



CONFIRM THE STATUS OF VCG *Fusarium oxysporum* f. sp. *Cubense* IN WEST SUMATERA INDONESIA

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ABSTRACT

The objective of this experiment was to confirm the status VCGs of *Foc* in West Sumatera by identifying of *Fusarium oxysporum* f. sp. *Cubense* isolates with Vegetative Compatibility Group test. A total 47 isolates of *Foc* were collected from Indonesian Tropical Fruits Research Institute collection that preserved in paper sterile technique. Isolation, isolate purification and VCG analysis were held at Pest and Disease Laboratory of Indonesian Tropical Fruit Research Institute and Department of Primary Industry, Plant Pathology section, Indooroopili, Australia. The experiment started from July 2008 until December 2009. The testers of VCGs used were from Australia, namely: VCG 0120, VCG 0120/15, VCG 01215, VCG 0123, VCG 0124, VCG 0124/5, VCG 0125, VCG 0126, VCG 0128, VCG 01211, VCG 01213, VCG 01213/16, VCG 01216, VCG 01219 and VCG 01220. The results showed that a total of 47 isolates of *Foc* collection from West Sumatra grouped into five VCG (01213/16, 0121, 01219, 01218 and 0120) and seven isolates are unknown VCGs. A total of 13 varieties were attacked by *Foc* are Raja/AAB, Raja serai/AAB, Buai/AAA, P. panjang/AAB, Barangan/AAA, Ambon kuning/AAA, Ambon hijau/AAA, Rejang/Aaw, Jantan/AAB, Kepok/ABB/BBB, Manih/AA, Cavendish/AAA, siem/ABB and Mas kirana./AA. Total of 28 isolates (59,5%) grouped into VCG 01213/16 (TR4) attacked 11 varieties of bananas (Raja/AAB, Raja serai/AAB, Buai/AAA, Panjang/AAB, Barangan/AAA, Ambon kuning/AAA, Ambon hijau/AAA, Rejang/Aaw, Jantan/AAB, Kepok/ABB/BBB, and Mas kirana/AA).

Keywords: banana, *Fusarium oxysporum* f. sp. *Cubense*, vegetative compatibility groups, Indonesia.

INTRODUCTION

Fusarium wilt of bananas is caused by *Fusarium oxysporum* f. sp. *cubense* (E.F. Smith) Snyder dan Hansen (*Foc*) generally considered being the most dangerous diseases in agriculture world history (Moore *et al.*, 1995, Ploetz, 1990, Simmonds, 1966). The disease was first reported in 1876 in Queensland Australia (Ploetz, 1990, 2006), moreover the pathogen has been widespread by planting material to all banana production regions in the world, except those bordering the Mediterranean, Melanesia, Somalia and some island in Pacific (Jeger *et al.*, 1996). In Indonesia this disease has been found since 1916 in West Java (Stover, 1957). Nasir *et al.*, (2002) reported that this pathogen became severe losses of banana production in West Sumatra since 1980s to 1990s. Furthermore Hermanto *et al.*, (2009) reported that *Fusarium* wilt attack several banana varieties and extensive losses were caused by *Foc* get to all provinces in Indonesia.

Foc is complex pathogen, which has specified character and different of population structure (Visser *et al.*, 2010; Ploetz, 1990), this character is showed by variation of race and strain and then level of virulence among different race (Su *et al.*, 1986; Ploetz, 1990). Several methods have been developed for improvement of knowledge about this pathogen characters.

One of them is vegetative compatibility group (VCGs) (Ploetz and Correll, 1988; Brake *et al.*, 1990; Moore *et al.*, 1993). According to Kistler (1997) this technique is very objective for analyzing genetic similarity of the isolate. The similarity genetic of one isolate is grouped into the same VCGs. Ploetz (1990) and Leslie

(1990) explained that genetic exchange never happened between different isolate which is paired. It showed that categorization by VCG is very specify and could determine strain of *Foc*. This is very useful for banana resistance revise and grouped of *Foc* according to virulence of this disease among different variety of banana (race) (Correll *et al.*, 1987; Leslie 1990). Ploetz and Correll (1988), Moore *et al.* (1993), Pegg *et al.* (1995) and Katan (1999) reported that 24 VCGs of *Foc* were found in the world, 15 of VCGs were found in Asia. Hermanto *et al.* (2009) identified 11 groups VCGs of *Foc* in Indonesia and most of them were VCGs 01213/16 known as Tropical Race 4, that attacked more than 50% from all variety infected. Nasir *et al.* (2003) reported that 9 VCGs of *Foc* found in 6 district of West Sumatera and most of isolates were VCGs 01213/16. It is believed that co-evolution between *Foc* and banana/host most probable happens in nature (Bentley *et al.* 1998; Fourie *et al.*, 2009) and based on Molina *et al.*, (2011) reported that number of isolate VCGs of *Foc* in Philippines increased after 15th year. Therefore, the objective of this study was to confirm the status VCGs of *Foc* in West Sumatera by identifying *Fusarium oxysporum* f. sp. *cubense* isolates with Vegetative Compatibility Group test.

