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Research Article

Evaluation of Some Specific Primer Sets Development for Detecting *Fusarium oxysporum* f. sp. *cubense* Tropic Race 4 (*Foc* TR4) Originating from Indonesia

Evaluasi Pengembangan Beberapa Primer Spesifik untuk Deteksi Fusarium oxysporum *f. sp.* cubense *Ras 4 Tropika (*Foc *TR4) Asal Indonesia*

Yudha Pratama[1)]*, Arif Wibowo[1)], Ani Widiastuti[1)], Siti Subandiyah[1,2)], Sri Widinugraheni[3,4)], & Martijn Rep[4)]

[1)]Department of Crop Protection, Faculty of Agriculture, Universitas Gadjah Mada
Jln. Flora No. 1, Bulaksumur, Sleman, Yogyakarta 55281 Indonesia

[2)]Research Center for Biotechnology, Universitas Gadjah Mada
Jln. Teknika Utara, Sleman, Yogyakarta 55281 Indonesia

[3)]Faculty of Agriculture, Nusa Cendana University
Jln. Adisucipto, Penfui-Kupang, Nusa Tenggara Timur 85228 Indonesia

[4)]Molecular Plant Pathology, University of Amsterdam
Science Park 904, 1098XH Amsterdam, Netherlands

**Corresponding author. E-mail: yudha.pratama1992@gmail.com*

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ABSTRACT

Fusarium oxysporum f. sp. *cubense* tropic race 4 (*Foc* TR4) strain which belong to Vegetative Compatibility Group (VCG) 01213 is the most devastating disease in global banana production. Validation of specific primer sets using the positive control (*Foc* TR4). In total, 50 isolates of *Foc* are collected from several banana production regions in Indonesia represent the group of VCG, races, genotype, cultivars, which are confirmed as *Foc* based on the tested using FocEf3 primer set, except Cjr-2 and Lmp-4 isolates. Foc-1/Foc-2 could amplify 34 *Foc* isolates included in *Foc* race 4. Three specific primer sets i.e. TR4-F/TR4-R, Six-1c, and TR4-F2/TR4-R1 are used to classify *Foc* isolates into *Foc* tropic race 4. TR4-F/TR4-R is known have the highest specificity as it could amplify 35 *Foc* isolates including positive controls (*Foc* TR4) compared to the other primer sets (Six-1c and TR4-F2/TR4-R1). This research indicates that there are a large number of diversity strains found in *Foc* isolates to be studied for further research. Race 4 of *Foc* (STR4 or TR4) is known to be widespread in several regions in Indonesia. Therefore, specific primer set development needs to be done to detect *Foc* TR4 and the most damaging strains on *Foc* TR4 based on molecular data.

Keywords: design primer, *Fusarium oxysporum* f. sp. *cubense*, molecular detection

INTISARI

Fusarium oxysporum *f. sp. cubense ras 4 tropika (*Foc *TR4) yang termasuk ke dalam kelompok VCG 01213 merupakan patogen yang paling merusak dalam produksi tanaman pisang secara global. Validasi primer spesifik berbasis PCR menggunakan kontrol positif (*Foc *TR4). Total, 50 isolat* Foc *dikoleksi dari beberapa daerah produksi pisang di Indonesia mewakili VCG, ras, genotipe dan kultivaryang dikonfirmasi sebagai isolat* Foc *berdasarkan pengujian menggunakan primer FocEf3, kecuali isolat Cjr-2 dan Lmp-4. Foc-1/Foc-2 dapat mengamplifikasi 34 isolat* Foc *yang termasuk ke dalam* Foc *ras 4. Selanjutnya tiga pasang primer spesifik yaitu TR4-F/TR4-R, Six-1c, dan TR4-F2/TR4-R1 digunakan untuk mengelompokkan isolat-isolat tersebut ke dalam isolat* Foc *ras 4 tropika. TR4-F/TR4-R diketahui memiliki spesifisitas tertinggi karena dapat mengamplifikasi sebanyak 35 isolat* Foc *termasuk kontrol positif (*Foc *TR4) dibandingkan dengan primer lainnya (Six-1c dan TR4-F2/TR4-R1). Penelitian ini menunjukkan bahwa terdapat sejumlah besar keragaman strain yang terlihat pada isolat-isolat* Foc *tersebut untuk dapat dipelajari lebih lanjut. Ras 4 dari* Foc *(STR4 atau TR4) diketahui tersebar luas pada beberapa daerah di Indonesia. Oleh karena itu, perlu dilakukan pengembangan primer spesifik untuk mendeteksi* Foc *TR4 dan strain yang paling merusak pada* Foc *TR4 berdasarkan data molekuler.*

