Kasetsart J. (Nat. Sci.) 45 : 451 - 460 (2011)

Vegetative Compatibility Groups of *Fusarium oxysporum f.sp. cubense*

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ABSTRACT

Vegetative compatibility groups (VCGs) of *Fusarium oxysporum* f.sp. *cubense* (FOC) were investigated by collecting 117 isolates from banana plantations in 25 provinces in five parts of Thailand and *nit* mutants were regenerated and identified for each isolate. VCGs were assessed on the basis of heterokaryon formation between *nit1* and *NitM* of different isolates paired on specific nutrient media in complementary tests. The ability of heterokaryon formation among isolates and VCGs testers was evaluated and the existing physiological races were also discussed.

The results indicated that almost 117 isolates of FOC were identified from the Klaui Namwa cultivar and they were classified to 6 VCGs: VCG 0123, VCG 0124, VCG 0125, VCG 0124/0125, VCG 01218 and VCG 01221. VCG 0123 represented the predominant population of FOC in Thailand with 76.0% of total isolates found in all parts of Thailand. VCG 01221 showed the lowest FOC population with 1.7% of total isolates. VCG 0124/0125 was classified as a cross compatible group to which some isolates in VCG 0124 and VCG 0125 were closely related and divided into subgroups. On the basis of pathogenic ability on banana genomics, the FOC race in Thailand was classified to be race 2 and it was less virulent than others worldwide. In this study, the FOC population of Thailand was the primative population and exhibited specific pathogens on one cultivar of banana—namely, Klaui Namwa (ABB genome).

Keywords: vegetative compatibility group, VCGs, *F. sarium oxysporum* f.sp. *cubense,* FOC, banana fusarium wilt

INTRODUCTION

Fusarium wilt is the most serious diseases affecting banana (*Musa* spp.) production worldwide. In Thailand, *F. oxysporum* f.sp. *cubense* (FOC) has been identified as the causal pathogen of the disease (Pitakpaiwan, 1985; Singburaudom, 1991). It has been found on the Pisang Awak group (ABB) and only on the variety Klaui Namwa. It has not been found on other types of banana varieties, especially the Gros Michel (AAA group), such as the Klaui Hom Thong variety. Singburaudom (1999) reported the status of Banana Fusarium wilt in Thailand spreading into the banana growing areas in more than 37 provinces in 1999. The symptoms appeared from 5 mth in the younger stage to the fruit stage. An external symptom is a yellowing of the leaf blade. An internal symptom exhibits as a reddish brown discoloration of the internal pseudostem. Infected plants collapse before maturity; even though they can grow, their yield and the quality of fruit is decreased.

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Received date : 07/06/10 Accepted date : 01/12/10

Traditionally, FOC has been classified into four physiological races using the reaction of banana cultivars. Race 1 includes Gros Michel, race 2 Bluggoe, race 3 *Heliconia* sp. and race 4 includes the Carvendish cultivar and all susceptible cultivars of banana to race 1 and 2. FOC in Thailand can infect only Klaui Namwa. As the race classification of FOC isolates was considered to inadequately reflect the pathogenic potential, numerous *in vitro* methods have been used to characterize FOC isolates into pathotypes.

FOC is a highly variable pathogen. It is a complex of genetically isolated asexual populations that can be defined on the basis of vegetative compatibility. Vegetative compatibility is a stable genetic marker which can be used to determine genetic relationships among populations of the pathogen. It is assessed on the basis of heterakaryon formation between isolates and paired in complementation tests. Using the method developed by Puhalla (1985) and refined by Correl *et al*. (1987), metabolic mutants can be generated for each isolate and paired on specific nutrient media. Heterokaryon formation can then be easily scored by the line of dense, nutrient sufficient heterokaryon growth which develops where the advancing margins of genetically complementary colonies meet. (Correl *et al*., 1987; Leslie, 1990). If no line of heterokaryon growth is observed, isolates are considered vegetatively incompatible. For isolates to be vegetatively compatible, they must possess identical alleles at all the nuclear gene loci governing vegetative compatibitity; such isolates form discretely via asexual breeding populations known as vegetative compatibility groups (VCGs).

