Persistent Identifier: urn:nbn:de: 0294-sp-2017-Reinh-32-2

Ishii H, Kurosaki A, Sensitivity of Fungal Strains Isolated from Rice Sheath Blight Symptom to the SDHI Fungicides Furametpyl and Benzovindiflupyr. In: Deising HB; Fraaije B; Mehl A; Oerke EC; Sierotzki H; Stammler G (Eds), "Modern Fungicides and Antifungal Compounds", Vol. VIII, pp. 193-196. © 2017 Deutsche Phytomedizinische Gesellschaft, Braunschweig, ISBN: 978-3-941261-15-0

Sensitivity of Fungal Strains Isolated from Rice Sheath Blight Symptom to the SDHI Fungicides Furametpyl and Benzovindiflupyr

Ishii H, Kurosaki A

Kibi International University, Sareo 370-1, Shichi, Minami-awaji, Hyogo, 656-0484 Japan
Email:hi481204@yahoo.co.jp

INTRODUCTION

The three QoI fungicides metominostrobin, azoxystrobin, and orysastrobin have been used for the control of two major diseases blast and sheath blight, caused by *Magnaporthe oryzae* and *Rhizoctonia solani* (= *Thanatephorus cucumeris*), respectively, on rice in Japan. Isolates of *M. oryzae* resistant to QoI fungicides have been detected since 2012 and they are widely distributed now in Japan (Miyagawa & Fuji 2013). In the summer 2015, fungal strains were isolated from sheath blight and related symptoms naturally developing on rice at Minami-awaji, Hyogo, Japan. The isolates were tested for sensitivity to azoxystrobin on fungicide-amended potato dextrose agar (PDA) plates and it was indicated that less QoI-sensitive strains were present in the sampling area (Kurosaki & Ishii 2016).

In the same area, the SDHI fungicide furametpyl has been continuously applied once a year for sheath blight control as nursery box treatment over the last ten years. The sensitivity of *R. solani* and other fungal strains isolated from sheath blight and related symptoms was then examined on YBA agar plates supplemented with furametpyl and a novel SDHI fungicide benzovindiflupyr.

MATERIALS AND METHODS

Rice plants bearing symptoms of sheath blight and related diseases such as pseudo-sheath blight were sampled from Minami-awaji, Hyogo, Japan in August and September 2015. Fungal isolates were obtained from surface-sterilized symptomatic rice tissue and maintained on PDA medium. The reference isolate of *R. solani*, MAFF237257, was given by Genebank Project, NARO, Tsukuba, Ibaraki, Japan. The formulations of furametpyl and benzovindiflupyr (supplied by Syngenta) were used for fungicide sensitivity tests.

Fungal isolates were precultured on PDA plates at 25ºC for three to four days in darkness, mycelial discs, 4 mm in diameter, were cut from actively growing colony margins and transferred onto YBA agar plates containing furametpyl or benzovindiflupyr at 0, 0.1, 1, 10 and 100 mg L^{-1} of active ingredient (a.i.). After incubation at 25ºC for three days in the dark, the colony diameter was measured, and EC_{50} values were calculated by regressing percentage

Persistent Identifier: urn:nbn:de: 0294-sp-2017-Reinh-32-2

growth inhibition against the log of fungicide concentration using a software (a gift from So K, ZEN-NOH).

Total DNA of fungal isolates was extracted as described by Saitoh et *al.* (2006) with slight modifications (Ishii et *al.* 2016). Identification and assignment of fungal isolates were performed by amplifying ITS1, 5.8S, and ITS2 regions of rDNA using a primer pair of ITS5 and ITS4 (White et *al.* 1990). A quantity of 50 µL of PCR reaction mixtures contained 1 µL of total DNA, a set of forward and reverse primers (0.2 µM for each) and premixed Go Taq Green Master Mix (Promega, Madison, WI, USA). PCR reactions were programmed for 1 min at 94ºC, followed by 35 cycles of 1 min at 94ºC, 1 min at 55ºC, 2 min at 72ºC, a final extension for 10 min at 72ºC and holding at 10ºC. PCR products were separated by electrophoresis on a 1.5% agarose gel in 89 mM Tris-borate (pH 8.0) + 2 mM EDTA (TBE) buffer and stained with GelRed^{TM} (Biotium, Hayward, CA, USA). PCR products were cleaned up using ExoSAP-IT (Affymetrix, Santa Clara, CA, USA) according to the instructions supplied by the manufacturer. Sequencing was conducted at Macrogen Japan Corp. (Kyoto, Japan) and the sequences were analysed by NCBI BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

