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IDENTIFICATION AND DISTRIBUTION OF *Fusarium oxysporum* f. sp. *cubense* ISOLATES THROUGH ANALYSIS OF VEGETATIVE COMPATIBILITY GROUP IN LAMPUNG PROVINCE, INDONESIA

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ABSTRACT

The use of Vegetative Compatibility Group (VCG) analysis in this study was to identify the isolates and the distribution of *Fusarium oxysporum* f. sp. *cubense* (*Foc*) as well as the infected banana varieties. Samples of *Foc* isolates were collected from the diseased banana plants in Lampung province. While isolating and purifying the isolates with single spore techniques and analyzing the VCG were performed in the laboratory. Fifteen testers of VCGs used were from Australia. The results revealed that 30 isolates of the pathogen *Foc* have been found at all surveyed locations on 10 banana varieties. These varieties were seven varieties of dessert bananas i.e., Seribu (AA), Muli (AA), Ambon Kuning, Ambon Hijau and Cavendish (AAA), Raja Sere and Raja Bulu (AAB) and three varieties of cooking bananas i.e., Nangka (AAB), Jantan (AAB) and Kepok (ABB/BBB). Seventeen of these 30 isolates were VCGs 01213/16 (TR4) found in seven banana varieties. The other isolates comprised five isolates of VCGs 0120/15 (subTR4) in three banana varieties, three isolates of VCGs 01216 (TR4) in two varieties, one isolate of VCG 01213 (TR4) in one variety and four isolates of unknown VCGs in three varieties. Since VCGs 01213/16 (TR4) was the most prevalent, managing and preventing further spread of the disease in Lampung province should be taken into account.

Keywords: banana, vegetative compatibility groups, *Fusarium oxysporum* f. sp. *cubense*, distribution, lampung.

INTRODUCTION

Banana is one of the most important economic crops in Indonesia where about 66.56% of national banana production come from Java and Lampung provinces. Its contribution to the overall fruit production reached 31.0%, being the first rank among the main fruit crops (Department of Agriculture, Indonesia 2009). In Province of Lampung alone, banana has been programmed by Department of Agriculture, Indonesia to be a priority commodity for the development of horticultural region in order to increase production and competitive ability of national fruits.

Like other commodities, banana plant faces pests and diseases problem for its development. Wilt disease caused by *Fusarium oxysporum* Schlecht f. sp. *cubense* (*Foc*) is so far still the most dreaded disease of banana throughout the world. This disease is also well known as Panama disease (Stover 1957 and 1972; Moore *et al*., 1993; Pegg *et al*., 1996). In Indonesia, the disease has widely spread from Aceh province in western part through Papua province in eastern part. In 1992-1995, *Foc* disease had destroyed banana var. Barangan as large as approximately 1.300 ha in North Sumatera and 300 ha in Riau province. In Lampung province the *Foc* was firstly detected to attack banana only on one plant in the commercial orchard owned by National Tropical Fruit (NTF) in February 1993, yet by 2002 this disease had spread to the larger area. As a result, around 1700 ha out of 2100 ha of the existing banana orchard had been wiped out (Nasir and Jumjunidang, 2002). Nurhadi *et al.* (1994) estimated that crop loss caused by wilt disease (*Foc* and bacterial disease) could reach IDR 2.4 billion equal to US$ 1039,861.00 in 1992/1993.

