Specific Detection of the Wheat Blast Pathogen (*Magnaporthe oryzae* Triticum) by Loop-Mediated Isothermal Amplification

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Abstract

Wheat blast, caused by the *Magnaporthe oryzae* Triticum pathotype, is an economically important fungal disease of wheat. Wheat blast symptoms are similar to Fusarium head scab and can cause confusion in the field. Currently, no in-field diagnostic exists for *M. oryzae* Triticum. Loop-mediated isothermal amplification (LAMP) primers were designed to target the PoT2 and MoT3 loci, previously shown to be specific for *M. oryzae* and *M. oryzae* Triticum, respectively. Specificity was determined using 158 *M. oryzae* strains collected from infected wheat and other grasses and representing geographic and temporal variation. Negative controls included 50 *Fusarium* spp. isolates. Sensitivity was assessed using 10-fold serial dilutions of *M. oryzae* Triticum gDNA. PoT2- and

MoT3-based assays showed high specificity for *M. oryzae* and *M. oryzae* Triticum, respectively, and sensitivity to approximately 5 pg of DNA per reaction. PoT2 and MoT3 assays were tested on *M. oryzae* Triticum-infected wheat seed and spikes and identified *M. oryzae* and *M. oryzae* Triticum, respectively, using a field DNA extraction kit and the portable Genie II system. The mitochondrial NADH-dehydrogenase (*nad5*) gene, an internal control for plant DNA, was multiplexed with PoT2 and MoT3 and showed results comparable with individual assays. These results show applicability for *M. oryzae* Triticum field surveillance, as well as identifying nonwheat species that may serve as a reservoir or source of inoculum for nearby wheat fields.

Wheat (*Triticum* spp.) is a grass grown for diverse applications, including biofuels, livestock feed, and human food (Shewry 2009). Wheat is highly adaptable and can grow in a variety of climates and elevations (Feldman 1995; Saunders 1994), making it an important food staple for many countries and cultures (Shewry 2009) and resulting in world trade often greater than all other crops combined (Curtis et al. 2002). It is one of the top three cereal crops produced worldwide and is grown on approximately 220 million ha (approximately 544 million acres), which is larger than the area for any other

crop. In 2014, worldwide wheat production was approximately 730 million tons and valued at approximately US\$192 billion, with the top four wheat-producing countries being China (approximately 126 million tons), India (approximately 96 million tons), Russia (approximately 60 million tons), and the United States (approximately 55 million tons) (FAOSTAT 2014). Large-scale losses to wheat production can have devastating impacts on the global economy and food security.

Wheat blast, one of many diseases affecting wheat, is caused by the Triticum pathotype of *Magnaporthe oryzae* (synonymous with *Pyricularia oryzae*) (Couch and Kohn 2002; Zhang et al. 2016) and is considered a major disease affecting wheat production, with losses of up to 100% (Kohli et al. 2011; Peng et al. 2011) attributed to reduced yield and grain quality (Goulart et al. 2007) under conducive conditions (Anderson et al. 1947; Cardoso et al. 2008; Cruz and Valent 2017; Goulart et al. 2007; Urashima et al. 2009). Wheat blast disease was initially observed in 1985 in Paraná, Brazil (Igarashi et al. 1986; Maciel 2011) and subsequently in other areas in Brazil (Dos Anjos et al. 1996; Goulart et al. 1990; Igarashi 1990; Picinini and Fernandes 1990), Bolivia (Barea and Toledo 1996), Paraguay (Viedma 2005), and then Argentina (Perelló et al. 2015, 2017). The wheat blast pathogen was confined to South America until 2016, when it caused a widespread outbreak in Bangladesh (Callaway 2016; Malaker et al. 2016). Analyses of the strain causing the outbreak in Bangladesh suggested a close relation to aggressive *M. oryzae* Triticum isolates originating in South America (Islam et al. 2016; Malaker et al. 2016). These findings, combined with the knowledge that *M. oryzae* Triticum is seedborne (Goulart and Paiva 1990), raises concerns for widespread dissemination of *M. oryzae* Triticum to major wheat-producing areas of the world (Sadat and Choi 2017) such as India, China, and Russia, which are the top three wheat producers worldwide.

In the United States, wheat is one of the top four crop commodities (FAOSTAT 2014) and wheat blast disease caused by *M. oryzae* Triticum has not been reported to date. *M. oryzae* is subdivided into different pathotypes based on primary-host specificity (Couch et al. 2005; Farman 2002; Heath et al. 1990; Kato et al. 2000; Oh et al. 2002; Tosa et al. 2004; Urashima et al. 1993); however, *M. oryzae* pathotypes do infect and develop lesions on non-primary-host species (Dos Anjos and Charchar 2000; Farman 2002; Kohli et al. 2011; Tosa et al. 2004), causing confusion when surveying areas

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Table 1. *Magnaporthe oryzae* strains used in this study and corresponding loop-mediated isothermal amplification (LAMP) assay data

Genotype group, isolate ^a	Host of isolation	Country	State or province	Year collected	Collector or source	MoT3 ^b	LAMP data	
							PoT2	MoT3
Brachiaria								
Bm88324	<i>Brachiaria mutica</i> (Mauritius signal grass)	Philippines	Cabanatuan, Nueva Ecija	1988	R. Nelson	NT	+	–
Cynodon ^c								
Cd88215 ^c	<i>Cynodon dactylon</i> (Bermuda grass)	Philippines	Cabanatuan, Nueva Ecija	1988	R. Nelson	NT	+	–
Digitaria ^c								
Er88271 ^c	<i>Eragrostis</i> spp.	Philippines	Los Baños, Laguna	1988	R. Nelson	NT	+	–
Echinochoa ^c								
Ec88443 ^c	<i>Echinochoa colona</i> (jungle rice)	Philippines	Los Baños, Laguna	1988	R. Nelson	NT	+	–
Eleusine								
B51	<i>Eleusine indica</i> (goose grass)	Bolivia	Quirusillas	2012	Stack/Cruz	–	+	–
G22 (WGG-FA40)	<i>E. coracana</i> (finger millet)	Japan	...	1976	Yaegashi/Valent	–	+	–
Eragrostis								
G17 (K76-79)	<i>Eragrostis curvula</i> (weeping lovegrass)	Japan	...	1976	Yaegashi/Valent	–	+	–
Leersia ^c								
LhA8401 ^c	<i>Leersia hexandra</i> (southern cutgrass)	Philippines	Los Baños, Laguna	1984	R. Nelson	NT	+	–
Leptochloa ^c								
LcA8401 ^c	<i>Leptochloa chinensis</i> (Chinese sprangletop)	Philippines	Los Baños, Laguna	1984	R. Nelson	NT	+	–
Lolium-Festuca								
CHRF	<i>Lolium perenne</i> (perennial rygrass)	United States	Siler Springs, MD	1996	P. Dernoeden	–	+	–
CHW	<i>L. perenne</i>	United States	Annapolis/Severna Park, MD	1996	P. Dernoeden	NT	+	–
FH	<i>L. perenne</i>	United States	Fountain Head/Hagerstown, MD	1997	P. Dernoeden	NT	+	–
GG11	<i>L. perenne</i>	United States	Lexington, KY	1997	P. Vincelli	–	+	–
HO	<i>L. perenne</i>	United States	Richmond, PA	1996	P. Vincelli	–	+	–
LpKY97	<i>L. perenne</i>	United States	Lexington, KY	1997	P. Dernoeden	–	+	–
P28	<i>Bromus tectorum</i> (cheat grass)	Paraguay	Itapúa	2012/2014	M. Kohli	–	+	–
Pg1213-22	<i>Festuca arundinaceum</i> (tall fescue)	United States	Georgia	1999/2000	Tredway	–	+	–
PL2-1	<i>L. multiflorum</i> (Italian ryegrass)	United States	Pulaski County, KY	2002	P. Vincelli	–	+	–
PL3-1	<i>L. multiflorum</i>	United States	Pulaski County, KY	2002	J. Beale	–	+	–
WBKY11-15	<i>Triticum aestivum</i> (common wheat)	United States	Lexington, KY	2011	D. Hershman	–	+	–
Oryza								
GUY11	<i>Oryza sativa</i> (Asian rice)	French Guyana	...	1988	A. Ellingboe	NT	+	–
IA1 (ARB114)	<i>O. sativa</i>	United States	Arkansas	2009	Y. Jia	NT	+	–
IB49 (ZN61)	<i>O. sativa</i>	United States	Arkansas	1992	J. Correll	NT	+	–
IC17 (ZN57)	<i>O. sativa</i>	United States	Arkansas	1992	J. Correll	NT	+	–
IE1K (TM2)	<i>O. sativa</i>	United States	Arkansas	2003	T. Marchetti	NT	+	–
INA168	<i>O. sativa</i>	Japan	Aichi	1985	H. Kato	NT	+	–
ML33	<i>O. sativa</i>	Mali	...	1995	A. Ellingboe	NT	+	–
Panicum ^c								
PrA8202 ^c	<i>Panicum repens</i> (torpedograss)	Philippines	Los Baños, Laguna	1982	R. Nelson	NT	+	–
Setaria								
Arcadia	<i>Setaria viridis</i> (green foxtail)	United States	Lexington, KY	1998	M. Farman	NT	+	–