Analysis of VCGs has been used to subdivide the Australian populations of FOC (Brake *et al*., 1990). Some 400 isolates have been placed in six VCGs and there is a high degree of correlation for VCG and race. All putative race 1 isolates belonged to VCGs 0124 and 0125. VCGs 1021, 0129 and 01211 contained race 4 competent strains, while VCGs 0128 contained race 2 competent strains. Isolates of VCGs 0124 and 0125 were often cross compatible and a small number of isolates in VCGs 0120 and 0129 have been found to form weak heterokaryons with VCGs 01211. Although the reasons for cross compatibility were not known, it was thought that these VCGs were closely related and represented divergent sub-populations of the same VCG that had lost the ability to form heterokaryons consistently (Ploetz, 1990).

To date, more than 20 VCGs of FOC have been reported (Ploetz, 1997). Some of the VCGs have been recovered from a variety of cultivars and genomes, some have come from specific banana genomes and others have been found on single cultivars. Bananas are interspecific and intraspecific hybrids of *M. acuminata* and *M. balbisiana* (Zingiberales: Musaceae) and they are a perennial herb that originated in Southeast Asia. It is very interesting to study the genetic relationships among FOC populations in Thailand to get more information on why FOC can infect only Klaui Namwa and not other types of banana.

MATERIALS AND METHODS

FOC isolations

One hundred and seventeen isolates of FOC were collected from infected banana plants in five banana growing areas in Thailand, including the north, northeast, west, central plain and the south. Infected pseudostem tissues were isolated by a tissue transplanting method. Pure cultures of fungus were transferred onto potato dextrose agar (PDA).

VCGs Testers

Known VCGs of FOC obtained from Professor Randy C. Ploetz, University of Florida, USA, were used as standard testers. *NitM* of standard testers VCGs 0123, 0124, 0125, 01218 and 01221 were paired to *nit1* mutants of Thailand

FOC isolates in a complementation test.

Single conidia isolation

Single conidia were isolated from a conidia suspension in sterile distilled water. Mycelia that had been grown on PDA for 7 d were used to make conidia suspension in 10 mL sterile distilled water at an approximate conidia concentration of 10 conidia per low power (10×) microscopic field. One loop of conidia suspension was spread on the surface of water agar (WA) medium. The spread Petri dish was incubated for 16-24 h to obtain conidia germination and then the germinating conidia were transferred to PDA. Morphological studies on the microconidia, conidiophores and chlamydospores were conducted to identify FOC by the Nelson method (Nelson, 1983) and kept for future use.

Induction and isolation of *nit* mutants

FOC isolates were cultured on PDA for 5 d and mycelia were cut and subcultured onto 1.5% potassium chlorate ($KClO_3$) supplemented PDA. They were incubated at 25–27 °C (room temperature) under light fluorescence for 7–14 d.

A normal mycelial sector (resistant sector) of an FOC isolate on the medium was identified to be the crn mutant type, whilst any unusual mycelium sector was identified as the wild type. Characterization between the crn mutant and wild type was done intensively to obtain the real mutant for vegetative compatibility group (VCG) analysis. All resistant sectors were collected.

Characterization of nit mutants

All *nit* mutants were transferred to *nit* mutant identification media. Minimal medium (MM) was used for *nit1* mutant identification while MM containing hypoxanthine was used for *NitM* mutant identification. MM is composed of basal medium (2 g) with $NaNO_3$ in 1 L of medium (Correl *et al*., 1986). An amount of trace element (0.2 mL) was added to the MM. For *NitM* identification, MM was added with 0.2 g hypoxanthine into 1 L of medium. Typical wild type showed thick mycelia on MM while *nit1* mutant and *NitM* mutant colonies produced thin mycelia. The mutant colonies were collected for the next study.

Identification of VCGs

A complementation test was undertaken by pairing cultures of the *nit1* mutant of FOC from Thai isolates and *NitM* tester of known VCGs on MM in the same Petri dish at 1.5–2.0 cm distance between isolates. The cultures were incubated for 14–21 d at 25–27 °C under fluorescence light. Heterokaryon formation was observed and the VCGs were identified.

