± 0.31 e	73.35 ± 2.21 c
T3	0.95 ± 0.07 cd	65.45 ± 1.14 d	1.92 ± 0.03 cd	80.90 ± 1.52 c	5.45 ± 0.10 c	65.62 ± 2.12 c	0.94 ± 0.04 d	58.22 ± 3.23 d	2.45 ± 0.07 d	79.62 ± 2.11 c	6.85 ± 0.92 d	63.85 ± 4.09 d
T6	1.04 ± 0.10 c	62.18 ± 4.94 d	2.45 ± 0.06 c	75.62 ± 2.65 d	7.80 ± 0.33 b	50.79 ± 2.93 d	1.25 ± 0.10 c	44.44 ± 5.08 e	5.50 ± 0.11 c	54.24 ± 5.58 d	8.50 ± 1.03 c	55.15 ± 6.12 e
T5	1.25 ± 0.07 b	54.55 ± 2.55 e	4.95 ± 0.31 b	50.75 ± 7.41 e	8.35 ± 0.61 b	47.32 ± 6.41 d	1.55 ± 0.10 b	31.11 ± 4.02 f	7.05 ± 0.11 b	41.35 ± 6.99 e	10.33 ± 0.87 b	45.49 ± 5.66 f
T9	2.75 ± 0.13 a	-	10.05 ± 0.90 a	-	15.85 ± 0.88 a	-	2.25 ± 0.14 a	-	12.02 ± 1.19 a	-	18.95 ± 0.58 a	-

<sup>a</sup> The percentage of RBD-infected rice plants during the rice growing season were counted at the seedling, jointing and heading stages, respectively. The field control effect of fungicide products against RBD was evaluated according to percentage infection. There were no significant differences between the mean values with the same letters in the same column ( $P > 0.05$ )

identification based on BLAST analysis in GenBank database (performed on 4 March 2018). The 122 strains were identified as *F. fujikuroi*, *F. verticillioides*, *F. proliferatum* or *F. andiyazi* using the BLAST similarity search. Overall, among the 122 GFSC strains, *F. fujikuroi* (74.59%, 91 out of 122 identified strains) and *F. verticillioides* (18.03%, 22/122) were predominant, followed by *F. proliferatum* (5.74%, 7/122) and *F. andiyazi* (1.64%, 2/122) (Table S1).

Phylogenetic analysis was based on the *TEFI-α* sequences of 122 identified strains and 12 best-hit related strains (Table S1). The phylogenetic tree obtained from analysis of *TEFI-α* sequences showed broad genetic diversity among the strains, which were clearly grouped into four different clusters, *i.e.*, *F. fujikuroi*, *F. proliferatum*, *F. andiyazi*, and *F. verticillioides* (Fig. 2). The results proving that *TEFI-α* sequences was a good marker for species identification of the GFSC associated with RBD.

### Field Control Effect of Fungicides against RBD

Two year field trials data showed that the field control effects of eight fungicides against RBD were in the following order: penflufen > phenamacril > mixture of fludioxonil and metalaxyl-M > mixture of thiophanate-methyl, metalaxyl, and azoxystrobin > prochloraz > fludioxonil > mixture of MBC and thiram > hymexazol. Moreover, the field control effect from strong to weak could be roughly divided into the

following three significantly different echelons. (i) This echelon included two products, *i.e.*, penflufen and phenamacril. These were the latest relevant products registered by ICAMA and applied in the field in China. Field trials at the recommended dose of both products, at the seedling, jointing and heading stages of rice provided a >97% field control effect. (ii) This echelon included two products, *i.e.*, mixture of fludioxonil and metalaxyl-M and mixture of thiophanate-methyl, metalaxyl and azoxystrobin. These two products are compounding agents from well-known international companies, and at the recommended dose at the rice heading stage provided a ~80–90% field control effect. (iii) This echelon included four products, *i.e.*, prochloraz, fludioxonil, mixture of MBC and thiram, and hymexazol. Prochloraz, fludioxonil and mixture of MBC and thiram, which represent about 86% of the relevant products registered by ICAMA, both provided weak field control against RBD, especially with MBC as the main component, such as for mixture of MBC and thiram. The field control effect of fludioxonil was significantly lower than that of mixture of fludioxonil and metalaxyl-M. Hymexazol had a <50% field control effect at its recommended dose at the rice heading stage (Table 2).

### Discussion

Species identification of the GFSC associated with RBD is of great significance in many aspects, such as RBD

management, screening of resistant varieties and fungicide resistance management. *TEF-1a* is the most widely-used marker for molecular identification of *Fusarium* spp. due to its ability to resolve most *Fusarium* species (Leyva-Madriral *et al.*, 2014). In this study, 122 strains of *Fusarium* spp. were identified and phylogenetic analysis was carried out based on *TEF-1a*. The results showed that *TEF-1a* was a good marker for species identification among the GFSC. For phylogenetic analysis of *Fusarium* species,  $\beta$ -*tubulin* is commonly combined with *TEF-1a* because both genes have been shown to provide more robust and high-resolution tree branching patterns. For instance, the monophyly of *Fusarium* strains from coffee wilt was not well supported using *TEF-1a* but the strains were shown to be monophyletic in combined sequences of *TEF-1a* and  $\beta$ -*tubulin* (Geiser *et al.*, 2005). Thus, in some cases the phylogenetic analysis additional gene sequences to establish phylogenetic relationships within a species.