Foc possesses biological characters that are both very specific and various such as the different virulence between and within races and strains as well as the persistent ability in the baring soil up to 40 years (Su *et al*., 1986; Ploetz 1990 b). Several methods have been used to characterize *Foc* such as vegetative compatibility group test (VCG) (Ploetz and Correll, 1988; Ploetz, 1990 a and b; Moore *et al*., 1993; Ploetz and Pegg, 2000), volatile aldehydes production (Stover, 1962; Moore *et al.*, 1991; Batlle and Pérez. 2003), electrophoretic karyotyping (Boehm *et al*., 1994), RAPD-PCR analysis (Bentley *et al*., 1995; Bentley *et al*., 1998), RFLP analysis (Koenig *et al*., 1997) and AFLP analysis (Groenewald *et al*., 2004). The VCG test has fast developed and been used to study of the diversity, genotypes, ecology and population of pathogenic fungi (Puhalla 1985; Ploetz 1990 b). This technique is based on the genetic exchange between different isolates that are paired (Leslie, 1990; Ploetz, 1990 b) or based on the reproductive compatibility of different strains of the fungus (Davis, 2005). Furthermore, Davis (2005) pointed out that by this technique differentiation between and within strains could be more accurate. From the application of VCG technique, *Foc* isolates in the similar VCG will group in the same clone albeit from different geographic origin (Leslie, 1990).

To date, there have been classified 23 VCGs *Foc* throughout the world where 19 of them are in Asia (Moore *et al*., 1993; Pegg *et al*., 1995; Ploetz 2000). Bentley *et al*. (1998) reported 16 new genotypes that were not group in any of the previously known VCG. In 1993, of 21 *Foc*

isolates that were collected from West Java it had been found three VCGs belonging to VCG 0120, 0120-01215 and 01213 (Pegg *et al*., 1993). After that, Nasir and Jumjunidang (2003) had found seven VCGs *Foc* that were obtained from 15 varieties of banana in West Sumatera. While in Lampung province banana is the major commodity developed commercially, yet it has never been known how the variation in Fusarium disease is. Therefore, the current study aims to identify the *Foc* isolates using Vegetative Compatibility Group method and their distribution, as well as the infected banana varieties in Lampung province.

MATERIALS AND METHODS

Survey, collection, and purification of *foc* isolates

Survey and collection of *Foc* infected banana plants were carried out in Lampung province in June 2008. Isolation and purification of isolate as well as analysis of VCG were performed in the laboratory of plant protection of Indonesian Tropical Fruit Research Institute from July to December, 2009. Selection of locations in terms of district, sub district and village was based on the largeness of banana orchard. In this course two districts where each was taken two or three sub-districts and each sub-district was taken one village to be determined as locations for survey. Those districts were Tanggamus comprising two sub-districts (Sukoharjo and Pugung) and Lampung Selatan comprising three sub-districts (Natar, Bakauheuni and Kalianda). The isolates were also collected from banana plants on both sides all along the road that was passed through.

Foc samples were collected from banana plants showing external specific symptoms of *Foc* like wilting and a yellow coloring of the leaves, collapsed leaf petiole, and a longitudinal splitting of the lower portion of the pseudo-stem (10-15 cm above ground). From these diseased plants, it was taken partly pseudo-stem around 20-30 cm from rhizome. The interior of affected pseudo-stem will appear reddish, blackish, brownish or yellowish vascular lines. This pseudo-stem was cut into 5 cm x 15 cm, then put it in plastic bag, labeled, and stored in the cool box. Afterward the vascular lines were drawn to be separated from tissues, and then wind dried and kept in the sterile tissue paper.

Isolation of pathogen was carried out in the laboratory. The dried vascular lines were cut in 0.5-1.0 cm long and then cultured on 1/3 potato dextrose agar (PDA) supplemented with 50 ppm streptomycin. The culture was incubated at room temperature for 2-3 days. Colony growing and pink in color was selected as wild type of F. oxysporum under 40-watt neon light (black light). To ensure that colony is *Foc* if it has microscopic characters such as thin-walled macro-conidia, 3-4 septa and usually found on conidiophore branch, while micro-conidia is oval or kidney shaped and found on short-conidiophore petiole. Subsequently, purification of isolate was performed using single spore technique (sst) as described by Pittaway *et al*. (1999). Purified *Foc* isolates were then analyzed by Vegetative Compatibility Group technique.