MATERIALS AND METHODS

Forty seven (47) isolates of *Foc* were collected from Indonesia Tropical Fruits Research Solok that preserved in paper sterile technique. Isolation, isolate purification and VCG analysis were held at Pest and Disease Laboratory of Indonesian Tropical Fruit Research Institute and Department of Primary Industry, Plant



Pathology Section, Indooroopili, Australia. The experiment started from July 2008 to December 2009.

A 7 days purified isolate on PDA media was planted to Chlorate Potassium Media (CPM). 5-12 days later, sector will emerge at the edge of colony signing *Nit*-mutant has been formed. If *Nit*-mutant was not formed within 5-12 days, procedure must be repeated. The sector, growing on CPM media, cut into small size (0.5 cm) and moved into new minimal media (MM). Testers used in the test were from Department of Primary Industry, Plant Pathology Section, Indooroopili, Australia, its VCG code have been known, which were as follows: VCG 0120, VCG 0123, VCG 0126, VCG 0124/5, VCG 01211, VCG 01213, VCG 01213/16, VCG 01216, VCG 0120/15, VCG 01219, VCG 0124, VCG 0125, VCG 0128, and VCG 01220. Testers, come from storage media, re-cultured on MM media for rejuvenation, and were then labeled similar to tester's code.

VCG was determined using the technique by Puhala (1985). In this technique, heterokaryon formation between complementary nitrate-nonutilizing (*nit*) mutants produced on media supplement with chlorate on isolates was assigned to VCGs. Media used in the VCG analysis was Minimal Media (MM). Each mutant from the isolate tested, were paired with all *Nit*-mutant tester. After *Nit*-mutant tester grew on MM media, *Nit*-mutant was cut in small size (0.5 cm) which then cultured on new MM

media. The culture was put on Petri dish (in center part of Petri dish). Meanwhile, *Nit*-mutant isolate test, from MM media, was cut in small size (0.5 cm). A three pieces of *Nit*-mutant isolate test was put surrounding *Nit*-mutant tester in triangle formation. Couple of colony, isolated on MM testing, which was capable of heterokaryon formation, is called compatible to the tester, in the contrary, as was incompatible. Heterokaryon form grew on media, has the shape of hyphae, got thicker and white in color, it was growing among *Nit*-mutant tester and *Nit*-mutant isolate test. The compatibility and incompatibility meant that isolate identified was identical or unidentical to the tester.

RESULTS AND DISCUSSIONS

Isolates used in VCG test were *Foc* isolates collected from ITFRI. All isolates collected were originated from six districts of West Sumatera namely Pariaman, Solok, Agam, Dharmasraya, Tanah Datar and 50 Kota. Isolates were analyzed at ITFRI laboratory and 33 isolates were also analyzed at DPIH Indooroopili. Based on the result of VCG test, 40 isolates were capable of heterokaryon formation with 5 VCGs, characterized by thickening of white hypae zone between nit mutants and isolates tested. These isolates grouped into *Foc* VCGs 01213/16, 0121, 01219, 01218 and 0120. While 7 isolates did not form heterokaryon with 16 strains of VCGs.

Table-1. The VCG test result of some *Foc* isolates attacked varieties of banana in several locations of west Sumatera.