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^a Based on data provided by Gladieux et al. (2018).^b Based on data provided by Pieck et al. (2017); NT = was not tested.^c Based on new whole genome sequence analyses (M. Farman, unpublished data).^d Strains not grouped by whole-genome sequencing.^e Only non-*M. oryzae* Triticum isolates found to possess the MoT3 locus by whole-genome sequencing. Strains appear to be hybrids of two genotype groups: Brachiaria and Stenotaphrum (M. Farman, unpublished data).

Table 1. (Continued from previous page)

Genotype group, isolate ^a	Host of isolation	Country	State or province	Year collected	Collector or source	LAMP data		
						MoT3 ^b	PoT2	MoT3
GRF52	<i>S. viridis</i>	United States	Lexington, KY	2001	M. Farman	NT	+	–
Stenotaphrum SSFL02-1	<i>Stenotaphrum secundatum</i> (St. Augustine grass)	United States	Disneyworld, FL	2002	M. Farman	NT	+	–
Triticum B2	<i>Triticum aestivum</i>	Bolivia	Quirusillas	2011	Stack/Cruz	+	+	+
B71	<i>T. aestivum</i>	Bolivia	Quirusillas	2012	Stack/Cruz	NT	+	+
Br80	<i>T. aestivum</i>	Brazil	...	1991	D. Tharreau	NT	+	+
P29	<i>B. tectorum</i>	Paraguay	Itapúa	2012/2014	M. Kohli	+	+	+
P-3	<i>T. durum</i> (durum wheat)	Paraguay	Canindeyu	2012	G. Peterson	+	+	+
PY36.1	<i>T. aestivum</i>	Brazil	Brasília	2007	J. Maciel	+	+	+
Py22.1 ^c	<i>T. aestivum</i>	Brazil	Paraná	2007	J. M. Fernandes	+	+	+
PY5020 ^c	<i>T. aestivum</i>	Brazil	Londrina	2005	J. M. Fernandes	+	+	+
PY5003	<i>T. aestivum</i>	Brazil	Londrina	2005	J. Maciel	+	+	+
T25	<i>T. aestivum</i>	Brazil	Paraná	1988	S. Igarashi	+	+	+
Not grouped ^d								
BmA8309	<i>B. mutica</i>	Philippines	Los Baños, Laguna	1983	R. Nelson	NT	+	–
Bm88315	<i>B. mutica</i>	Philippines	Cabanatuan, Nueva Ecija	1988	R. Nelson	NT	+	–
Bm88509	<i>B. mutica</i>	Philippines	Cabanatuan, Nueva Ecija	1988	R. Nelson	NT	+	–
Bm88509	<i>B. mutica</i>	Philippines	Cabanatuan, Nueva Ecija	1988	R. Nelson	NT	+	–
Bm88510	<i>B. mutica</i>	Philippines	Cabanatuan, Nueva Ecija	1988	R. Nelson	NT	+	–
Bm88511	<i>B. mutica</i>	Philippines	Cabanatuan, Nueva Ecija	1988	R. Nelson	NT	+	–
Bm88512	<i>B. mutica</i>	Philippines	Cabanatuan, Nueva Ecija	1988	R. Nelson	NT	+	–
Bm88513	<i>B. mutica</i>	Philippines	Cabanatuan, Nueva Ecija	1988	R. Nelson	NT	+	–
P-0030	<i>Brachiaria</i> spp.	Paraguay	Itapúa	2014	G. Peterson	–	+	–
Br35 ^e	<i>Brachiaria</i> spp.	Brazil	Paraná	1990	S. Igarashi	NT	+	+
Cd88217	<i>C. dactylon</i>	Philippines	Cabanatuan, Nueva Ecija	1988	R. Nelson	NT	+	–
CrA8401	<i>Cyperus rotundifolia</i> (Cyperus root)	Philippines	Los Baños, Laguna	1984	R. Nelson	NT	+	–
Dc8301	<i>Digitaria ciliaris</i> (southern crabgrass)	Philippines	Los Baños, Laguna	1983	R. Nelson	NT	+	–
P-0026	<i>D. insularis</i> (sourgrass)	Paraguay	Canindeyú	2014	G. Peterson	–	+	–
B57	<i>Digitaria</i> spp.	Bolivia	Quirusillas	2012	Stack/Cruz	–	+	–
U167	<i>Digitaria</i> spp.	Uruguay	Las Achiras, Lavalleja	2010	S. Martinez	NT	+	–
U227	<i>Digitaria</i> spp.	Uruguay	Pueblo del Barro, Tacuarembó	2017	S. Martinez	NT	+	–
Ec883	<i>E. colona</i>	Philippines	Bay, Laguna	1988	R. Nelson	NT	+	–
U170	<i>Echinochloa</i> spp. (barnyard grass)	Uruguay	Zapata, Treinta y Tres	2010	S. Martinez	NT	+	–
EC4J	<i>Eleusine coracana</i>	Japan	...	2015	Y. Tosa	–	+	–
EiA8303	<i>E. indica</i>	Philippines	Los Baños, Laguna	1983	R. Nelson	NT	+	–
Ei8927	<i>E. indica</i>	Philippines	Bukidnon	1989	R. Nelson	NT	+	–
Ei88435	<i>E. indica</i>	Philippines	Tanauan, Batangas	1988	R. Nelson	NT	+	–
Ei88365	<i>E. indica</i>	Philippines	Sto. Tomas, Batangas	1988	R. Nelson	NT	+	–
Ei88366	<i>E. indica</i>	Philippines	Sto. Tomas, Batangas	1988	R. Nelson	NT	+	–
Ei88367	<i>E. indica</i>	Philippines	Sto. Tomas, Batangas	1988	R. Nelson	NT	+	–
Ei88368	<i>E. indica</i>	Philippines	Sto. Tomas, Batangas	1988	R. Nelson	NT	+	–
Ei88369	<i>E. indica</i>	Philippines	Sto. Tomas, Batangas	1988	R. Nelson	NT	+	–
Ei88370	<i>E. indica</i>	Philippines	Sto. Tomas, Batangas	1988	R. Nelson	NT	+	–
Ei88424	<i>E. indica</i>	Philippines	Sto. Tomas, Batangas	1988	R. Nelson	NT	+	–
Ei88425	<i>E. indica</i>	Philippines	Sto. Tomas, Batangas	1988	R. Nelson	NT	+	–
Ei88448	<i>E. indica</i>	Philippines	Tanauan, Batangas	1988	R. Nelson	NT	+	–
U228	<i>E. indica</i>	Uruguay	Valle Alto, Treinta y Tres	2017	S. Martinez	NT	+	–
U229	<i>E. indica</i>	Uruguay	Valle Alto, Treinta y Tres	2017	S. Martinez	NT	+	–
U231	<i>E. indica</i>	Uruguay	Valle Alto, Treinta y Tres	2017	S. Martinez	NT	+	–
U169	<i>Eleusine</i> spp.	Uruguay	Río Branco, Cerro Largo	2010	S. Martinez	NT	+	–
P-0025	<i>Eragrostis</i> spp.	Paraguay	Canindeyú	2014	G. Peterson	–	+	–
Pg1213-2	<i>F. arundinaceum</i>	United States	Georgia	1999/2000	L. Tredway	–	+	–
TFEGA	<i>F. arundinaceum</i>	United States	Kentucky	2013	M. Farman	–	+	–
FPH2015-44 ^e	<i>Hakonechloa macra</i> (Japanese forest grass)	United States	Ohio	2015	F. Hand	NT	+	+
Lh8841	<i>Leersia hexandra</i>	Philippines	Cabanatuan, Nueva Ecija	1988	R. Nelson	NT	+	–
Lh8842	<i>L. hexandra</i>	Philippines	Cabanatuan, Nueva Ecija	1988	R. Nelson	NT	+	–
Lh8844	<i>L. hexandra</i>	Philippines	Cabanatuan, Nueva Ecija	1988	R. Nelson	NT	+	–