For inoculation tests, rice seeds (cultivar: Koshihikari) were germinated in distilled water (DW) at 32ºC for two days in the dark, planted in plastic pots containing soil, and kept in a glasshouse until 3-4 leaf stage. The plants were sprayed with furametpyl at 125 mg L^{-1} (a.i.). DW was used as a control. On the following day, mycelial discs of fungal isolates, 4 mm in diameter, cut from actively growing colony margins on PDA plates were placed beneath the leaf sheath of treated plants and covered with aluminum foil to maintain humidity (Park et *al.* 2008). The inoculated plants were incubated at 25ºC in a moist plastic container until desease development was assessed by measuring the lesion length seven days after inoculation.

RESULTS

In mycelial growth tests conducted on YBA agar medium, the EC_{50} of furametpyr and benzovindiflupyr was lower than 0.1 mg L^{-1} for a sensitive reference isolate. All of the *R. solani* isolates (identified in the present study based on their sequences of rDNA-ITS and colony morphology on PDA plates) examined were also sensitive (EC_{50} < 0.1 mg L^{-}) to both SDHI fungicides. However, many isolates other than *R. solani* showing various colony morphology and growth speed on PDA plates were less sensitive to furametpyr (Table 1). Out of 30 isolates tested, 17 isolates were less sensitive to this fungicide (EC_{50}: 9.9 mg L^{-} to >100 mg L^{-}) as compared with the reference isolate. In contrast, almost all of them were highly sensitive to benzovindiflupyr (EC_{50} < 0.1 mg L^{-}) with the exception of one isolate (4-3) for which the EC_{50} of furametpyr and benzovindiflupyr was >100 mg L^{-} and 1.6 mg L^{-}, respectively.

The species of fungal isolates used for mycelial growth tests were identified based on the nucleotide sequence of rDNA-ITS. They were identified to *R. solani*, *Ceratorhiza oryzae-sativae*, *Nigrospora oryzae*, *Fusarium equiseti*, and *N. sphaerica* with high identity to the sequences registered in NCBI GenBank database.

Persistent Identifier: urn:nbn:de: 0294-sp-2017-Reinh-32-2

Table 1 Differential sensitivity of fungal strains isolated from sheath blight and related symptoms on rice to furametpyr and benzovindiflupyr (mycelial growth tests)

Species	Isolate	EC_{50} (mg L^{-1})	
		Furametpyr	Benzovindiflupyr
Rhizoctonia solani	MAFF237257*	< 0.1	< 0.1
R. solani	RSKIU-3	< 0.1	< 0.1
R. solani	RSKIU-5	< 0.1	< 0.1
R. solani	RSKIU-6	< 0.1	< 0.1
R. solani	Ama-5	< 0.1	< 0.1
R. solani	2-4	< 0.1	< 0.1
R. solani	2-7	< 0.1	< 0.1
R. solani	2-9	< 0.1	< 0.1
Ceratorhiza oryzae-sativae	5-4	< 0.1	< 0.1
C. oryzae-sativae	9-7	< 0.1	< 0.1
Nigrospora oryzae	1-6	24.7	< 0.1
Unidentified	3-1	> 100	< 0.1
Fusarium equiseti	4-3	> 100	1.6
N. sphaerica	6-7	9.9	< 0.1
N. oryzae	8-4	> 100	< 0.1
N. oryzae	Ama-2	> 100	< 0.1
N. oryzae	Ama-4	> 100	< 0.1
N. oryzae	Ama-6	> 100	< 0.1

*Sensitive reference isolate.

In inoculation tests using rice seedlings grown in plastic pots, the three isolates of *R. solani* (2-4, 2-7, and 2-9) were highly suppressed their lesion development when seedlings were sprayed with furametpyr at 125 mg L^{-1} (a.i.) priot to inoculation preventively (Table 2). On the contrary, the efficacy of furametpyr on the two isolates of *Nigrospora oryzae* (1-6 and 8-4) was lower than that on *R. solani* isolates under the same treatment conditions.