No.	Location (district: sub district)	Isolate code	Variety/Genom	VCGs	Race
1	Pariaman				
	Pasar Usang	ITFRI09	P. Panjang (AAB)	01213/16	4
2	Solok				
	Kubung	09.03.01	Siem (ABB)	01218	1
		09.03.02	Kepok/(ABB/BBB)	01213/16	4
		09.03.04	Buai(AAA)	01213/16	4
		09.03.06	Buai(AAA)	01213/16	4
		09.03.07	Kepok(ABB/BBB)	'0120	1
		09.03.08	Manih(AA)	Not found	
	X Koto Singkarak	ITFRI 001	Barangan(AAA)	Not found	
		ITFRI 002	Cavendish/A.Hijau(AAA)	Not found	
		ITFRI 003	Kepok(ABB/BBB)	Not found	
		ITFRI 004	Barangan(AAA)	Not found	
		ITFRI 005	Kepok(ABB/BBB)	Not found	
		ITFRI 06	Kepok(ABB/BBB)	01219	4
		ARP01	Ambon kuning(AAA)	01213/16	4
		ITFRI 07	Barangan (AAA)	01213/16	4
		SMN01	Mas kirana (AA)	01213/16	4
		SMN02	Ambon hijau (AAA)	01213/16	4
	ARP02	Ambon hijau(AAA)	01213/16	4	
	ARP03	Ambon hijau(AAA)	01213/16	4	
3	Dharmasraya				
	Sitiung	09.01.01.26	Siem (ABB)	01218	1
		09.01.01A.27	Siem (ABB)	01218	1
		09.01.01A.04	Raja Serai(AAB)	01213/16	4



	Pulau Punjung	09.01.01B.26	Kepok(ABB/BBB)	01219	4	
		09.01.01B.27	Buai(AAA)	01213/16	4	
		09.01.02A.26	Raja(AAB)	01213/16	4	
		09.01.02B.27	Buai(AAA)	01213/16	4	
4	Agam					
		Lubuk basung	09.02.01A.01	Jantan(AAB)	01213/16	4
			09.02.01A.04	Kepok(ABB/BBB)	01213/16	4
			09.02.01A.05	Raja Serai(AAB)	01213/16	4
			09.02.01A.26	Buai(AAA)	01213/16	4
			09.02.01A.27	Kepok(ABB/BBB)	01213/16	4
			09.02.01B.27	Rejang(AAw)	01213/16	4
			09.02.01B.28	Rajo(ABB)	not found	
			09.02.01B.29	Raja(ABB)	01213/16	4
	Baso		09.02.02A.01	Buai(AAA)	0121	4
		09.02.02A.26	P.Panjang(AAB)	01213/16	4	
		09.02.02A.27	Buai(AAA)	01213/16	4	
		09.0202A.28	Buai(AAA)	0121	4	
		09.02.02B.01	Buai(AAA)	0121	4	
		09.02.02B.03	Buai(AAA)	01213/16	4	
		09.02.02B.26	Buai(AAA)	0121	4	
		DP01	Barangan(AAA)	01213/16	4	
		ITFRI11	P.Panjang (AAB)	01213/16	4	
5	Tanah Datar					
	Sei Tarab	004	Buai(AAA)	01213/16	4	
		042	Raja serai(AAB)	0121	4	
Tanjung Alam	IM01	Buai(AAA)	01213/16	4		
6	50 Kota					
	Bukit Barisan	046	Buai(AAA)	0121	4	

Foc attack different varieties of bananas from a wide spread location in West Sumatera just grouped into five VCGs (Table-1). Based on these results that development of *Foc* VCG status in West Sumatera when compare with isolates characterized by Nasir *et al.*, (2003) showed significantly differ. Nasir *et al.*, (2003) only analyzed 37 isolates originated form 6 districts in west Sumatera and these isolates grouped into 9 VCGs namely; 01216, 01213, 01219, 01218, 01215, 0120, 01213/16, 0120/15 and 0124. Decreased numbers of VCGs were found in this study presumably because of several isolates not characterized yet. Pegg *et al.*, (1994) reported several numbers of *Foc* isolates originated from Asia (Indonesia, Malaysia, Filipina dan Thailand) that did not yet identified their group in VCGs. Furthermore Vicente (2004) reported that 16th group of *Foc* isolates were unknown VCGs and several isolates originated from Indonesia include in 16 group were unknown VCGs. Thus VCGs were detected by Nasir *et al.*, (2003) will be probable found in this study. Another possibility caused dominance of *Foc* VCGs 01213/16 that attack bananas in this area, the number, location and distribution of banana varieties were attacked. Numbers of *Foc* VCGs 01213/16 were 59.5% from all isolates were analyzed. In the same location, if there is high number of VCGs 01213/16 was identified, others VCGs will be rarely found. This assumed based on Ploetz *et al.*, (2003, 2006) that VCGs 01213/16 known as

Tropical race 4 is the most destructive strain of *Foc*, this strain attacked most of banana varieties and also attack banana which is susceptible to another strain. Moreover this fact probably caused by varieties which is collected susceptible to VCG 01213/16, thus assumed this VCGs more probably found than the other VCGs. *Foc* VCG 01213/16 has proven as most virulent strain than the others and attack most of banana variety were attacked by race 1 and 2 (Su *et al.*, 1986; Buddenhagen, 1990; Ploetz, 2001; Nasir *et al.*, 2003; Ploetz, 2006). Consequently, if a location area has 2 strain of VCG, one of which is the *Foc* VCG 01213/16, immediately the faster strain of VCG attacking is *Foc* VCG 01213/16. Brake *et al.*, (1990) report VCG associated with virulence of certain varieties. 148 isolates were collected from susceptible variety to race 1 (lady finger) grouped in VCG 0124 and VCG 0125 except 1 isolate. However, after plantation it was replaced with Cavendish variety, VCG 0120 and VCG 01219 was found.