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Table 1. (Continued from previous page)

Genotype group, isolate ^a	Host of isolation	Country	State or province	Year collected	Collector or source	LAMP data		
						MoT3 ^b	PoT2	MoT3
Lh8982	<i>L. hexandra</i>	Philippines	Los Baños, Laguna	1989	R. Nelson	NT	+	–
Lh88405	<i>L. hexandra</i>	Philippines	Los Baños, Laguna	1988	R. Nelson	NT	+	–
Lh88504	<i>L. hexandra</i>	Philippines	Cabanatuan, Nueva Ecija	1988	R. Nelson	NT	+	–
U221	<i>L. multiflorum</i>	Uruguay	Berachi, Cerro Largo	2017	S. Martinez	NT	+	–
U234	<i>L. multiflorum</i>	Uruguay	18 de Julio, Rocha	2017	S. Martinez	NT	+	–
U235	<i>L. multiflorum</i>	Uruguay	18 de Julio, Rocha	2017	S. Martinez	NT	+	–
U236	<i>L. multiflorum</i>	Uruguay	18 de Julio, Rocha	2017	S. Martinez	NT	+	–
U237	<i>L. multiflorum</i>	Uruguay	UEPL, Treinta y Tres	2017	S. Martinez	NT	+	–
U238	<i>L. multiflorum</i>	Uruguay	UEPL, Treinta y Tres	2017	S. Martinez	NT	+	–
LpNC17-1	<i>L. perenne</i>	United States	Asheville, NC	2017	M. Farman	NT	+	–
MoLFP136	<i>L. perenne</i>	United States	Marysville, KS	2007	M. Kennedy	NT	+	–
MoLFP137	<i>L. perenne</i>	United States	Trampis, KS	2007	M. Kennedy	NT	+	–
MoLFP138	<i>L. perenne</i>	United States	Prairie Dunes, KS	2007	M. Kennedy	NT	+	–
PgVC1.4.2	<i>L. perenne</i>	United States	New York	2001	W. Uddin	–	+	–
U168	<i>Luziola</i> spp. (watergrass)	Uruguay	Río Branco, Cerro Largo	2010	S. Martinez	NT	+	–
U171	<i>Luziola</i> spp.	Uruguay	Zapata, Treinta y Tres	2010	S. Martinez	NT	+	–
O-249	<i>O. sativa</i>	India	Cuttack, Orissa	1986	Bonman/Valent	NT	+	–
O-250	<i>O. sativa</i>	India	Hydrabad	1986	Bonman/Valent	NT	+	–
O-260	<i>O. sativa</i>	Nepal	Kathmandu Valley	1987	Bonman/Valent	NT	+	–
O-261	<i>O. sativa</i>	Nepal	Kathmandu Valley	1987	Bonman/Valent	NT	+	–
O-282	<i>O. sativa</i>	Brazil	Goiania	1987	Bonman/Valent	NT	+	–
O-283	<i>O. sativa</i>	Brazil	Goiania	1987	Bonman/Valent	NT	+	–
RB-1	<i>O. sativa</i>	United States	Glen County, CA	2009	Kosta	–	+	–
RB-2	<i>O. sativa</i>	Korea	...	2011	K. Pedley	–	+	–
RB-3	<i>O. sativa</i>	Philippines	...	2011	K. Pedley	–	+	–
SSID116	<i>O. sativa</i>	United States	Louisiana	2013	Y. Jia	NT	+	–
U75	<i>O. sativa</i>	Uruguay	UEPL, Treinta y Tres	2005	S. Martinez	NT	+	–
UB107	<i>O. sativa</i>	Uruguay	Charqueada, Treinta y Tres	2009	S. Martinez	NT	+	–
U198	<i>O. sativa</i>	Uruguay	Cinco Sauces, Tacuarembó	2014	S. Martinez	NT	+	–
Pd8824	<i>Paspalum distichum</i> (knotgrass)	Philippines	Cabanatuan, Nueva Ecija	1988	R. Nelson	NT	+	–
Pd8825	<i>P. distichum</i>	Philippines	Cabanatuan, Nueva Ecija	1988	R. Nelson	NT	+	–
Pd8826	<i>P. distichum</i>	Philippines	Cabanatuan, Nueva Ecija	1988	R. Nelson	NT	+	–
Pd8827	<i>P. distichum</i>	Philippines	Cabanatuan, Nueva Ecija	1988	R. Nelson	NT	+	–
Pd8828	<i>P. distichum</i>	Philippines	Cabanatuan, Nueva Ecija	1988	R. Nelson	NT	+	–
Pd8829	<i>P. distichum</i>	Philippines	Cabanatuan, Nueva Ecija	1988	R. Nelson	NT	+	–
Pd8830	<i>P. distichum</i>	Philippines	Cabanatuan, Nueva Ecija	1988	R. Nelson	NT	+	–
Pd8831	<i>P. distichum</i>	Philippines	Cabanatuan, Nueva Ecija	1988	R. Nelson	NT	+	–
Pd8832	<i>P. distichum</i>	Philippines	Cabanatuan, Nueva Ecija	1988	R. Nelson	NT	+	–
Pd8833	<i>P. distichum</i>	Philippines	Cabanatuan, Nueva Ecija	1988	R. Nelson	NT	+	–
Pd8834	<i>P. distichum</i>	Philippines	Cabanatuan, Nueva Ecija	1988	R. Nelson	NT	+	–
Pd8835	<i>P. distichum</i>	Philippines	Cabanatuan, Nueva Ecija	1988	R. Nelson	NT	+	–
Pd8836	<i>P. distichum</i>	Philippines	Cabanatuan, Nueva Ecija	1988	R. Nelson	NT	+	–
Pd8837	<i>P. distichum</i>	Philippines	Cabanatuan, Nueva Ecija	1988	R. Nelson	NT	+	–
Pd8838	<i>P. distichum</i>	Philippines	Cabanatuan, Nueva Ecija	1988	R. Nelson	NT	+	–
Pd8841	<i>P. distichum</i>	Philippines	Cabanatuan, Nueva Ecija	1988	R. Nelson	NT	+	–
Pd8843	<i>P. distichum</i>	Philippines	Cabanatuan, Nueva Ecija	1988	R. Nelson	NT	+	–
Pr88171	<i>P. repens</i>	Philippines	Cabanatuan, Nueva Ecija	1988	R. Nelson	NT	+	–
Pr889	<i>P. repens</i>	Philippines	Bay, Laguna	1988	R. Nelson	NT	+	–
Pr8988	<i>P. repens</i>	Philippines	Los Baños, Laguna	1989	R. Nelson	NT	+	–
Pr88165	<i>P. repens</i>	Philippines	Cabanatuan, Nueva Ecija	1989	R. Nelson	NT	+	–
Pr88167	<i>P. repens</i>	Philippines	Cabanatuan, Nueva Ecija	1988	R. Nelson	NT	+	–
Pr88168	<i>P. repens</i>	Philippines	Cabanatuan, Nueva Ecija	1988	R. Nelson	NT	+	–
Pr88169	<i>P. repens</i>	Philippines	Cabanatuan, Nueva Ecija	1988	R. Nelson	NT	+	–
Pr88170	<i>P. repens</i>	Philippines	Cabanatuan, Nueva Ecija	1988	R. Nelson	NT	+	–
GF7 (SS1)	<i>Setaria faberi</i> (giant foxtail)	United States	Lexington, KY		M. Farman	–	+	–