Heterokaryon formed between isolates where both isolates were grouped in the same VCG. The degree of heterokaryon formation was also evaluated into five categories: none, slight, moderate, high and very high formation of robust mycelial growth at the interface of the two developing colonies (Figures 3 and 4).

RESULTS

***nit* mutant induction**

MM containing 1.5% $KClO_3$ had different percentages of *nit* mutants characterized as *nit1* and *NitM* with 100 and 52.1%, respectively (Table 1). The result suggested that *nit1* was easier to obtain than *NitM*. The proportion of isolates showing both *nit1* and *NitM* was 52.1% whereas those showing *nit1* was 47.9%.

Vegetative compatibility ability

In the complementary test, *nit1* of Thai isolates were paired with *NitM* of tester VCGs on specific media and the variation in the degree of heterokaryon formation was scored to indicate the degree of vegetative compatibility existing among the FOC population. The vegetative compatibility degree of the 117 FOC isolates was divided into

five groups and is summarized in Tables 2–5. There was a large range in the degree of heterokaryon formation in the FOC population of the northern banana plantation of Thailand, with slight, moderate, high and very high heterokaryon formation. The other FOC population showed a narrow range with only high and very high heterokaryon formation. Cross compatibility was found in the FOC populations from the north and the central plain of Thailand. The degree of cross compatibility between different VCGs was very high in the FOC population of the central plain, whereas the FOC population of the north showed a lower degree of cross compatibility. Most of the cross compatible isolates were found between VCGs 0124 and 0125.

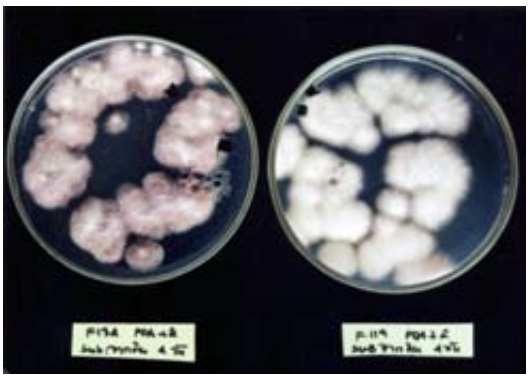

Figure 1 Wild type colonies of *Fusarium oxysporum* f.sp. *cubense*.

Identification of VCGs

The complementary tests between the *nit1* isolates of 117 Thai FOC that had unknown VCGs and *NitM* of known VCGs tester were investigated. The result revealed that the 117 FOC of Thai isolates could be identified into 6 VCGs as shown in Table 6.

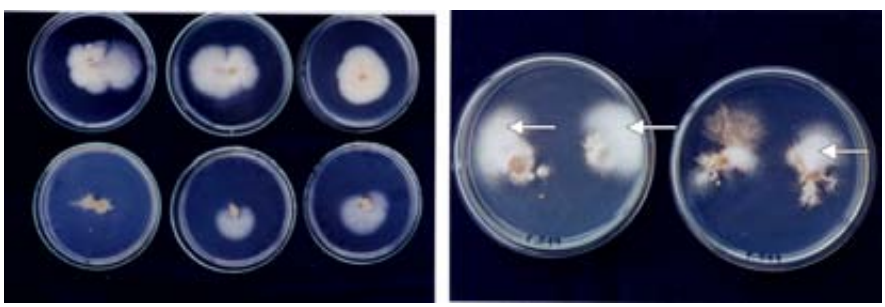

Figure 2 Induction of *nit* mutant sectors on MM medium containing 1.5% $KClO_3$. (Arrows show mutant sectors).