Growing *nit*-mutant and preparing tester

Pure *Foc* isolates, seven days on PDA media, were cultured on potassium chlorate media (PCM). Sector will usually emerge at the tip of colony in 5-12 days, indicating *nit*-mutant has been formed. If *nit*-mutant is not formed in 5-12 days, this activity needs to be repeated. The tips of hyphae on sector growing on PCM medium were cut in 0.5 cm long and moved to a new Minimal Medium (MM), and then labeled as respective code of isolate.

Testers used for assessment were from Department of Primary Industry, Plant Pathology Section, Indooroopilli, Australia, namely *nit*-mutant in which the codes of VCGs had already been known. These testers were 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 testers taken from the storage were re-cultured on MM medium in order to rejuvenate and then labeled as respective tester code.

Analysis of vegetative compatibility group

Medium used in the analysis of Vegetative Compatibility Group was Minimal Medium (MM). In this medium a small cutting of each *nit*-mutant tester (0.5 cm) was put in the center of petri dish and then three small cuttings of each *nit*-mutant tested (0.5 cm) were put in surrounding *nit*-mutant tester (see Figure-1). The paired colonies growing to form *heterokaryon* on the MM indicated that mutant tested was compatible similar to mutant tester and vice versa. *Heterokaryon* on the medium was in the form of hyphae that appeared to thicken white in color in area between tester and tested *nit*-mutant.

RESULTS AND DISCUSSIONS

From the survey in two districts (Tanggamus and Lampung Selatan) and at two sides (right and left) along the passed road, it was found Fusarium wilt infecting 10 varieties of banana and spread at all locations observed (Table-1). The spread of Fusarium wilt in Lampung province might occur because of easily spreading disease through irrigation water, agricultural tools, and planting materials (suckers or rhizome pieces).

A number of 30 isolates of *Foc* had been collected from the various banana varieties during the survey. After growing on the potassium chlorate medium, all *Foc* isolates were able to form *sectors*, i.e., colonies fast growing at the tips of thickening hyphae (Figure-1), indicating that *nit*-mutants (nitrate non-utilizing mutants) had been formed. These *nit*-mutants were then moved to the minimal media (MM) to be used for VCG analysis.

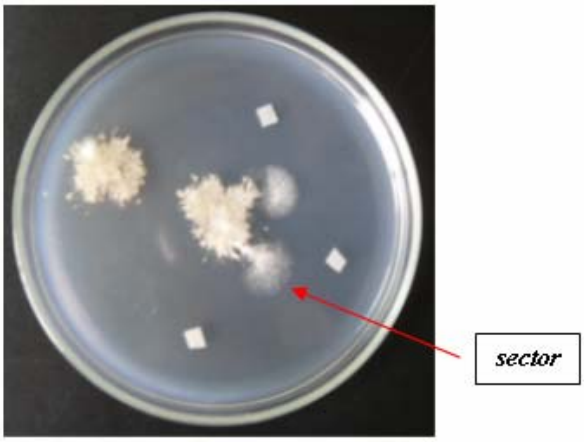

Figure-1. Sector or colony growing fast at hyphae tip knotted, indicating the *nit*-mutant forming.

The results of VCG analysis indicated that 26 of 30 *Foc* isolates tested on minimal media (MM) were able to form heterokaryon or compatible to VCGs 01213 (TR4), 01216 (TR4), 01213/16 (TR4), and 0120/15. Isolates of *Foc* attacking various varieties of banana in Lampung province are presented in Table-1. Heterokaryon or compatibility between tested isolate and tester VCG was attributed with the formation of white, thickening hyphae in zone between tested *nit*-mutant isolate and tester VCG. There were four isolates of *Foc* not able to form heterokaryon with 15 VCGs used. Figure-2a shows the isolates forming heterokaryon while Figure-2b shows the isolates not forming heterokaryon.

