Specific Detection of *Fusarium oxysporum* f. sp. *cubense* and its Races by Loop-Mediated Isothermal Amplification (LAMP)

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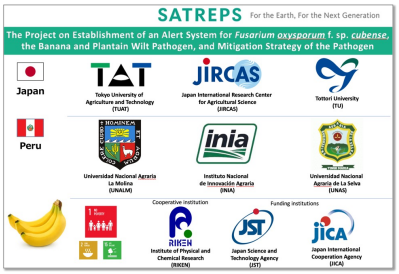
Abstract

Loop-mediated isothermal amplification (LAMP) can be used for rapid, simple and easy detection of plant pathogens. In this study we established LAMP for specific detection of the banana wilt pathogen *F. oxysporum* f. sp. *cubense* (*Foc*) and its races, based on the genomic information. *Foc*-specific detection primer set was designed on a candidate effector gene *ce15*. The gene was present in all *Foc* isolates tested, but absent in other formae speciales of *F. oxysporum* and non-pathogenic *Fusarium* spp. A *Foc* race 1 isolate 160527 possessed *ce15* on the contig 2, and its contig 2-partly-deficient mutant lost pathogenicity to banana (cv. Shima-banana; Matsui 2022). Races of *Foc* can be distinguished by the retention pattern of the putative effector genes. Race SR4 (subtropical race 4) possesses *SIX7* and *SIX8*, TR4 (tropical race 4) possesses only *SIX8*, and race 1 has neither. We designed primer sets for the specific detection of *SIX7* and *SIX8*, respectively. We could propose the rapid detection of *Foc* and its races by combination of LAMP with these primer sets. Extraction of genomic DNA, used as a template, from plant tissues and fungal mycelial cake took about 10 min using Template Prepper kit, LAMP reaction was completed in about 30 min and the determination can be made immediately after the reaction. The detection limit of the template DNA was 0.5 x 10^{-2} ng/µl reaction mixture for all the primer sets.



Form- and race-specific DNA regions

Genome comparisons of various forms and races of *Fusarium oxyspourm* have revealed form- and race-specific DNA regions. In many cases, form- and race-specific regions exist on so-called accessory chromosomes. In the banana wilt pathogen, *F. oxyspourm* f. sp. *cubense* (*Foc*) 160527, for example, the contig 2 (ctg. 2) seems to be an accessory chromosomal region, and genes on such regions can be used to design primers for the specific detection by PCR and LAMP (Fig. 1). *Ce15* is present in all *Foc* isolates, but absent in other formae speciales of *F. oxyspourm* and non-pathogenic *Fusarium* spp. (Table 1). Race SR4 (subtropical race 4) possesses *SIX7* and *SIX8*, TR4 (tropical race 4) possesses only *SIX8*, and race 1 has neither (Table 1).

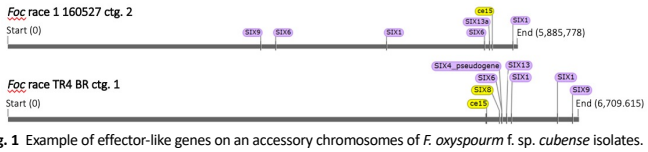


Fig. 1 Example of effector-like genes on an accessory chromosomes of *F. oxyspourm* f. sp. *cubense* isolates.

Table 1 Candidate genes used for specific detection of *Foc* and its races

	Ce15	*SIX7*	*SIX8*
F. oxyspourm f. sp. *cubense*			
race 1	+	—	—
race SR4	+	+(a)	+(a,b)
race TR4	+	—	+(a)
Other forms of *F. oxyspourm*	—	±	±
Non-pathogenic *Fusarium* spp.	—	—	—

+indicates possession; ±indicates some isolates possesses and the others not.

Loop-mediated isothermal amplification (LAMP) and primer sets

Loop-mediated isothermal amplification (LAMP) is an isothermal nucleic acid amplification technique (Fig. 2). In contrast to the polymerase chain reaction (PCR) technology, in which the reaction is carried out quickly with a series of alternating temperature steps or cycles, isothermal amplification is carried out at a constant temperature and does not require a thermal cycler. LAMP has been used to detect certain diseases.

In this study, primer sets were designed for *Ce15*, *SIX7* and *SIX8* and used for the specific detection of *Foc* and its races (Fig. 3).

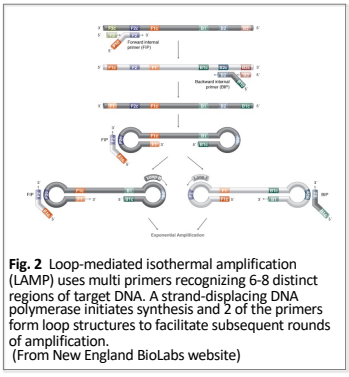


Fig. 2 Loop-mediated isothermal amplification (LAMP) uses multi primers recognizing 6-8 distinct regions of target DNA. A strand-displacing DNA polymerase initiates synthesis and 2 of the primers form loop structures to facilitate subsequent rounds of amplification.
(From New England BioLabs website)

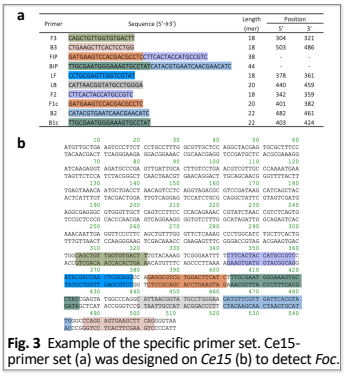


Fig. 3 Example of the specific primer set. Ce15-primer set (a) was designed on *Ce15* (b) to detect *Foc*.