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Table 1. (Continued from previous page)

Genotype group, isolate ^a	Host of isolation	Country	State or province	Year collected	Collector or source	LAMP data		
						MoT3 ^b	PoT2	MoT3
U232	<i>S. italica</i> (foxtail millet)	Uruguay	Minas, Lavalleja	2017	S. Martinez	NT	+	–
GrF57	<i>S. viridis</i>	United States	Lexington, KY	2001	M. Farman	NT	+	–
YF DC	<i>S. viridis</i>	United States	Lexington, KY	2013	M. Farman	–	+	–
A-0005	<i>S. secundatum</i>	United States	Spanish Plaza, AL	2012	Olive	–	+	–
LD2	<i>S. secundatum</i>	United States	FL	2006	L. Datnoff	–	+	–
Pg1054	<i>S. secundatum</i>	United States	Parrot, GA	2000	L. Tredway	NT	+	–
SSTX16-1	<i>S. secundatum</i>	United States	College Station, TX	2016	D. Ebbole	NT	+	–
U217	<i>S. secundatum</i>	Uruguay	Valle Alto, Treinta y Tres	2015	S. Martinez	NT	+	–
U224	<i>S. secundatum</i>	Uruguay	Santa Teresa, Rocha	2014	S. Martinez	NT	+	–
U233	<i>S. secundatum</i>	Uruguay	Covidef 1, Florida	2017	S. Martinez	NT	+	–
UF0822	<i>S. secundatum</i>	United States	FL	2008	L. Datnoff	–	+	–
B50	<i>T. aestivum</i>	Bolivia	Quirusillas	2012	Stack/Cruz	NT	+	+
B53	<i>T. aestivum</i>	Bolivia	Quirusillas	2012	Stack/Cruz	NT	+	+
B54	<i>T. aestivum</i>	Bolivia	Quirusillas	2012	Stack/Cruz	NT	+	+
P-16	<i>T. aestivum</i>	Paraguay	Alto Paraná	2012	Peterson/Kohli	NT	+	+
Py22.1	<i>T. aestivum</i>	Brazil	Paraná	2007	J. M. Fernandes	+	+	+
PY5020	<i>T. aestivum</i>	Brazil	Londrina	2005	J. M. Fernandes	+	+	+
T-0002	<i>T. aestivum</i>	Brazil	PR	1987	A. S. Prabhu	+	+	+
T-0050	<i>T. aestivum</i>	Brazil	MG	1989	S. Igarashi	+	+	+

for disease or potential sources of inoculum. A fitting example occurred in the United States in 2011, when *M. oryzae* was isolated from a single wheat spike showing symptoms of wheat blast in Kentucky (Farman et al. 2017). *M. oryzae* Triticum was not reported in the United States; thus, this finding was cause for concern. Extensive analyses of the infecting isolate (WBKY11-15) revealed that it was more closely related to native ryegrass populations of *M. oryzae* (Lolium pathotype) than to South American *M. oryzae* Triticum strains (Farman et al. 2017). Further disease surveys did not identify a second occurrence (Cruz and Valent 2017). However, these findings suggested a close relationship between Lolium and Triticum pathotypes (Farman 2002; Farman et al. 2017; Tosa et al. 2004; Viji et al. 2001) and revealed a potential risk not only from importing aggressive strains of *M. oryzae* Triticum from South America but also from cross-host infection by indigenous Lolium pathotypes (Cruz and Valent 2017).

Control measures for this disease are limited and require a multifaceted approach. There are many unanswered questions regarding the factors that enable epidemics. Surveying fields and nearby areas for disease and latent infections could identify or prevent potential outbreaks. This would also involve screening and identifying alternative hosts harboring the pathogen as potential sources of inoculum. This method requires the proper identification of the disease as well as the causal agent. Additionally, screening seed and certifying it *M. oryzae* Triticum-free will reduce the risk of further international spread. These approaches require a powerful diagnostic tool and there is no standardized molecular detection assay for identifying *M. oryzae* Triticum in the field. There is a need for such an assay, because in-field whole-genome sequence (WGS) analysis for identification is not yet practical.