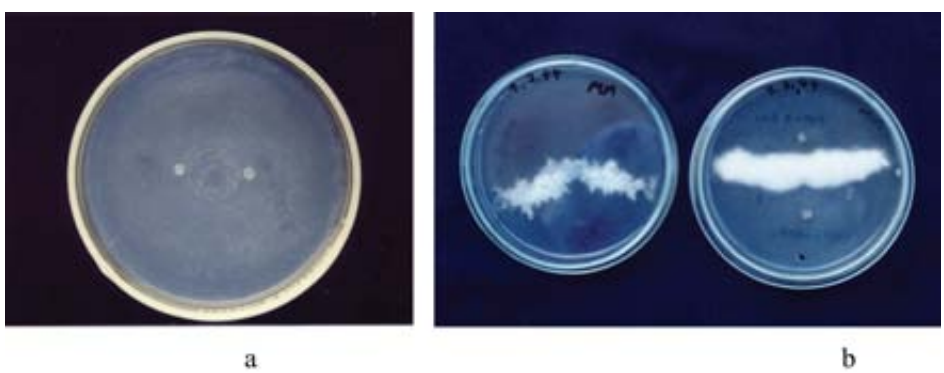

Figure 3 Complementary test on MM; a. No heterokaryon formation; b. Heterokaryon formation.

VCG 0123 represented the predominant population of FOC in Thailand with 76.0% of total FOC identified as VCG 0123, whereas VCG 01218 and VCG 01221 had the two lowest population proportions with 2.6 and 1.7% of the total population, respectively. VCG 0124 and VCG 0125 were small to medium groups of the FOC population, with proportions of 7.7 and 5.0%, respectively. The cross compatible population was VCG 0124 and VCG 0125 with a proportion of 7.0%. The cross compatible group showing the ability to form heterokaryons has been found in India, China, Malaysia, Australia and Africa (Ploetz, 1997).

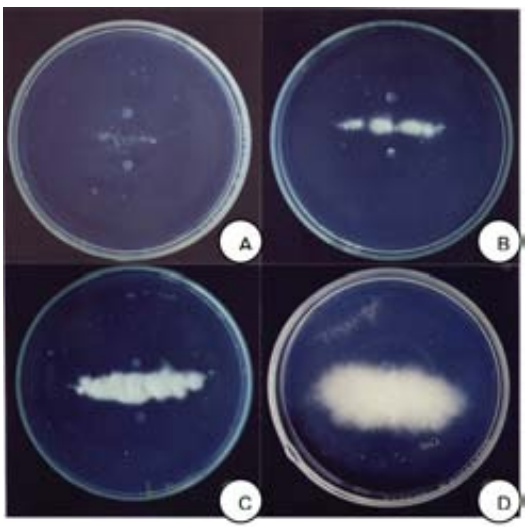

Figure 4 Degree of heterokaryon formation between different isolates in the same VCG. (A) Slight heterokaryon formation; (B) Moderate heterokaryon formation; (C) High heterokaryon formation; and (D) Very high heterokaryon formation.

The distribution of VCGs was considered on the basis of banana plantations. VCG 0123 was present in all parts of Thailand with a large proportion found in the northeast and central plain with 32.5 and 30.4% of total population, respectively. VCG 0124, VCG 0125, and VCG 0124 and VCG 0125, a cross compatible group, was present in the north and central plain. There were two VCGs detected from specific banana growing areas, with VCG 01218 found in the south while VCG 01221 was found in the north of Thailand. The results of this study revealed that VCG 0123 had the largest FOC population based on both population frequency and area of distribution. The greatest diversity of VCG 0123, VCG 0124, VCG 0125, VCG 0124/0125 and VCG 01221 was discovered in the north and on the central plain. The population of FOC in the northeast was homogeneous in genetic identification because only VCG 0123 was found. VCG 01218 and VCG 01221 were interesting because both were found in specific areas of the south with low frequency.

DISCUSSION

The biological species concept (BSC) identifies species as groups of a population that potentially can interbreed with each other and has been defined as the largest and most inclusive reproductive community of sexual and cross-fertilizing individuals sharing a common gene pool (Taylor *et al*., 2000). BSC shifts the focus from

Table 1 *nit1* and *NitM* induction of *Fusarium oxysporum* f.sp. *cubense* (FOC) on MM.

Nit mutants	No. of isolates	% induction
nit1	117	100
NitM	61	52.1
nit1 without *NitM*	56	47.9
nit1 and *NitM*	61	52.1
Total	117	100

Table 2 Degree of vegetative compatibility of 25 representative Thai isolates of FOC from northern banana plantations.