Kata kunci: desain primer, deteksi molekuler, Fusarium oxysporum *f. sp*. cubense

INTRODUCTION

Fusarium wilt or Panama disease caused by *Fusarium oxysporum* f. sp. *cubense* (*Foc*) (E.F. Smith) Snyder and Hansen is the most destructive pathogen of banana in the world. Four races of *Foc* have been reported based on their pathogenicity to different banana cultivars, except race 3 which is not a pathogen of banana as it only infects *Heliconia* spp. Race 1 infects Gros Michel (AAA), Silk and Pome varieties (AAB). Race 2 causes disease in cooking banana cultivars such as Bluggoe (ABB) (Ploetz, 2006). Race 1 of *Foc* gained prominence when it almost destroyed Gros Michel bananas, in Central America in the first half of the 1900s. As a result, Gros Michel cultivar is replaced by resistant Cavendish cultivar (AAA) in the early 1960s (Stover, 1962). Unfortunately, a new race of *Foc*, that is, race 4 is the most recently evolved and most virulent strain infecting the Cavendish cultivar as well as both race 1 and race 2 susceptible cultivars (Stover, 1972). Hence, fusarium wilt continues to be a constraint to susceptible varieties and is still considered a major threat to banana production because it cannot be controlled with fungicides.

Race 4 of *Foc* is first designated in Taiwan by Su in 1977 on the basis of wilt in Cavendish cultivar (Su *et al*., 1986). Before 1990, isolates that are classified as race 4 only caused serious losses in Cavendish genotypes in sub-tropic regions of Australia, the Canary Islands and Taiwan (Ploetz, 1990). Since then, a new variant that severely affects Cavendish cultivar in the tropics is identified. Thus, two types of *Foc* race 4, subtropic race 4 (STR4) and tropic race 4 (TR4) are designated. However, while *Foc* STR4 isolates cause disease in Cavendish cultivar in the subtropics, mainly when plants are exposed to abiotic stress, *Foc* TR4 isolates are pathogenic under both tropic and subtropic conditions (Buddenhagen, 2009).

Since its appearance, Foc TR4 has caused severe damage to Cavendish cultivars in TR4 had been confirmed in Australia (Northern Territory and Queensland), China (Hainan, Hunan, Guangdong, and Guangxi), Indonesia (Bali, Halmahera, Kalimantan, Java, Papua Province, Sulawesi, and Sumatra), Jordan, Lebanon, Malaysia (Peninsular and Sarawak), Mozambique, Oman, Pakistan, the Philippines (Mindanao), and Taiwan (Butler, 2013; Garcia *et al*. 2013; Molina *et al*., 2010; Ordonez *et al*., 2015; Ploetz, 2006; Ploetz *et al.*, 2015). Control strategies of this pathogen are based on visual monitoring for early symptom appearance, eradication of infected plants and isolation of infested areas to reduce pathogen dissemination. However, these strategies are often impractical and therefore does not carried out (Dita *et al*., 2010).

Based on Vegetative Compatibilty Group (VCG), *Foc* have been separated into 24 vegetative compatibility groups (VCGs) (Ghag *et al*., 2015). Finally, various identification tools have been used to separate *Foc* into a number of clonal lineages to their grouping based on VCGs (Bentley *et al*., 1998). Till now, *Foc* separated into two clades and eight lineages with the majority of groups present in Asia (Ghag *et al*., 2015). Phenotypic and genotypic analyses of worldwide collections of *Foc* suggest that Southeast Asia is the centre of origin of *Foc* (Fourie *et al.*, 2009) and the pathogen has been introduced into new regions from there (Stover, 1962). The tropic race 4 strain of *Foc* belongs to a single group of VCG 01213/16 complex, whereas the subtropic race 4 isolates belong to VCG 0120, 0121, 0122, 0129 and 01211 (Buddenhagen, 2009).