Fusarium head blight (FHB), or scab of wheat, caused by *Fusarium graminearum*, is another disease that impacts global wheat production and produces symptoms similar to wheat blast (McMullen et al. 1997). A proper diagnostic tool would differentiate between *F. graminearum* and *M. oryzae*. Recently, the PoT2 transposon (Farman 2002; George et al. 1998; Harmon et al. 2003; Kachroo et al. 1994) was used in a polymerase chain reaction (PCR)-based diagnostic platform to detect *M. oryzae* at the species level (Pieck et al.

2017) and discriminate *M. oryzae* from other genera of fungi. However, the PoT2 locus does not differentiate among pathotypes of *M. oryzae*. A diagnostic assay is needed to distinguish the Triticum pathotype from all other *M. oryzae* pathotypes. Correct identification of *M. oryzae* Triticum will allow proper phytosanitary measures to be implemented to prevent further spread of *M. oryzae* Triticum, as well as to track or trace outbreaks originating from foreign sources. An end-point and real-time PCR assay using the MoT3 locus of the retinol dehydrogenase gene for detection of *M. oryzae* Triticum has been published (Pieck et al. 2017). In the current study, the PoT2 transposon and MoT3 locus were used to develop loop-mediated isothermal amplification (LAMP) assays for the specific detection of *M. oryzae* Triticum in-lab and in the field. LAMP is a technology that allows nucleic acid amplification under isothermal conditions, enabling rapid detection in the laboratory and field using a variety of methods and instrumentation for running reactions and viewing results (Notomi et al. 2000).

Materials and Methods

Fungal specimens and DNA isolation. All work with active *M. oryzae* Triticum strains was performed in a BSL-3 biocontainment laboratory at the Biosecurity Research Institute in Pat Roberts Hall at Kansas State University in compliance with United States Department of Agriculture–Animal and Plant Health Inspection Service Plant Protection and Quarantine permit conditions (P526P-15-01607); only *M. oryzae* Triticum DNA was used outside of containment. *M. oryzae* strains used in this study are shown in Table 1 and all other strains tested are shown in Table 2. *Magnaporthe* strains were grown as described previously (Pieck et al. 2017). DNA was isolated from lyophilized fungal tissue using a phenol chloroform extraction or the OmniPrep for Fungi DNA extraction kit (G-Biosciences, St. Louis). *Fusarium* strains were grown and DNA extracted according to a method published previously (Leslie and Summerell 2006). DNA was quantified using the Qubit 3.0 Fluorometer (ThermoFisher Scientific Life Technologies, Waltham, MA, USA) and working solutions were made at 1 ng/μl.

LAMP primer design and reaction conditions. Based on previous studies, the PoT2 locus (Farman 2002; George et al. 1998;

Harmon et al. 2003; Kachroo et al. 1994; Pieck et al. 2017) and MoT3 locus (Pieck et al. 2017) were chosen as targets for specific detection of all *M. oryzae* and only *M. oryzae* Triticum strains, respectively. The mitochondrial NADH-dehydrogenase (*nad5*) gene (Ecke et al. 1990) was chosen as an internal control for plant DNA. LAMP primers (Table 3) were designed using PrimerExplorer V5 (Eiken Chemical Co., Ltd., Tokyo), available at <http://primerexplorer.jp/e/>. Individual LAMP reactions contained 5 µl of sample, 5 µl of primer master mix (1 µM F3, 1 µM B3, 8 µM FIP, 8 µM BIP, 4 µM each Loop, 0.4 µM Probe, and 0.8 µM Quencher Probe [Kubota et al. 2011]) and 15 µl of Isothermal Mastermix ISO-001nd (OptiGene, West Sussex, UK). Multiplexed Nad5/PoT2 LAMP reactions used 2.5 µl of primer master mix (modified concentrations of 3 µM Nad5-LoopB, 3.2 µM PoT2-LoopB, 1 µM each Nad5-Probe, 0.8 µM each PoT2-Probe, and 3.6 µM Quencher Probe [Kubota et al. 2011]) and 17.5 µl of Isothermal Mastermix ISO-001nd. Multiplexed Nad5/MoT3 LAMP reactions used 2.5 µl of primer master mix (modified concentrations of 3 µM Nad5-LoopB, 2 µM MoT3-LoopB, 1 µM each Nad5-Probe, 2 µM each MoT3-Probe, and 6 µM Quencher Probe [Kubota et al. 2011]) and 17.5 µl of Isothermal Mastermix ISO-001nd. Positive and negative controls used 5 µl of *M. oryzae* or *M. oryzae* Triticum DNA and double-distilled water (ddH₂O) or pathogen-free sample, respectively. All LAMP reactions, except Nad5/MoT3, were run and analyzed using the CFX96 Real-Time System (Bio-Rad, Hercules, CA, USA) at 65°C for 30 min, with fluorescence readings taken at 1-min intervals. Nad5/MoT3 assays were run for 50 min, to account for delayed or shifted reaction curves. Individual reactions containing carboxyfluorescein (FAM) probes were also run in the Genie II (OptiGene).

Assay specificity and sensitivity testing. Primer specificity was determined using 40 *M. oryzae* strains assigned to 14 host-specialized groups (M. Farman, unpublished data) and 118 unassigned strains (Table 1). Fifty-one isolates of other fungi composed the exclusivity panel (Table 2), which included several *Fusarium* spp. (Sarver et al. 2011) commonly found on wheat spikes. The sensitivity of these primers was determined using 10-fold serial dilutions of DNA working solution (*M. oryzae* Triticum strain T25) at 1 ng/µl down to 1 fg/µl, which corresponded to DNA at 5 ng down to 5 fg/reaction, respectively. LAMP reactions were performed as individual and multiplexed assays, as described above. Reactions were run in triplicate. This process was repeated to include wheat DNA in each reaction to assess any possible effects of extraneous DNA on sensitivity.

Plant inoculation and sample testing. *M. oryzae* Lolium-inoculated (MoLFP136) seed were prepared according to a protocol established by J. Kiyuna (personal communication). Total DNA was extracted using the Quick-DNA Fungal/Bacterial Miniprep Kit and a field extraction kit (Plant Material Lysis Kit; OptiGene). *M. oryzae* Triticum-inoculated (B71) plant material (wheat spikes) was prepared, according to a protocol established previously (Cruz et al. 2012, 2016). Seed infected naturally with *M. oryzae* Triticum from Quirusillas, Santa Cruz, Bolivia in 2014 was also tested. Symptomatic spike samples and naturally infected seed (1 and 5 seeds) were processed using the Plant Material Lysis Kit. Noninoculated seed or plant materials were used as negative controls. Processed samples were tested by individual and multiplexed LAMP assays, as described above.