Although China is the largest rice-producing country, little is known about the GFSC species that have infected rice locally in recent years. This study was the first report on the population structure of the GFSC associated with RBD in Jiangsu Province. Four species of the GFSC were isolated and identified from RBD-infected rice plants: *F. fujikuroi* (representing 74.59%), *F. verticillioides* (18.03%), *F. proliferatum* (5.74%) and *F. andiyazi* (1.64%). Thus, *F. fujikuroi* was the most abundant species of the GFSC associated with RBD in Jiangsu, similar to results in most studies worldwide (Amatulli *et al.*, 2010; Nur and Salleh, 2010; Wulff *et al.*, 2010). In addition, this was the second record of the isolation of *F. andiyazi* (two out of 122 identified strains) from RBD-infected rice plants in China and the first record has been reported by Rong *et al.* (2018). *F. andiyazi* is closely related to *F. thapsinum* (Marasas *et al.*, 2001) and has been reported to cause human infection (Kebabci *et al.*, 2013). It should be noted that the results of population structure identification may not fully reflect the current situation in Jiangsu Province, due to the few samples collected.

Currently, RBD management has always relied on seed treatment with fungicides in China. For management of RBD in Jiangsu Province, MBC has been discontinued for more than 30 years, but the frequency of field-resistant strains of MBC remains high, and the frequency of double-resistant strains for MBC and prochloraz was 58.42% (Chen *et al.*, 2017). In this study, mixture of MBC and thiram at the recommended dose at the rice heading stage provided a  $50.79 \pm 2.93$  and  $55.15 \pm 6.12\%$  field control effect in 2016 and 2017, respectively; prochloraz correspondingly provided a  $68.14 \pm 1.88$  and  $73.35 \pm 2.21\%$  field control effect, respectively (Table 2). Up to 20 Jul 2018, the above-mentioned two fungicides have represented about 66% (90 out of 137 registered products) of the relevant products registered by ICAMA, and their continued use is of little importance in RBD management

in Jiangsu Province. The field control effects of fludioxonil were slightly weaker than that of prochloraz, but mixture of fludioxonil and metalaxyl-M was stronger than that of prochloraz. This indicates that some fungicides such as fludioxonil may provide a stronger field control effect by optimizing combinations, and could play a greater role in RBD management.

As a *Fusarium*-specific fungicide, phenamacril has excellent inhibitory activity against the GFSC associated with RBD, as well as excellent efficacy in RBD management (Chen *et al.*, 2012). In this study, phenamacril at the recommended dose at the rice heading stage provided a  $97.48 \pm 0.21$  and  $97.63 \pm 0.23\%$  field control effect in 2016 and 2017, respectively, which was an excellent result for RBD management (Table 2). In recent years, phenamacril has become the most effective fungicide for RBD management in Jiangsu Province, but the resistance of field pathogen populations would develop rapidly (Hou *et al.*, 2018). Therefore, when phenamacril is used for RBD management, appropriate strategies must be taken to avoid or delay development of resistance.

Penflufen has excellent inhibitory activity against a variety of pathogenic fungi (Tonin *et al.*, 2013). Penflufen is one of the latest relevant fungicide products registered by ICAMA for RBD management; it has been valid since 18 March 2017 and still only has temporary registration (Registration no. LS20150048). Penflufen at the recommended dose at the rice heading stage provided a  $98.42 \pm 0.11$  and  $98.26 \pm 0.11\%$  field control effect in 2016 and 2017, respectively, and had the strongest field control effect on RBD among the eight tested fungicides (Table 2). In addition, field trials of seeds of infected grains of "Huaidao 11" treated by soaking in SDW for 48 h, and then dressing in the recommended dose of penflufen, showed excellent field control effects against RBD (data not shown). Penflufen is excellent for controlling RBD, but its resistance risk and dynamics in rice are unknown and should be further studied.

## Conclusion

The study carried out the species identification and phylogenetic analysis of 122 strains of *Fusarium* spp. collected from 15 counties (cities) in Jiangsu Province based on *TEF-1a* gene sequences. Results provided insight into the population structure of the GFSC associated with RBD in Jiangsu Province, *i.e.*, *F. fujikuroi* (representing 74.59%), *F. verticillioides* (18.03%), *F. proliferatum* (5.74%) and *F. andiyazi* (1.64%). In addition, this was the second record of *F. andiyazi* associated with RBD in China. The study showed the current situation of control effects of fungicides against RBD in Jiangsu Province, which had high frequency of field-resistant strains of MBC and prochloraz. Currently, penflufen and phenamacril are the most effective fungicides for RBD management in Jiangsu Province.

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