The development of a rapid and reliable PCR diagnostic for *Foc* TR4 (VCG 01213) using Random Amplification of Polymorphic DNA (RAPD), Restriction Fragment Length Polymorphisms (RFLPs), Sequence Characterized Amplified Region (SCAR), Elongation Factor (EF1α) gene, Single Nucleotide Polymorphisms (SNPs) based on the Intergenic spacer (IGS) and small secreted protein (Six, Secret in xylem) have been developed for the identification of *Foc* using diagnostic traits that are directly linked to pathogenicity or virulence would provide another powerful approach for identification of soil-borne pathogenic fungi such as *Foc* TR4 (Lin *et al*., 2008; Liao *et al.*, 2009; Dita *et al*., 2010; Lin *et al.*, 2012; Li *et al*., 2013; Guo *et al.*, 2014). Therefore, the main objective of this study is to evaluate the specific primer sets development as well as its specificity for *Foc* TR4 (VCG 01213) detection originating from different banana production areas in Indonesia.

MATERIALS AND METHODS

Isolates collection

In total, 50 monosporic cultures of *Foc* isolates originating from different banana production areas in Indonesia (Bangka, West Java, Central Java, East Java, Kalimantan, and Sumatera) are analysed.

The cultivars, genotypes and VCG of all *Foc* isolates are presented in Table 2. VCG tests are performed for all of the *Foc* isolates from different banana production areas have been done by Wibowo *et al*. (2011), including VCG 01213 which are belong to *Foc* TR4 (Table 2).

DNA Extraction

To obtain the mycelial mass, the isolates are grown on Potato Dextrose Broth medium (PDB) in 100 ml Erlenmeyer flasks for 7 days at 25ºC. The isolates are shaken continuously for a period depending on each isolate. After 7 days, the mycelial mass of each isolate is filtered for the extraction of DNA. Filter paper is used to collect the mycelia and they are grinded in CTAB buffer using a mortar and pestle. The extraction of DNA from the isolates is performed using the CTAB method with some modification. PCR product is visualized on a 1% agarose gel stained with ethidium bromide viewed under UV light.

Identification Foc Isolates Using FocEf3 Primer Set

Firstly, to make sure that DNA samples of fifty isolates are *Foc*, FocEf3 primer set that is designed by Widinugraheni *et al*. (2015) used for *Foc* detection yield an amplification product of 600 bp using the following programme: 94ºC for 5 min followed by 30 cycles consisting of 94ºC for 30 sec, 57ºC for 30 sec, and 72ºC for 2 min 30 sec followed by an additional extension time at 72°C for 5 min.

Evaluation Test of Foc Isolates Using Specific Primer Design of Foc Race 4 and Foc TR4

All isolates are tested using specific primer Foc-1/Foc-2 (Lin *et al*., 2008) to detect *Foc* race 4. This primer yield an amplification product of 242 bp using the following programme: 95ºC for 5 min followed by 33 cycles consisting of 95ºC for 1 min, 55ºC for 1 min, and 72ºC for 3 min followed by an additional extension time at 72ºC for 10 min. The amplified isolates using Foc-1/Foc-2, are tested using specific primer sets to detect *Foc* TR4 i.e. TR4-F/TR4-R (Dita *et al*., 2010), Six-1c (Widinugraheni *et al*., 2015) and TR4-F2/TR4-R1 (Bentley *et al.*, 2003). TR4-F/TR4-R yield an amplification product of 463 bp with the following programme: 95ºC for 5 min followed by 30 cycles consisting of 95ºC for 1 min, 60°C for 1 min, and 72ºC for 3 min followed by an additional extension time at 72ºC for 10 min. Six-1c yield an amplification product of 884 bp with the following programme: 94ºC for 5 min followed by 30 cycles consisting of 94ºC for 30 s, 57ºC for 30 s, and 72ºC for 2 min 30 s followed by an additional extension time at 72ºC for 5 min. TR4-F2/TR4-R1 primer set that also used in this study according the combination and modification of methods developed by Bentley *et al.* (2003), yield an amplification product of 1400 bp with the following programme: 95ºC for 2 min followed by 30 cycles consisting of 95ºC for 30 sec, 68ºC for 1 min 30 sec, and 72ºC for 3 min followed by an additional extension time at 72ºC for 10 min.