LAMP reaction is completed in 30 min

DNA extraction from the pseudostem tissues of diseased banana plants and from fungal mycelia takes about 10 min using Template Prepper Kit (Nippon Gene). LAMP reaction using LAMP MASTER for Turbidity (Visible Dye) Kit (Nippon Gene) or DryADD LAMP Master Mix (Turibidity / Visible Dye) (Nippon Gene Material) and each primer set at 65˚C is completed in about 30 min and the determination can be made immediately after the reaction. The detection limit of the template DNA was 0.5 x 10^{-2} ng/µl reaction mixture for all the primer sets (data not shown). Detection finishes in about 40 min in total (Fig. 4).

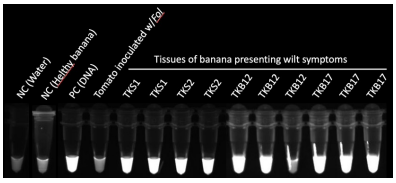


Fig. 4 Example of LAMP reaction. DNA was purified from the tissues of banana presenting wilt symptoms in the Kansai-1 and -2 fields, and tested with the Ce15-primer set to detect *Foc*.

Diagnoses of Peruvian and Japanese isolates

F. oxysporum isolates were subjected to LAMP with Ce15-, SIX7- and SIX8-primer sets to determine if it is *Foc* and its races.

Table 2 Detection of *Foc* and its races by the combination of LAMP-reactions using specific primer sets

Primer set for LAMP			Determination
Ce15	*SIX7*	*SIX8*	
+	—	—	→ *Foc* race 1
+	+	+	→ *Foc* race SR4
+	—	+	→ *Foc* race TR4
—	NT	NT	→ Not *Foc*

+indicates positive reaction; —indicates negative reaction.

Peruvian isolates were determined to be race 1 or SR4 (Table 3). No race TR4 was found.

Japanese isolates from Okinawa, Amami, and Kansai-2 fields were race 1; however, those obtained from Kanto-1 and Kansai-1 fields seemed to be race TR4 (Table 3). These observation was confirmed by the phylogeny based on the combined sequence of *EF1*α and rDNA-IGS (Fig. 5).

Table 3 Isolates of banana wilt pathogen tested in this study and race determined by LAMP

Country / Isolate	Place of sampling	Year	LAMP reaction *Ce15*	*SIX7*	*SIX8*	Determined race
Indonesia						
FocBR	Unknown	Unkown	+	—	+	TR4
Japan						
160527	Miyako, Okinawa	2016	+	—	—	1
220608	Okinawa, Okinawa	2022	+	—	—	1
22YN1-1	Amami, Kagoshima	2022	+	—	—	TR4
FKS-2	Kanto-1	2022	+	—	+	TR4
KH1-1	Amami, Kagoshima	2022	+	—	—	1
KH1-1	Amami, Kagoshima	2022	+	—	—	1
TKB12-2	Kansai-2	2022	+	—	—	1
TKB17-2	Kansai-2	2022	+	—	—	1
TKS1-1	Kansai-1	2022	+	—	+	TR4
TKS2-2	Kansai-1	2022	+	—	+	TR4
Peru						
11-Cvi	Sullana, Peru	2018	+	+	+	SR4
13a-Ma	Chanchamayo, Peru	2018	+	+	+	SR4
14a-Ma	Chanchamayo, Peru	2018	+	+	+	SR4
TMB1-1	Tingo Maria, Peru	2022	+	—	—	1
TMB1-1	Tingo Maria, Peru	2022	+	+	+	SR4
TMD1-1	Tingo Maria, Peru	2022	+	+	+	SR4
TMD1-2	Tingo Maria, Peru	2022	+	—	—	1
TME1-1	Tingo Maria, Peru	2022	+	+	+	SR4

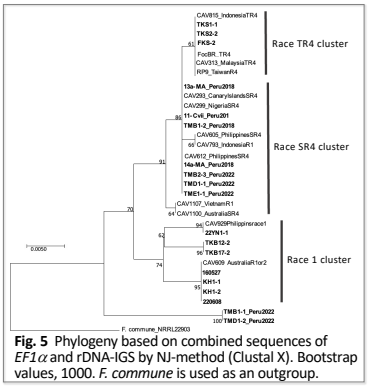


Fig. 5 Phylogeny based on combined sequences of *EF1*α and rDNA-IGS by NJ-method (Clustal X). Bootstrap values, 1000. *F. commune* is used as an outgroup.

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