Interlaboratory reproducibility testing. LAMP reagents for individual PoT2 and MoT3 assays (FAM) and representative DNA samples were sent to three National Plant Diagnostic Network (Stack et al. 2014) laboratories: the Institute of Food and Agricultural Sciences Plant Diagnostic Center at the University of Florida, Michigan State University, and the School of Integrated Plant Sciences at Cornell University. Ten samples were tested under blind conditions. Assays were performed in the QuantStudio3 (Applied Biosystems, ThermoFisher Scientific, Waltham, MA, USA), QuantStudio6 (Applied Biosystems, ThermoFisher Scientific), and StepOnePlus (Applied Biosystems, ThermoFisher Scientific) at Cornell University, the University of Florida, and Michigan State University, respectively. Results were reported from these laboratories and then assessed by comparing data to known strain or assay information.

Results

Assay specificity and sensitivity. Results from this study corroborate those produced by Pieck et al. (2017) using end-point PCR. The PoT2 LAMP primer set was specific at the species level and reacted with all *M. oryzae* strains. The MoT3 LAMP primer set was specific for the *M. oryzae* Triticum pathotype (Table 1), with two exceptions (isolates FPH-2015-44 and Br35). Although not true wheat blast pathogens, isolates FPH-2015-44 and Br35 possess the MoT3 locus (M. Farman, unpublished data). Additionally, these two strains grouped between *Brachiararia* and *Stenotrophum* groups, suggesting these strains are hybrids of the two pathotypes (M. Farman, unpublished data). Thus far, WGS has not identified other non-*M. oryzae* Triticum strains containing the MoT3 locus (M. Farman, unpublished data). No positive reactions were observed with the negative controls or with non-*M. oryzae* strains (Table 2).

The PoT2 and MoT3 LAMP assays could detect as few as 5 pg of DNA per reaction, even in the presence of wheat DNA. There were no differences in sensitivity when FAM was used instead of rhodamine X (ROX) and cyanine 5 (Cy5) for PoT2 and MoT3, respectively. Additionally, the sensitivity of these primer sets was identical when tested in the CFX96 Real-Time System and Genie II. Multiplexed assays were sensitive to as few as 50 pg of DNA per reaction and resulted in a slight delay in reaction time. This shift in sensitivity is typical in multiplexed reactions.

Plant sample testing. The PoT2 and MoT3 LAMP assays functioned properly when testing seed and spike samples, regardless of the DNA preparation kit used. Sensitivity of the PoT2 LAMP assay was sufficient to yield positive detection of *M. oryzae* Lolium from single seeds, using both the Quick-DNA Fungal/Bacterial Miniprep Kit and Plant Material Lysis Kit. Both reactions failed to detect *M. oryzae* Triticum in a single naturally infected seed processed with the Plant Material Lysis Kit, even using a modified protocol to prevent overdilution of the sample (40 µl of sample instead of 10 µl). Both assays were able to detect *M. oryzae* Triticum from the naturally infected seed lot when 5 seeds were processed together, using the manufacturers' protocol. No reactions were observed for any of the negative controls, which included healthy wheat seed and spikes, rye seed, and oat seed, as well as ddH₂O.

The Nad5 LAMP primers were used as a plant DNA control and positive reactions were only observed in samples containing plant material. The Nad5/PoT2 assay was able to detect *M. oryzae* Lolium in a single inoculated seed when processed by the Quick-DNA Fungal/Bacterial Miniprep Kit but not the Plant Material Lysis Kit. This was most likely due to the reduction in sensitivity of the multiplexed reaction. Detection of *M. oryzae* Lolium in a single inoculated seed processed with the Plant Material Lysis Kit was obtained by modifying the manufacturer's protocol to prevent overdilution of the sample (40 µl of sample instead of 10 µl). Both multiplex reactions were able to detect *M. oryzae* Triticum from an infected spike. Multiplexed assays also detected *M. oryzae* Triticum from 5 infected seeds processed with the Plant Material Lysis Kit, using a modified protocol (40 µl of sample instead of 10 µl). The Nad5/PoT2 assay produced predicted results using the 30-min protocol, most likely due to it being a multicopy target; however, the Nad5/MoT3 assay needed a 50-min protocol, being a single-copy target in a multiplex reaction. These results demonstrate applicability for field studies using a field-isolation kit and portable real-time amplification system, because field samples will include multiple infected seed and plant material, ensuring enough pathogen present in samples to allow detection.

Interlaboratory reproducibility testing. All data reported from each of the three laboratories (Supplementary Table S1) were congruent with each other and data presented in Table 1, reflecting the accuracy of the PoT2 and MoT3 LAMP assays in identifying *M. oryzae* and *M. oryzae* Triticum, respectively. Additionally, each laboratory performed the assays in a SmartCycler (Cepheid, Sunnyvale, CA, USA) for 30 min with optics off for 53 s and optics on for 7 s. The SmartCycler was able to produce accurate results; however, further optimization is required for use in this platform. Based on these results, its small sample capacity ($n = 16$), and the fact that Cepheid

will no longer support this platform, it is recommended that this system not be used.

Discussion

M. oryzae is subdivided into pathotypes based on primary host specificity: *Brachiaria* (signal grass), *Eleusine* (finger millet), *Eragrostis* (lovegrass), *Lolium* (turfgrasses), *Oryza* (rice), *Setaria* (foxtail millet), *Stenotaphrum* (St. Augustine grass), and *Triticum* (wheat) pathotypes (Couch et al. 2005; Farman 2002; Gladieux et al. 2018; Heath et al. 1990; Kato et al. 2000; Oh et al. 2002; Tosa et al. 2004; Urashima et al. 1993). The strains used in this study were chosen to represent a wide range of isolates from different hosts, times, and geographic locations, as well as inclusion in previous studies (Gladieux et al. 2018; Pieck et al. 2017). Strains used by Pieck et al. (2017) were included to check if the LAMP primers could produce specificity results comparable with those produced by PCR primers developed in that study. However, a major problem facing proper identification of *M. oryzae* Triticum is the fact that *M. oryzae* pathotypes can also infect or reside on hosts that are not their primary host. Therefore, host of isolation does not accurately represent pathotype and extensive pathogenicity tests have not been performed on many

M. oryzae isolates. Strains used by Gladieux et al. (2018) were included in this study to take advantage of WGS genome-group data, which were used in the absence of pathogenicity data to suggest pathotype, regardless of host of isolation.

The PoT2 and MoT3 primers designed in this study demonstrated high specificity for *M. oryzae* and *M. oryzae* Triticum, respectively. Three isolates from wheat (WBKY11-15, PY5010, and PY86.1; the latter two not tested in this study) showed potential false negatives with the MoT3 assay; although isolated from wheat, these strains grouped with the *Lolium-Festuca* clade by WGS (Gladieux et al. 2018) and, consequently, are likely true negatives. Only one true false negative has been identified; wheat blast isolate Br32 does not possess the MoT3 locus and should not be detectable with either the PCR or LAMP assays (M. Farman, unpublished data). Two potential false positives were identified by LAMP (isolates FPH-2015-44 and Br35), and any strain producing positive results in a region without wheat blast would have to be confirmed by in-depth methods such as WGS. Taken together, the specificity data obtained in this study suggest that these primers, as well as the PoT2 and MoT3 loci, are useful tools for identification of the wheat blast pathogen (*M. oryzae* Triticum) in the field.