RESULTS AND DISCUSSION

Identification Foc Isolates Using FocEF3 Primer Set

In total, 48 isolates are confirmed as *Foc* isolates based on the test using FocEf3 primer set which is designed by Widinugraheni *et al*. (2015) for *Foc* except Cjr-2 and Lmp-4 isolates that probably contaminant (Table 1).

Evaluation Test of Foc Isolates Using Specific Primer Design of Foc Race 4 and Foc Tropic Race 4

Lin *et al*. (2008) designed a specific primer set which is derived from RAPD fragment specific to *Foc* race 4 isolates from Taiwan are amplified by the random primer OPA02404 and designed a specific primer set called Foc-1/Foc-2 (Foc242) from the OPA02404 nucleotide sequences is used in this study. The Foc-1/Foc-2 (Foc242) had been confirmed with high specificity to detect *Foc* race 4 (*Foc* race 4) (Lin *et al*., 2008). From 50 *Foc* isolates, Foc-1/Foc-2 could amplify 34 isolates with a percentage of 68%, but not to the other 16 isolates (Table 1).

To classify the isolates into *Foc* tropic race 4 (*Foc* TR4), then some specific primer sets for *Foc* TR4 detection are used. In this study 3 specific primer sets are developed to amplify *Foc* TR4 (VCG 01213) i.e. TR4-F/TR4-R (Dita *et al.*, 2010), Six-1c (Widinugraheni *et al*., 2015), and TR4-F2/TR4-R1 (Bentley *et al*., 2003).

TR4-F/TR4-R primer set derived from sequences of IGS region to study genetic diversity of *Foc*, with the aim of identifying Single Nucleotide Polymorphisms (SNPs) for specific primer design for *Foc* TR4 detection. However, the results showed that the higher SNP frequency of the IGS region provides a rich source of genetic diversity in *Foc* (Dita *et al*., 2010). From 50 *Foc* isolates have been tested by TR4-F/TR4-R, 35 *Foc* isolates could be amplified by TR4-F/TR4-R with a percentage of 70%, but not to the other 15 isolates (Table 1).

Table 1. Total amplified isolates and not amplified using general primer set of *Foc*, specific primer sets of *Foc* race 4 and *Foc* TR4

Primer name	References	Primer specificity	Amplification target	Total of amplified isolates	Total of not amplified isolates
FocEf3	Widinugraheni *et al.*, 2015	General *Foc*	600 bp	48	2
Foc-1/Foc-2	Lin *et al*. 2008	*Foc* race 4	242 bp	34	16
TR4-F/TR4-R	Dita *et al*. 2010	*Foc* tropic race 4	463 bp	35	15
Six-1c	Widinugraheni *et al*., 2015	*Foc* tropic race 4	884 bp	25	25
TR4-F2/TR4-R1	Bentley *et al.* 2003	*Foc* tropic race 4	1400 bp	17	33

Six-1c specific primer set that is designed by Widinugraheni *et al*. (2015) with some selection of several effectors on *Foc* TR4 (II5) strain genome based on the homology of Sixgeneis used in this study also capable in detecting *Foc* TR4. From the result, Six-1c could amplify 25 *Foc* isolates with a percentage of 50%, but not to the other 25 isolates (Table 1).

TR4-F2/TR4-R1 primer set that also used in this study according the combination and modification of methods developed by Bentley *et al*. (2003) is also specific to *Foc* TR4, have the lowest specificity which could only amplify 17 *Foc* isolates with a percentage of 34%, but not to the other 33 isolates. Beside of that Bdg-1A, Cms-1, Grt-1, Grt-3, Tsk-2, Bgl-3, Sdt-1, U-14, Batu-2, Kbr-1, Kdg-1, Ksp-1, Pjn-5, Btp-1, Ktr-1, Kd-2, Kp-1, and Kp-4 isolates could be amplified by TR4-F/TR4-R, but they are not using TR4-F2/TR4-R1 (Table 2).