Area code	*nit1* of isolates	*NitM* of tester VCGs				
		0123	0124	0125	01218	01221
N 1	F055	++++	-	-	-	-
N 2	F082	-	++++	++++	-	-
	F083	-	+	++	-	-
	F084	-	+	++	-	-
	F103	-	-	+++	-	-
	F105	-	+++	-	-	-
	F106	-	+++	-	-	-
	F107	-	+++	-	-	-
	F108	-	++++	++	-	-
	F109	-	++	+	-	-
	F110	-	++++	-	-	-
	F111	-	+	-	-	-
	F112	-	++	++++	-	-
N 3	F008	-	-	-	-	++++
	F010	-	-	-	-	++++

- = no heterokaryon formation
+ = slight heterokaryon formation
++ = moderate heterokaryon formation
+++ = high heterokaryon formation
++++ = very high heterokaryon formation

Table 3 Degree of vegetative compatibility of 38 representative Thai isolates of FOC in northeastern banana plantations.

Area Code	*nit1* of isolates	*NitM* of tester VCGs				
		0123	0124	0125	01218	01221
NE1	F001	+++	-	-	-	-
	F026	++++	-	-	-	-
	F087	+++	-	-	-	-
NE2	F025	++++	-	-	-	-
NE3	F038	++++	-	-	-	-
NE4	F004	+++	-	-	-	-
	F020	+++	-	-	-	-
	F041	+++	-	-	-	-
	F046	++++	-	-	-	-
	F048	+++	-	-	-	-
	F052	+++	-	-	-	-

- = no heterokaryon formation
+ = slight heterokaryon formation
++ = moderate heterokaryon formation
+++ = high heterokaryon formation
++++ = very high heterokaryon formation

Table 4 Degree of vegetative compatibility of 47 representative Thai isolates of FOC in central plain banana plantations.

Area Code	*nit1* of isolates	*NitM* of tester VCGs				
		0123	0124	0125	01218	01221
C1	F012	++++	-	-	-	-
	F005	++	-	-	-	-
C2	F016	-	+++	-	-	-
	F018	-	++++	++++	-	-
	F023	-	+++	+++	-	-
	F062	-	++++	-	-	-
	F075	-	-	+++	-	-
	F090	-	+++	-	-	-
	F100	-	-	++++	-	-
C3	F076	-	-	+++	-	-
	F077	-	-	++++	-	-
	F088	-	+++	-	-	-
	F101	-	-	+++	-	-

- = no heterokaryon formation
+ = slight heterokaryon formation
++ = moderate heterokaryon formation
+++ = high heterokaryon formation
++++ = very high heterokaryon formation

Table 5 Degree of vegetative compatibility of 7 representative Thai isolates of FOC in southern banana plantations.

Area Code	*nit1* of isolates	*NitM* of tester VCGs				
		0123	0124	0125	01218	01221
S1	F078	++++	-	-	-	-
	F080	+++	-	-	-	-
S2	F117	-	-	-	++++	-

- = no heterokaryon formation
+ = slight heterokaryon formation
++ = moderate heterokaryon formation
+++ = high heterokaryon formation
++++ = very high heterokaryon formation

Table 6 Vegetative compatibility groups of 117 FOC Thai isolates.

VCGs	No of isolates	%
0123	89	76.0
0124	9	7.7
0125	6	5.0
0124/0125	8	7.0
01218	3	2.6
01221	2	1.7
	117	100

Table 7 Vegetative compatibility groups and their distribution of 117 isolates *F. oxysporum* f.sp. *cubense* from banana plantations in Thailand.

VCG	Area	No. of provinces found	% of distribution	No. of isolates	% of VCGs
0123	north	4	16	11	9.4
	northeast	7	28	38	32.5
	central plain	8	32	36	30.8
	south	1	4	4	3.4
	VCG Total	20	80	89	76.0
	Totals	25	100	117	100
0124	North	1	4	5	4.3
	central plain	3	12	4	3.4
	VCG Total	4	16	9	7.7
	Totals	25	100	117	100
0125	North	1	4	1	0.8
	central plain	2	8	5	4.3
	VCG Total	3	12	6	5.1
	Totals	25	100	117	100
0124 / 0125	north	1	4	6	5.1
	central plain	2	8	2	1.7
	VCG Total	3	12	8	6.8
	Totals	25	100	117	100
01218	south	3	12	3	2.6
	VCG Total	3	12	3	2.6
	Totals	25	100	117	100
01221	North	1	4	2	1.7
	VCG Total	1	4	2	1.7
	Totals	25	100	117	100