Table 2. Non-*Magnaporthe oryzae* strains used in this study and corresponding loop-mediated isothermal amplification data

Organism ^a	Isolate	Host	Country	State or province	Year collected	Collector	PoT2	MoT3
<i>Fusarium aethiopicum</i>	46710	<i>Triticum aestivum</i>	...	...	2012	O'Donnell/ Bowden	–	–
<i>F. aethiopicum</i>	46718	<i>T. aestivum</i>	...	...	2012	O'Donnell/ Bowden	–	–
<i>F. aethiopicum</i>	46726	<i>T. aestivum</i>	...	...	2012	O'Donnell/ Bowden	–	–
<i>F. asiaticum</i>	NRRL6101	<i>T. aestivum</i>	United States	Peoria, IL	2010		–	–
<i>F. asiaticum</i>	NRRL28720	<i>T. aestivum</i>	United States	Peoria, IL	2010		–	–
<i>F. boothii</i>	NRRL26916	<i>T. aestivum</i>	United States	Peoria, IL	2010		–	–
<i>F. gerlachii</i>	36905	<i>T. aestivum</i>	...	...	2012	O'Donnell/ Bowden	–	–
<i>F. gerlachii</i>	38380	<i>T. aestivum</i>	...	...	2012	O'Donnell/ Bowden	–	–
<i>F. gerlachii</i>	38405	<i>T. aestivum</i>	...	...	2012	O'Donnell/ Bowden	–	–
<i>F. graminearum</i>	00-540M	<i>T. aestivum</i>	...	...	2004	L. Gale	–	–
<i>F. graminearum</i>	00-566M	<i>T. aestivum</i>	...	...	2004	L. Gale	–	–
<i>F. graminearum</i>	00-649M	<i>T. aestivum</i>	...	...	2004	L. Gale	–	–
<i>F. graminearum</i>	3653	<i>T. aestivum</i>	United States	Nemaha County, KS	1990		–	–
<i>F. graminearum</i>	5 kernel	<i>T. aestivum</i>	United States	Shawnee County, KS	2012	E. DeWolff	–	–
<i>F. graminearum</i>	COX 66	<i>T. aestivum</i>	Brazil	Coxilha, Passo Fundo	...	...	–	–
<i>F. graminearum</i>	CX101	<i>T. aestivum</i>	Germany	...	2004	T. Miedaner	–	–
<i>F. graminearum</i>	CX153	<i>T. aestivum</i>	Germany	...	2004	T. Miedaner	–	–
<i>F. graminearum</i>	CX38	<i>T. aestivum</i>	Brazil	Coxilha, Passo Fundo	...	...	–	–
<i>F. graminearum</i>	D036	<i>T. aestivum</i>	United States	Elkland, IL	2008	B. Bowden	–	–
<i>F. graminearum</i>	FG108	<i>T. aestivum</i>	Germany	...	2004	T. Miedaner	–	–
<i>F. graminearum</i>	FG3211	<i>T. aestivum</i>	Germany	...	2004	T. Miedaner	–	–
<i>F. graminearum</i>	FG96	<i>T. aestivum</i>	Germany	...	2004	T. Miedaner	–	–
<i>F. graminearum</i>	FRC-R-6221	<i>T. aestivum</i>	Australia	New South Wales	2006	...	–	–
<i>F. graminearum</i>	H001	<i>T. aestivum</i>	United States	New York	1999	B. Bowden	–	–
<i>F. graminearum</i>	MRC4712	<i>T. aestivum</i>	Germany	...	2004	W. Marassas	–	–
<i>F. graminearum</i>	MRC6010	<i>T. aestivum</i>	Germany	...	2004	W. Marassas	–	–

(Continued on next page)

^a The *F. graminearum* species complex contains 16 phylogenetically distinct species (Sarver et al. 2011).

^b GCP = Gulf coast population.

The sensitivities of the LAMP primers designed in this study were 5 pg/reaction, similar to those published in other studies. This level of sensitivity is expected when compared with LAMP assays designed to detect bacteria, which tend to be between 1 and 100 fg of DNA per reaction. Fungal genomes are much larger than bacterial genomes and usually comprise multiple chromosomes; therefore, the target gene/DNA mass ratio will be substantially lower, thus resulting in a lower relative sensitivity.

The cumulative results of this study demonstrate the utility of the PoT2- and MoT3-based LAMP assays for laboratory and field applications. The PoT2 assay could be used as a general screening assay to detect all *M. oryzae* pathotypes. Upon finding a positive reaction, the MoT3 assay could be used to confirm the presence of true *M. oryzae* Triticum. Under the current capabilities of the Genie II, the PoT2 and MoT3 assays would have to be run individually using FAM probes. The Nad5/PoT2 assay could be used in the field as a preliminary assay to detect the presence of *M. oryzae*, using a device capable of running two multiplexed reactions. The MoT3 or Nad5/MoT3 assay could then be used as a confirmatory assay to determine whether the detected *M. oryzae* strain is true *M. oryzae* Triticum. Additionally, the Nad5/MoT3 assay could be deployed

alone to detect *M. oryzae* Triticum in fields located in endemic areas where *M. oryzae* is established. Individual reactions cost less than US\$1 per reaction, while the multiplexed assays cost approximately US\$1.20. Cost per reaction could decrease following further optimization.

Future work will involve field testing in South America, particularly in Bolivia and Brazil, where wheat blast is common and many *M. oryzae* pathotypes are present. Wheat fields could be surveyed for *M. oryzae* and *M. oryzae* Triticum using the LAMP assays described in this article. In addition, surrounding fields and plants could be checked for infection with *M. oryzae* and *M. oryzae* Triticum. In particular, *Brachiaria* fields that are grown as a forage crop and are located near commercial wheat fields could be surveyed for the presence of *M. oryzae* and *M. oryzae* Triticum, because this plant has been identified as a possible primary *M. oryzae* Triticum-inoculum source for wheat fields located in Brazil (J. M. C. Fernandes, personal communication). These assays could also be used to survey surrounding areas to determine the extent of spread of *M. oryzae* Triticum from diseased fields. This would be particularly useful in areas surrounding outbreaks in Bangladesh to determine areas affected by spreading *M. oryzae* Triticum from diseased regions.