In identifying *Foc* TR4 isolates, 34 *Foc* isolates that amplified using Foc-1/Foc-2 are compared using three pairs of specific primer TR4-F/TR4-R, Six-1c and TR4-F2/TR4-R1. From 34 *Foc* isolates, there are 24 isolates that could be amplified by TR4-F/TR4-R (Bdg-1A, Bjr-2, Grt-1, Tsk-2, Bgl-3, Mln-1, Pbn-1, Prb-1, Sdt-1, Batu-2, Ksp-1, Pjn-5, A-13, Bnt-1, Bnt-2, Btp-1, Gnk-2, Kp-1, Kp-4, Kp-H, Slm-1, Slm-3, Lmp-1 and Lmp-3). Otherwise Six-1c could amplified 20 Foc isolates (Bdg-1, Bdg-1A, Bjr-2, Tsk-2, Mln-1, Pbn-1, Prb-1, Sdt-1, Batu-2, Batu-3B, A-13, Bnt-1, Bnt-2, Gnk-2, Kp-4, Kp-H, Slm-1, Slm-3, Lmp-1, and Lmp-3). Meanwhile TR4-F2/TR4-R1 could only amplify 13 isolates (Bjr-2, Mln-1, Pbn-1, Prb-1, A-13, Bnt-1, Bnt-2, Gnk-2, Kp-H, Slm-1, Slm-3, Lmp-1, and Lmp-3) (Table 2).

Because of the highest specificity of *Foc* TR4-F/TR4-R than the other primer sets (Six-1c and TR4-F2/TR4-R1), all of the amplified isolates using TR4-F/TR4-R, arecompared by Foc-1/Foc-2. From all of the *Foc* isolates showed that Bnk-12, Bnk-25, Cms-1, Grt-3, U-14, Kjg-2, Kbr-1, Kdg-1, Kd-2, Ktr-1, and Kp-3 that could be amplified by TR4-F/ TR4-R but they are not using Foc-1/Foc-2. Otherwise, Bdg-1, Bjr-1, Cjr-1, Grt-1, Grt-2, Prb-2, Skj-2, Tmg-4, Batu-3, Batu-3B, and Batu-4 isolates are not amplified by TR4-F/TR4-R but they are using Foc-1/Foc-2 (Table 2). In addition, there are some isolates that could be amplified by TR4-F/TR4-R, Six-1c, and TR4-F2/TR4-R1 but could not be amplified by Foc-1/Foc-2, in which these isolates should be included to *Foc* race 4 (Table 2).

Dita *et al.* (2010) also found similar case. In their study, TR4-F/TR4-R could only amplify *Foc* TR4 isolates (VCG 01213). Meanwhile, Foc-1/Foc-2 reacted with *Foc* tropic race 4 (VCG 01213) and others VCG known as *Foc* subtropic race 4, they are FocYB, Foc19508 (VCG 0120), NRRL36107 (VCG 0126), NRRL36101 (VCG 0123) and NRRL26029 (VCG 01210). Based on the source of the reference, these isolates are known as *Foc* race 1. This ambiguity illustrates the drawback inthe current race designation system for *Foc* in banana. An isolate may be classified as STR4 in subtropic areas (where it affects Cavendish), but in tropic areas, an isolate called as *Foc* race 1 as is the case in the isolates (where it is unable to affect Cavendish). For instance, genetically identical isolates of Foc are classified as race 4 isolates in the subtropics, because they cause disease to Cavendish bananas under subtropic conditions only but it designated as race 1 isolates in the tropics (Dita *et al*., 2010).