individuals to the population and defines a species in terms of how the members of the populations interact and relate to one another, rather than by comparisons with a static standard. The properties common to most BSC have been defined as: 1) shared characters within a species were more important than differences between species; 2) the population was required to clearly delimit and define the extent of variation within the species; and 3) interfertility between individuals in a population was not the definitive criterion for subdividing species. BSC treats species as categories defined by an actually or potentially shared gene pool rather than as a taxon or type as is applied by the morphological species concept and is commonly done with many phylogenetic species concepts. *F. oxysporum* fsp *cubense* is a highly variable plant pathogen. It shows high variation in morphology, pathogenecity and in genetic and secondary metabolites. Also it is very difficult to find examples where sexual reproduction occurs naturally. The vegetative compatibility group concept was defined as an alternative choice for studying the genetic relationship among populations of this plant pathogen. Vegetatively compatible fungi, those in the same VCG, can exchange genetic information, whereas incompatible fungi are genetically

isolated. VCG analysis has been used to subdivide the FOC population worldwide (Brake *et al.,* 1990; Ploetz, 1990). However, at least 21 known VCGs have been reported for *F oxysporum* f.sp *cubense (*Ploetz, 1997). VCG 0123 has been found in the Philippines, Malaysia, Taiwan and Thailand but it has not yet been detected in other banana growing areas of the world. As banana is a perennial herb that originated in Southeast Asia, it might be possible to say that VCG 0123 was the primative FOC population originating in Southeast Asia and that it distributed to other banana growing areas by transportation of agricultural produce.

The relationship between VCGs and the race of FOC has been reported by Ploetz (1990) and it was found that race 1 contained VCG 0124 and VCG 0125. It was very surprising that there was no mention of the relationship between VCG 0123 and race. Historically, the traditional classification of the physiological race of FOC worldwide was classified to be race 1 using the basis of pathogenicity on the Gros Michel cultivar (AAA group) and it was classified to be race 2 if it could infect an ABB genome such as Bluggoe and Pisang Awak. Singburaudom (1991) reported that most of the FOC isolates in Thailand had been found on Klaui Namwa, the most common cooking banana cultivar of Thailand, and to date, it has not been reported on any other type of banana. From the present study, the race of FOC in Thailand was classified to be race 2 containing VCG 0123, VCG 0124, VCG 0125, VCG 0124 and VCG 0125, a cross compatible group, VCG 01218 and VCG 01221. However, it was different from other race 2 populations because it was quite specific to only one banana cultivar, Klaui Namwa. It was classified to be a lower virulent race 2 because it infected one genome, ABB, compared with other race 2 populations that were virulent on genomes AAA, AAB and ABB. VCG 01218 was also different from the others because it infected only genome ABB, whereas the others infected both genomes AAB and ABB. VCG 01221 had a very narrow distribution of FOC population because it is found only in Thailand and it might be a primative FOC.

CONCLUSIONS

This investigation concluded that the population of *F. oxysporum* fsp *cubense* in Thailand was primative and originated in Southeast Asia. VCG 0123 was the predominant population measured by both population frequency and area distribution. It was not cross compatible with different VCGs and it could maintain genetic homogeniety and information within the population. VCG 01221 was the most primative FOC in Thailand because it was found only in Thailand and it was pathogenic on a specific banana cultivar (Klaui Namwa). On the basis of the relationship between VCG and race classification, all six VCGs found in Thailand were classified to be race 2. VCG 0123 and VCG 01221 were classified as being avirulent race 2. A pathogenecity test on different banana types and phylogenetic analysis among FOC populations might be important to study the genetics of the host-parasite relationship and the genetic lineage relationship among FOC populations. Secondary metabolite profiles of all VCGs might play a role in more intensive investigations to gain an understanding of the genetic diversity of this fungi.

ACKNOWLEDGEMENTS

The author would like to express sincere thanks to Professor Dr. Randy C. Ploetz, University of Florida, USA for providing a set of *nit1* and *NitM* tester.

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