

# Development and application of loop-mediated isothermal amplification for detection of the F167Y mutation of benzimidazole-resistant isolates in *Fusarium graminearum*

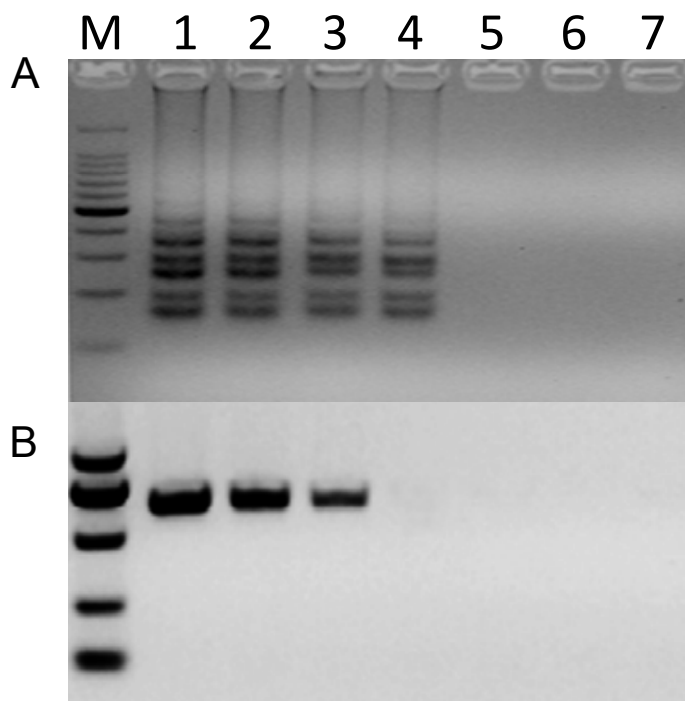
Yabing Duan, Xiaoke Zhang, Changyan Ge, Yong Wang, Junhong Cao, Xiaojing Jia, Jianxin Wang & Mingguo Zhou  
College of Plant Protection, Nanjing Agricultural University, Nanjing, 210095, China

Correspondence and requests for materials should be addressed to M. G. Z. (mgzhou@njau.edu.cn)

## Figure legend

Fig. S1 Sensitivity of LAMP vs. conventional PCR for detection of the F167Y mutation. (A) Gel electrophoresis of LAMP. (B) Gel electrophoresis of PCR. Concentrations of template DNA ( $\text{ng } \mu\text{L}^{-1}$ ) per reaction in (A), and (B) were: 1= $10^0$ , 2= $10^{-1}$ , 3= $10^{-2}$ , 4= $10^{-3}$ , 5= $10^{-4}$ , 6= $10^{-5}$ , and 7= $10^{-6}$ . In (A) and (B), M indicates 100-bp, and 250-bp ladder, respectively.

Figure S1



## Supplementary Tables

**Table S1. Information of the primers used in this study.**

**Table S2. *Fusarium graminearum* isolates used in specificity test of LAMP.**

**Table S3. *Fusarium graminearum* isolates used in repeatability test of LAMP**

### Tables

**Table S1**

Primers	Sequence (5'-3') <sup>a</sup>	Use
F3	TTCCAGCTGACGCACTCT	Forward outer primer for LAMP
B3	ACAGAAGGTCTCGTCAGAGT	Backward outer primer for LAMP
FIP	TGCGATCGGGGAACCTCTCG-TGGTACCGGTTCCGGTATG	Forward inner primer for LAMP
BIP1	CT <b>A</b> TTCCGTTATGCCCTCGCCC-ACGAGCTGGTTCAGAGACAA	Backward inner primers to distinguish the <i>Fusarium graminearum</i> genotypes (F167Y) for LAMP
BIP2	T <b>A</b> TTCCGTTATGCCCTCGCCC-ACGAGCTGGTTCAGAGACAA	
BIP3	<b>A</b> TTCCGTTATGCCCTCGCCC-ACGAGCTGGTTCAGAGACAA	
BIP4	GA <b>A</b> TTCCGTTATGCCCTCGCCC-ACGAGCTGGTTCAGAGACAA	
BIP5	A <b>A</b> TTCCGTTATGCCCTCGCCC-ACGAGCTGGTTCAGAGACAA	
BIP6	AA <b>A</b> TTCCGTTATGCCCTCGCCC-ACGAGCTGGTTCAGAGACAA	
BIP7	<b>A</b> AAACCGTTATGCCCTCGCCC-ACGAGCTGGTTCAGAGACAA	
Fgbeta637F	TCAGGGCGGCAACGACAAGTA	To amplify the partial fragments (637 bp) of the
Fgbeta637R	GTGAAGTCGGGGGAACGGAAT	$\beta_2$ -tubulin gene containing the F167Y position.

<sup>a</sup> Nucleotides in frames are modified from the sequence of the  $\beta_2$ -tubulin gene in the sensitive biotype and the resistant biotype. Nucleotides in bold are mismatches manually added specifically to distinguish *F. graminearum* genotypes (F167Y).