Fungi, like all living organisms, have the ability to adapt in response to changing or new environments. Environmental changes exert selection pressure on an organism (McDonald, 1997), and only individuals that adjust to change are able to succeed. The capacity

Table 2. Origin *Foc* isolates, VCG, race classification, cultivar, genotype and response to specific primer sets

No.	Origin	Isolate name	VCG	Race	Host or cultivar	Genotype	Response to specific primer sets				
							FocEf3	Foc1/Foc2	TR4-F2/TR4-R1	TR4-F/TR4-R	Six-1c
1	BANGKA	Bnk-12	nc		Ambon	AAA	+	-	+	+	+
2		Bnk-25	nc		Ambon	AAA	+	-	+	+	+
3	WEST JAVA	Bdg-1	nt		Ambon	AAA	+	+	-	-	+
4		Bdg-1A	nc		Ambon	AAA	+	+	-	+	+
5		Bdg-2	nt		Uter	ABB	+	-	-	-	-
6		Bjr-1	nt		Kepok	ABB	+	+	-	-	-
7		Bjr-2	nc		Uter	ABB	+	+	+	+	+
8		Cms-1	nt		Uter	ABB	+	-	-	+	-
9		Cjr-1	nt		Ambon	AAA	+	+	-	-	-
10		Cjr-2	nt		Kepok	ABB	-	-	-	-	-
11		Grt-1	nc		Ambon	AAA	+	+	-	+	-
12		Grt-2	nt		Uter	ABB	+	+	-	-	-
13		Grt-3	nc		Ambon	AAA	+	-	-	+	-
14		Tsk-1	nt		Uter	ABB	+	-	-	-	-
15		Tsk-2	nt		Ambon	AAA	+	+	-	+	-
16	CENTRAL JAVA	Bgl-3	01213/16	TR4	Kepok	ABB	+	+	-	+	+
17		Mln-1	01213/16	TR4	Kepok kerau	ABB	+	+	+	+	-
18		Pbn-1	nt		Ambon	AAA	+	+	+	+	+
19		Prb-1	nt		Ambon	AAA	+	+	+	+	+
20		Prb-2	nt		Ambon	AAA	+	+	-	-	+
21		Sdt-1	nc		Ambon	AAA	+	+	-	+	-
22		Skj-2	nc		Ambon	AAA	+	+	-	-	+
23		Tmg-4	nt		Uter	ABB	+	+	-	-	-
24		U-14	nt		Uter	ABB	+	-	-	+	-
25	KALIMANTAN	Kjg-2	nc		Raja	AAB	+	-	+	+	+

Table 2. Origin *Foc* isolates, VCG, race classification, cultivar, genotype and response to specific primer sets (continued)

No.	Origin	Isolate name	VCG	Race	Host or cultivar	Genotype	Response to specific primer sets				
							FocEf3	Foc1/Foc2	TR4-F2/TR4-R1	TR4-F/TR4-R	Six-1c
26	EAST JAVA	Batu-2	nt		Kepok	ABB	+	+	-	+	+
27		Batu-3	nc		Raja	AAB	+	+	-	-	+
28		Batu-3B	nc		Raja	AAB	+	+	-	-	-
29		Batu-4	nc		Kepok	ABB	+	+	-	-	+
30		Kbr-1	nc		Ambon	AAA	+	-	-	+	-
31		Kdg-1	nc		Kepok	ABB	+	-	-	+	+
32		Ksp-1	nt		Ambon	AAA	+	+	-	+	-
33		Pjn-4	0129	R1	Raja	AAB	+	-	-	-	-
34		Pjn-5	nc		Raja	AAB	+	+	-	+	-
35	YOGYAKARTA	A-13	01213/16	TR4	Ambon	AAA	+	+	+	+	-
36		Bnt-1	nc		Cavendish	AAA	+	+	+	+	+
37		Bnt-2	01213/16	TR4	Awak	ABB	+	+	+	+	+
38		Btp-1	nt		Ambon	AAA	+	+	-	+	+
39		Gnk-2	nc		Ambon	AAA	+	+	+	+	-
40		Kd-2	nt		Uter	ABB	+	-	-	+	+
41		Kp-1	nc		Ambon	AAA	+	+	-	+	-
42		Kp-3	nt		Uter	ABB	+	-	+	+	-
43		Kp-4	nc		Ambon	AAA	+	+	-	+	-
44		Kp-H	nc		Kepok	ABB	+	+	+	+	+
45		Ktr-1	nt		Uter	ABB	+	-	-	+	+
46		Slm-1	nt		Ambon	AAA	+	+	+	+	+
47		Slm-3	nt		Uter	ABB	+	+	+	+	+
48	SUMATERA	Lmp-1	01213/16	TR4	Raja nangka	AAB	+	+	+	+	+
49		Lmp-3	nc		Cavendish	AAA	+	+	+	+	+
50		Lmp-4	nt		Cavendish	AAA	-	-	-	-	-