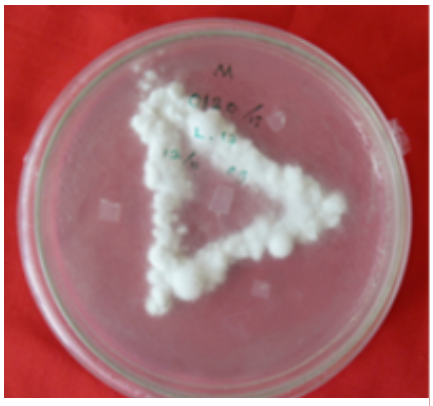

Figure 2a. *Foc* isolate L17 with tester of VCG 01213/16 forming *heterokaryon* (compatible)

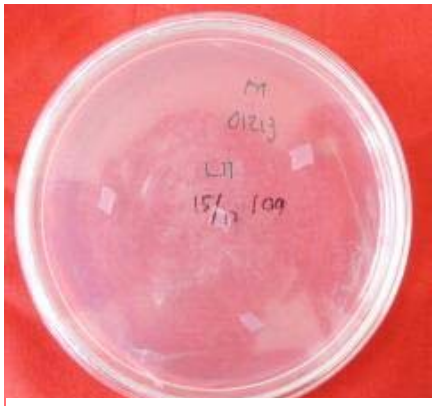

Figure 2b. *Foc* isolate L11 with tester of VCG 01213/16 not forming *heterokaryon* (incompatible)

Table-1. The result of VCG test on 30 isolates of *Foc* attacking various varieties of banana in Lampung province.

No.	Location (Sub-district: District)	Isolate code	Variety/Genom	VCGs	Race
1	Gunung Sugih: Kodya Metro	028	Seribu (AA)	01216	4[1]
2	Natar: Lampung Selatan	027	Raja Bulu (AAB) Muli (AA) Kepok (ABB/BBB) Ambon Kuning (AAA)	01213/16 01213 uk 01216	4[1)] 4[1)] uk 4[1)]
3	Bakauheni: Lampung Selatan	L20 L17 L19 L23A L23B	Ambon Kuning (AAA) Ambon Kuning (AAA) Cavendish (AAA) Nangka (AAB) Raja Bulu (AAB)	01213/16 0120/15 01213/16 uk 01213/16	4[1)] 4[2)] 4[1)] uk 4[1)]
4	Kalianda: Lampung Selatan	L28 L30 L.26, 31, 18 L25	Kepok (ABB/BBB) Jantan (AAB) Raja Bulu (AAB) Ambon Hijau (AAA)	0120/15 01213/16 01213/16 01213/16	4[2)] 4[1)] 4[1)] 4[1)]
5	Penengahan: Lampung Selatan	017	Jantan (AAB)	01213/16	4[1]
6	Jabung: Lampung Timur	020 022 032	Kepok (ABB/BBB) Raja Bulu (AAB) Cavendish (AAA)	01213/16 01213/16 01213/16	4[1)] 4[1)] 4[1)]
7	Padang Cermin: Pasawaran	015 016	Raja Bulu (AAB) Ambon Kuning (AAA)	01213/16 01213/16	4[1)] 4[1)]
8	Kedondong: Pasawaran	NN DPLP1	Ambon Kuning (AAA) Ambon Kuning (AAA)	uk 01216	uk 4[1)]
9	Sukoharjo: Tanggamus	L7 L11	Ambon kuning (AAA) Raja Sere (AAB)	0120/15 0120/15	4[2)] 4[2)]
10	Pugung: Tanggamus	L15 L8 L16 L12	Raja Sere (AAB) Kepok (ABB/BBB) Ambon Kuning (AAA) Nangka (AAB)	01213/16 01213/16 0120/15 uk	4[1)] 4[1)] 4[2)] uk