Table 2. (Continued from previous page)

Organism ^a	Isolate	Host	Country	State or province	Year collected	Collector	PoT2	MoT3
<i>F. graminearum</i>	NRRL13383	<i>T. aestivum</i>	United States	Peoria, IL	2010	...	–	–
<i>F. graminearum</i>	NRRL28063	<i>T. aestivum</i>	United States	Peoria, IL	2010	...	–	–
<i>F. graminearum</i>	NRRL28336	<i>T. aestivum</i>	United States	Peoria, IL	2010	...	–	–
<i>F. graminearum</i>	NRRL28439	<i>T. aestivum</i>	United States	Peoria, IL	2010	...	–	–
<i>F. graminearum</i>	NRRL6394	<i>T. aestivum</i>	United States	Peoria, IL	2010	...	–	–
<i>F. graminearum</i>	NZ425	<i>Chionochloa rubra</i> ssp. <i>cuprea</i> (Red tussock grass)	New Zealand	South Island	...	A. Bentley	–	–
<i>F. graminearum</i>	NZ427	<i>C. rubra</i> ssp. <i>cuprea</i>	New Zealand	South Island	...	A. Bentley	–	–
<i>F. graminearum</i>	PF00-30	<i>T. aestivum</i>	Brazil	Passo Fundo	...	...	–	–
<i>F. graminearum</i>	PF99-41	<i>T. aestivum</i>	Brazil	Passo Fundo	...	...	–	–
<i>F. graminearum</i>	PF99-47	<i>T. aestivum</i>	Brazil	Passo Fundo	...	...	–	–
<i>F. graminearum</i>	PT-1-04Fg	<i>T. aestivum</i>	United States	Pottawatomie County, KS	...	M. Davis	–	–
<i>F. graminearum</i>	VAC 84	<i>T. aestivum</i>	Brazil	Vacaria	...	...	–	–
<i>F. graminearum</i>	VAC-2	<i>T. aestivum</i>	Brazil	Vacaria	...	...	–	–
<i>F. graminearum</i> subpopulation GCP ^b	29149	<i>T. aestivum</i>	...	...	2012	O'Donnell/Bowden	–	–
<i>F. graminearum</i> subpopulation GCP ^b	38369	<i>T. aestivum</i>	...	...	2012	O'Donnell/Bowden	–	–
<i>F. graminearum</i> subpopulation GCP ^b	38371	<i>T. aestivum</i>	...	...	2012	O'Donnell/Bowden	–	–
<i>F. lousianense</i>	54196	<i>T. aestivum</i>	United States	Jefferson Davis Parish, LA	2012	O'Donnell/Bowden	–	–
<i>F. proliferatum</i>	Fp-70-2-5	<i>Z. mays</i> (corn)	United States	Manhattan, KS	...	Reyes-Gaige	–	–
<i>F. ussurianum</i>	45681	<i>T. aestivum</i>	...	...	2012	O'Donnell/Bowden	–	–
<i>F. ussurianum</i>	45795	<i>T. aestivum</i>	...	...	2012	O'Donnell/Bowden	–	–
<i>F. verticillioidea</i>	Fv-54-3-5	<i>Z. mays</i>	United States	Manhattan, KS	...	Reyes-Gaige	–	–
<i>F. vorosii</i>	37605	<i>T. aestivum</i>	...	...	2012	O'Donnell/Bowden	–	–
<i>F. vorosii</i>	38207	<i>T. aestivum</i>	...	...	2012	O'Donnell/Bowden	–	–
<i>F. vorosii</i>	38208	<i>T. aestivum</i>	...	...	2012	O'Donnell/Bowden	–	–
<i>Magnaporthe pennisetigena</i>	Ce88454	<i>Cenchrus echinatus</i> (southern sandbur)	Philippines	...	1988	...	–	–

Table 3. Oligonucleotide primers used in this study

Oligonucleotide primer	Sequence (5'–3') ^a	Source or reference
Nad5-F3	ACACATTCGACGATTGGC	This study
Nad5-B3	CCGGTCTTAACGCCCTA	This study
Nad5-FIP	ACCAACCAAAGCAGGTAGGCGAAAGTGGATCTGTTGTGCGA	This study
Nad5-BIP	GGCTCCTCCGTTGTGGGTAAACTGTGCAGCCTTTCCTCG	This study
Nad5-LoopF	CCACGGTTCTATAACGCACTCT	This study
Nad5-LoopB	CGGGAACCCGACTCTACG	This study
Nad5-Probe	/56-FAM/ACGCTGAGGACCCGGATGCGAATGCGGATGCGGATGCCGACGGGAAACCCGACTCTACG	This study
PoT2-F3	AATTACGCTTTTTTGCCGA	This study
PoT2-B3	GGTTTAATAGCGTTAATAACCGG	This study
PoT2-FIP	GAGCCAAAAACGAGTTATCCAACGACGAATTCTTCAAGCCGC	This study
PoT2-BIP	AATCCTTAAACCCAAAGGCCACCAAGATTTAATTACCTCCGT	This study
PoT2-LoopF	CCAAGGCCTTTTGTCTCTCCG	This study
PoT2-LoopB	CGTCGAATAGATAACGCCCGG	This study
PoT2-Probe ^b	/56-ROXN/ ACGCTGAGGACCCGGATGCGAATGCGGATGCGGATGCCGACGTGCGAATAGATAACGCCCGG	This study
MoT3-F3	CCAAGGATGTATGCCCTGAC	This study
MoT3-B3	GTTGGGGGCTTCGTATGC	This study
MoT3-FIP	CAAACCGTTAGAAAGCTGTACTTTCAGTTGTTACAACATCACGAATCG	This study
MoT3-BIP	TTGCACAAAACAACCCAGTTCAGAGACGTTGTTTATGAATTCCG	This study
MoT3-LoopB	CTTTTGCATCGCCGAGCC	This study
MoT3-Probe ^b	/5Cy5/ACGCTGAGGACCCGGATGCGAATGCGGATGCGGATGCCGACTTTTGCATCGCCGAGCC	This study
Quencher probe	TCGGCATCCGCATCCGCATTCGCATCCGGTCTCAGCGT/3BHQ_1/	Kubota et al. 2011

^a /56-FAM/ = 5'-carboxyfluorescein, /56-ROXN/ = 5'-rhodamine X, /5Cy5/ = 5' cyanine 5, and /3BHQ_1/ = 3' Black Hole Quencher 1.

^b FAM probes were substituted for ROX and Cy5 when using the Genie II.

In addition to *M. oryzae* Triticum, some *M. oryzae* Lolium strains cause disease in wheat, under laboratory conditions, with strain WBKY11-15 causing blast disease naturally; therefore, *M. oryzae* Lolium should not be overlooked (Farman et al. 2017). A LAMP assay was designed to detect *M. oryzae* Lolium from perennial ryegrass (*Lolium perenne*) (Villari et al. 2017). However, the *M. oryzae* Lolium LAMP assay specificity was determined against a small number of isolates that did not represent all major pathotypes of *M. oryzae*, with *M. oryzae* Triticum not being included in the study due to quarantine issues (Villari et al. 2017). Further studies should be done to optimize this *M. oryzae* Lolium LAMP assay for field detection. A multiplexed assay could then be produced that could detect *M. oryzae* and determine whether *M. oryzae* Lolium or *M. oryzae* Triticum was present in the sample. Additionally, the occurrence of the MoT3 locus in a few non-Triticum pathotype strains may suggest a potential for false-positive detections in practice. Hence, there is a need to identify additional *M. oryzae* Triticum-unique markers to complement the MoT3-based assay for identification of true *M. oryzae* Triticum. Moreover, extensive pathogenicity testing of isolates to determine virulence on different host species is needed to properly sort them into pathotypes and, thus, establish a level of agreement between WGS-based genomic-grouping and current molecular diagnostic assays.

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