Remark: Vegetative Compatibility Group (VCG) is performed for all *Foc* isolates by Wibowo *et al.* (2011), including VCG 01213 group known as *Foc* TR4, nt: not tested, and nc: not compatible with the entire VCG tester

of population of pathogens to adapt is determined, in part, by their diversity. As the gene pool that a population can sample increases, so does its adaptability to a changing environment or a new host genotype (McDonald & McDermott, 1993).

The evolutionary history of *Foc* is very complex. Based on the phylogenetic studies of *Foc* divided taxon into several lineage representing two of four *F. oxysporum* clade, indicating that the polyphyletic of *Foc* more closely related to other *F. oxysporum* forma specialist than *Foc* lineage. This led to a speculation that the formation of a new pathogen from *F. oxysporum* maybe come from a member of the pathogens and non-pathogens from this species (Fourie *et al*., 2011). Fourie *et al*. (2011) also confirm that co-evolution between plant and horizontal gene transfer is the evolutionary history of *Foc* and showed that there is a lot of diversity on *Foc* strain that should be studied and need further research and surveys against the evolution of this pathogen.

Genetically, *Foc* have ability to infect specific banana cultivar, so that it can be grouped into biological races (Waite & Stover, 1960; Su *et al*., 1986). Based on the tested *Foc* isolates from Indonesia and some previous research in grouping *Foc* based on the PCR-based methods using specific primer sets, it turns out that there is not enough to grouping different strains of *Foc* based on the races. *Foc* is classified into two clades A and B based on the evolutionary origins. Clade A group of *Foc*, co-evolved with bananas having A genome, whereas clade B evolved along with their host banana plants having purely B genome or mixed A and B genomes. Currently, the banana disease causing strains of *Foc* are evolved from both clade A and clade B lineages (Fourie *et al*., 2009). In order to characterize these genetically related populations, they have now been grouped into same VCGs based on the formation of a heterokaryon (Puhalla, 1985).

The VCG gives a fair idea of the genetic diversity and evolution of the pathogen. However, the use of VCG as a means to classify *Foc* is also considered incomplete as one race since it can comprise of more than one VCG (e.g. VCG 0124 and VCG 0125 belong to race 1) or one VCG may occur in multiple races (e.g. VCG 0124 occurs in both race 1 and race 2). In addition, few of the VCG groups are cross compatible, giving rise to VCG complexes making it more difficult for pathotype identification. Till now, 24 VCGs of *Foc* have been identified with further separation into two clades and eight lineages originated from Asia (Ghag *et al*., 2015).

This result suggested that there are a large amount of diversity seen in *Foc* strains have to be discovered and that additional surveys and research are needed for the full appreciation of the evolution of this pathogen. Therefore, in order to pinpoint potential species boundaries to elucidate the true relationships among the VCGs and lineages of *F. oxysporum* f. sp. *cubense*, then, the diversity of *F. oxysporum* complex needs to be fully characterized (Fourie *et al*., 2009).

CONCLUSION

It showed that tropic and subtropic race 4 of *Foc* have been widely distributed in some regions in Indonesia. Based on the tested *Foc* TR4 isolates (VCG 01213) as control, TR4-F/TR4-R have the highest specificity to *Foc* TR4 isolate detection. Meanwhile, TR4-F2/TR4-R1 has the lowest specificity to *Foc* TR4 isolate detection. Further research should develop a specific primer set for detecting *Foc* TR4 and the most damaging strain of *Foc* based on genetic diversity. Therefore, in studying *Foc* especially *Foc* TR4 strain should focus on the specificity of genetic based on molecular data.

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