Note: 1): tropical race 4 (TR4); 2): subtropical race 4 (subTR4); uk = unknown

Table-1 shows that the diversity in variety of banana attacked by *Foc* was relatively high enough (i.e., 10 banana varieties), conversely, the diversity of the *Foc* VCG was low (only four VCGs). In fact, 23 VCGs of *Foc* have been found throughout the world to date, whereby 19 of them are in Asia (Pérez, 2004). In Indonesia itself, there are 10 VCGs identified and the other 5 new VCGs that have not been identified. Different VCGs in any region are greatly determined by geographic origin Ploetz (1990b). Based on the diversity concept, VCGs identified from 30 isolates of *Foc* in this current study are different from VCG *Foc* infecting banana plants in other regions. In West Sumatera, Indonesia it had been found seven VCGs *Foc* infecting 15 varieties of banana (Nasir and Jumjunidang, 2003) whereas in Southern Philippines only three VCGs (Molina *et al*., 2008). The low diversity of VCG *Foc* might be not only caused by geographical factor but also by the unknown four VCGs and the dominance or aggressiveness of VCG *Foc*, in this case VCG 01213/16. VCG 01213/16 is the aggressive strain well known as "Topical Race 4 (TR4)" spreading in the tropical areas (Daly and Walduck, 2006) and causing many millions loss in banana production (Pérez, 2004). In this study, the VCG 01213/16 appeared to be dominant in the number, locality distribution, and diseased banana varieties.

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

No.	Variety/Genom	Number of isolates	VCG				Unknown
			0120/15	01213	01216	01213/16	
1	Ambon kuning AAA	8	3	-	2	2	1
2	Raja bulu AAB	**7**	-	-	-	**7**	-
3	Kepok BBB/ABB	4	1	-	-	2	1
4	Seribu AA	1	-	-	1	-	-
5	Muli AA	1	-	1	-	-	-
6	Cavendish AAA	2	-	-	-	2	-
7	Nangka AAB	2	-	-	-	-	2
8	Jantan AAB	2	-	-	-	2	-
9	Raja sere AAB	2	1	-	-	1	-
10	Ambon hijau AAA	1	-	-	-	1	-
	Total	**30**	**5**	**1**	**3**	**17**	**4**

From four VCGs of *Foc* identified, as many as 21 isolates (66.6%) belong to VCG 01213/16 infecting seven of the 10 banana varieties, whereas five isolates (16.6%) found in three banana varieties belong to VCG 0120/15 (Table-2). This indicated that VCG 01213/16 was considerable prevalent race/VCG in Lampung province. A similar result was reported by Nasir dan Jumjunidang (2003) that VCG 01213-01216 was the most widespread and could infect 14 out of 15 banana cultivars in West Sumatera province. Again, Molina *et al.* (2008) also reported that 14 of 17 *Foc* isolates (82.35%) attacking four of six banana varieties in high- and lowland in the Philippines was VCG 01213/16. According to Su *et al*. (1986) that VCG 01213/16 had the highest virulence and capability of infection among the existing VCGs.

The unidentified four VCGs obtained from this study might belong to the other seven VCGs out of 14 VCG testers used or even belong to among five VCGs of 16 new VCGs that not group in any of the previously known VCG (Bentley *et al*., 1998). This phenomenon likely occurred since Indonesia and the other Southeast Asian countries is the major center of origin of both banana diversity and *Foc* in the world (Pérez, 2004). Hence, as the diversity concept (Vavilov's theory) stating that the *Focs* have probably co-evolved with bananas in Asia, particularly Southeast Asia (Bentley *et al*., 1995), it has also probable taken place on the isolates of *Foc* originating from Lampung province.

CONCLUSIONS

a) Fusarium disease had been found at all surveyed locations in Lampung province, infected 10 varieties of banana;
b) Thirty isolates of *Foc* had been collected and grouped into four VCGs. These four VCGs were VCG 0120/15, VCG 01213, VCG 01216, and VCG 01213/16; and
c) VCG 01213/16 was the most prevalent since 17 of the 30 isolates were VCG 01213/16 (TR4), found on 7 of 10 diseased banana varieties and present at all surveyed locations.

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