In this study, VCG 0121 was found which was not detected by Nasir *et al.* (2003) (Table-1). However this VCG was detected in 1998 by Bentley *et al.* (1998) in West Sumatera. This VCG is not found by Nasir *et al.* (2003) because it does not use VCGs 0121 in analyzing and thus there are still opportunities to increase the number of VCG, detected in 2003.

**Table-2.** Distribution of *Foc* VCGs based on infected banana variety.

No.	Variety/ Genom	Number of isolates	VCG							Unknown
			01213/16	01213	01216	0121	01219	0120	01218	
1.	Panjang	3	3	-	-	-	-	-	-	-
2.	Barangan	4	2							2
3.	Siem	3	-	-	-	-	-		3	
4.	Kepok	8	3				2	1		2
5.	Buai	14	9			5				
6.	Mas kirana	1	1							
7.	Manih	1								1
8.	Cavendish	1	-	-	-	-	-	-	-	1
9.	Ambon kuning	1	1	-	-	-	-	-	-	-
10.	Ambon hijau	3	3							
11.	Raja serai	3	2	-	-	1	-	-	-	-
12.	Raja	3	2							1
13.	Jantan	1	1	-	-	-	-	-	-	-
14.	Rejang	1	1	-	-	-	-	-	-	-

Foc has been grouped into different strain, can attack different varieties of banana. 13 varieties were attacked by *Foc* are Raja/ABB, Raja serai/AAB, Buai/AAA, P. panjang/AAB, Barangan/AAA, Ambon kuning/AAA, Ambon hijau/AAA, Rejang/AAw, Jantan/AAB, Kepok/ABB/BBB, Manih/AA, Cavendish/AAA, Siem/ABB and Mas kirana/AA. Nasir *et al.* (2003) who conducted a survey in six districts in West Sumatera found 15 varieties attacked by *Foc*. These varieties are Ambon kuning, Ambon, Buai, Jantan, Kalek air, Kepok, Lidi, Manis, Mas, Raja, Raja kinalun, Raja serai, Siam, Randah and Udang. Although Nasir *et al.* (2003b) also reported that most of traditional plantation of banana in West Sumatra was attacked by *Foc* in 1980 to 1990 and caused declining of banana plantation there. Moreover Horticulture Processing and Marketing Department (2005) reported that banana harvesting area in West Sumatra declined very significantly from 1434 to 715 ha in 2000 until 2003. However damage caused by *Foc* is still going on and attacking different varieties of bananas in West Sumatra. According to Horticulture Plant Protection Department (2007) reported that epidemic rates of *Foc* race 4 in Sumatra and other province reach 100 km/year.

It can be seen that *Foc* VCG 01213/16 (TR4) attacked 11 of 14 banana varieties (Table-2). It is already discussed above that *Foc* VCG 01213/16 has a higher level of virulence and able to attack different varieties affected by other VCGs. Molina *et al.* (2008) reported that 14 of 17 isolates were collected in low and highland of

Philippines are grouped in to *Foc* VCG 01213/16 and the isolates collected from 4 varieties of banana.

CONCLUSIONS

- A total of 47 isolates of *Foc* collection from west Sumatra grouped into 5 VCG (01213/16, 0121, 01219, 01218 and 0120) and seven isolates are unknown VCGs;
- A total of 13 varieties were attacked by *Foc* are Raja/ABB, Raja serai/AAB, Buai/AAA, P.panjang, Barangan/AAA, Ambon kuning/AAA, Ambon hijau/AAA, Rejang/AAw, Jantan/AAB, Kepok/ABB/BBB, Manih/AA, Cavendish/AAA, Siem/ABB and Mas kirana; and
- A total of 28 isolates (59, 5%) grouped into VCG 01213/16 (TR4), attacked 11 varieties of bananas (Raja, Raja serai, Buai, P. panjang, Barangan, Ambon kuning, Ambon hijau, Rejang, Jantan, Kepok, and Mas kirana).

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