**Table S2**

Isolates	Genotype description	Origin	Resistance phenotype <sup>a</sup>	LAMP <sup>b</sup>
R9	Mutation at codon 167 of $\beta_2$ tubulin (F167Y)	Zhejiang province, China	MBC <sup>R</sup>	+
2021	Wild type	Zhejiang province, China	MBC <sup>S</sup>	—
Y50C	Mutation at codon 50 of $\beta_2$ tubulin (Y50C)	In our Lab.	MBC <sup>R</sup>	—
J-2	Mutation at codon 198 of $\beta_2$ tubulin (E198K)	Anhui province, China	MBC <sup>R</sup>	—
ZJ80	Mutation at codon 198 of $\beta_2$ tubulin (E198Q)	Anhui province, China	MBC <sup>R</sup>	—
NT-7	Mutation at codon 200 of $\beta_2$ tubulin (F200Y)	Hubei province, China	MBC <sup>R</sup>	—

<sup>a</sup> MBC<sup>S</sup> and MBC<sup>R</sup> indicate that the isolate is sensitive and resistant to carbendazim, respectively.

<sup>b</sup> ‘+’ and ‘—’ represent positive and negative results, respectively.

**Table S3**

Order	Isolates	Genotype description	Origin	Resistance phenotype <sup>a</sup>	LAMP <sup>b</sup>
1	YZ1001	Mutation at codon 167 of $\beta_2$ tubulin (F167Y)	Jiangsu province, China	MBC <sup>R</sup>	+
2	CZ1001	Mutation at codon 167 of $\beta_2$ tubulin (F167Y)	Jiangsu province, China	MBC <sup>R</sup>	+
3	LYG1209	Mutation at codon 167 of $\beta_2$ tubulin (F167Y)	Jiangsu province, China	MBC <sup>R</sup>	+
4	TZ1205	Mutation at codon 167 of $\beta_2$ tubulin (F167Y)	Jiangsu province, China	MBC <sup>R</sup>	+
5	HA1008	Mutation at codon 167 of $\beta_2$ tubulin (F167Y)	Jiangsu province, China	MBC <sup>R</sup>	+
6	WX1002	Mutation at codon 167 of $\beta_2$ tubulin (F167Y)	Jiangsu province, China	MBC <sup>R</sup>	+
7	YC1011	Mutation at codon 167 of $\beta_2$ tubulin (F167Y)	Jiangsu province, China	MBC <sup>R</sup>	+
8	JH1408	Mutation at codon 167 of $\beta_2$ tubulin (F167Y)	Jiangsu province, China	MBC <sup>R</sup>	+
9	JH1411	Mutation at codon 167 of $\beta_2$ tubulin (F167Y)	Jiangsu province, China	MBC <sup>R</sup>	+
10	JH1463	Mutation at codon 167 of $\beta_2$ tubulin (F167Y)	Jiangsu province, China	MBC <sup>R</sup>	+
11	XS1403	Mutation at codon 167 of $\beta_2$ tubulin (F167Y)	Jiangsu province, China	MBC <sup>R</sup>	+
12	XS1433	Mutation at codon 167 of $\beta_2$ tubulin (F167Y)	Jiangsu province, China	MBC <sup>R</sup>	+
13	XS1443	Mutation at codon 167 of $\beta_2$ tubulin (F167Y)	Jiangsu province, China	MBC <sup>R</sup>	+
14	XS1451	Mutation at codon 167 of $\beta_2$ tubulin (F167Y)	Jiangsu province, China	MBC <sup>R</sup>	+
15	CHZ1013	Mutation at codon 167 of $\beta_2$ tubulin (F167Y)	Anhui province, China	MBC <sup>R</sup>	+
16	SUZ1003	Mutation at codon 167 of $\beta_2$ tubulin (F167Y)	Anhui province, China	MBC <sup>R</sup>	+
17	LJ1403	Mutation at codon 167 of $\beta_2$ tubulin (F167Y)	Anhui province, China	MBC <sup>R</sup>	+
18	LJ1411	Mutation at codon 167 of $\beta_2$ tubulin (F167Y)	Anhui province, China	MBC <sup>R</sup>	+
19	SC1103	Mutation at codon 167 of $\beta_2$ tubulin (F167Y)	Sichuan province, China	MBC <sup>R</sup>	+
20	SC1105	Mutation at codon 167 of $\beta_2$ tubulin (F167Y)	Sichuan province, China	MBC <sup>R</sup>	+
21	SC1108	Mutation at codon 167 of $\beta_2$ tubulin (F167Y)	Sichuan province, China	MBC <sup>R</sup>	+
22	HB1002	Mutation at codon 167 of $\beta_2$ tubulin (F167Y)	Hubei province, China	MBC <sup>R</sup>	+
23	HB1003	Mutation at codon 167 of $\beta_2$ tubulin (F167Y)	Hubei province, China	MBC <sup>R</sup>	+
24	LY1102	Mutation at codon 167 of $\beta_2$ tubulin (F167Y)	Shandong province, China	MBC <sup>R</sup>	+
25	LY1108	Mutation at codon 167 of $\beta_2$ tubulin (F167Y)	Shandong province, China	MBC <sup>R</sup>	+
26	LY1110	Mutation at codon 167 of $\beta_2$ tubulin (F167Y)	Shandong province, China	MBC <sup>R</sup>	+
27	LH1201	Mutation at codon 167 of $\beta_2$ tubulin (F167Y)	Henan province, China	MBC <sup>R</sup>	+
28	LH1001	Mutation at codon 167 of $\beta_2$ tubulin (F167Y)	Henan province, China	MBC <sup>R</sup>	+

<sup>a</sup> MBC<sup>R</sup> indicate that the isolate is sensitive and resistant to carbendazim, respectively.

<sup>b</sup> ‘+’ represent positive results.