Table 2 Differential sensitivity of fungal strains isolated from sheath blight and related symptoms on rice to furametpyr (inoculation tests)

Species	Isolate	Suppression of lesion development (%)
Rhizoctonia solani	2-4	100
R. solani	2-7	90.7
R. solani	2-9	97.9
Nigrospora oryzae	1-6	-0.9
N. oryzae	8-4	-77.0

DISCUSSION

Fungal isolates obtained from sheath blight and related disease symptoms on rice in 2015 were tested for sensitivity to the two SDHI fungicides furametpyl and benzovindiflupyr. Mycelial growth of *R. solani* isolates was sharply inhibited by both fungicides on YBA agar medium. However, the furametpyl sensitivity of several isolates belonging to other species than *R.*

Persistent Identifier: urn:nbn:de: 0294-sp-2017-Reinh-32-2

solani, such as *N. oryzae* was much less. But interestingly, most of these isolates showed high sensitivity to benzovindiflupyr.

Results from inoculation tests on rice seedlings also showed the lack of furametpyl efficacy against the isolates of *N. oryzae*. 'Katsumon-byo' caused by *N. oryzae* is a minor disease on rice but it may not be very easy to distinguish this disease from others such as sheath blight and pseudo-sheath blight. In order to judge whether the low furametpyl sensitivity of these isolates was due to resistance development or not, the sensitivity of baseline isolates will be tested in the near future. The performance of benzovindiflupyr against the isolates of *N. oryzae* and *R. solani* will also be examined using fungicide sprayed rice plants.

Isolates of *R. solani* resistant to the SDHI fungicide thifluzamide have been obtained from a field and under laboratory conditions. In those isolates, the H249Y mutation conferring resistance was found in *sdhB* gene encoding the fungicide-targeted protein subunit SDHB (Mu et *al.* 2014). In our study, however, the wild type amino-acid histidine in *sdh*B was conserved in the two isolates of *N. oryzae*, less sensitive to furametpyr (data not shown).

Benzovindiflupyr is one of the SDHI fungicides most recently developed (Ishii et *al.* 2016) and this fungicide controls rusts, many different leaf spots, apple scab, powdery mildew and Rhizoctonia, and also available for use on wheat, corn, cucurbit and fruiting vegetables, grapevine, peanuts, pome fruit, potato and soybean in the United States. A mixture of benzovindiflupyr with azoxystrobin has also been developed to combat Asian rust on soybean in Brazil (http://www.syngentacropprotection.com/news).

REFERENCES

Ishii H; Zhen F; Hu M; Li X; Schnabel G (2016). Efficacy of SDHI fungicides including benzovindiflupyr against *Colletotrichum* species. *Pest Management Science* 72, 1844-1853.

Kurosaki A; Ishii H (2016). Reduced QoI-fungicide sensitivity in *Rhizoctonia solani* isolated from rice sheath blight symptoms. *Abstracts of PSJ Annual Meeting*, 88 (Japanese abstract).

Miyagawa N; Fuji M (2013). Occurrence of QoI fungicide-resistant strains of *Magnaporthe oryzae* on rice and fungicidal effective. *Abstracts of the 23rd Symposium of Research Committee on Fungicide Resistance, the Phytopathological Society of Japan*, 25-36 (in Japanese with English abstract).

Mu W; Li B; Chen C; Liu X; Hao J (2014). Molecular mechanisms of thifluzamide resistance in *Rhizoctonia solani*. *Phytopathology* 104 (1s), S1.4 (abstract).

Park DS; Sayler RJ; Hong YG; Nam MH; Yang Y (2008) A method for inoculation and evaluation of rice sheath blight disease. *Plant Disease* 92, 25-29.

Saitoh K; Togashi K; Arie T; Teraoka T (2006). A simple method for a mini-preparation of fungal DNA. *Journal of General Plant Pathology* 72, 348-350.

White TJ; Bruns T; Lee S; Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: *PCR Protocols: a guide to methods and applications*, eds MA Innis, DH Gelfand, JJ Sninsky & TJ White, pp. 315-322. Academic Press, New York, USA.