Evaluation of Fusarium wilt resistance among Thai banana cultivars (*Musa* spp.)

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ABSTRACT

Fusarium wilt disease (FWD) is caused by the soil-borne fungus *Fusarium oxysporum* f. sp. *cubense* (*Foc*) and results in severe devastation of global commercial banana production. *Foc*-resistant banana genotypes show promise for sustainable effective control of FWD. ScaU1001 and ScaS0901 markers and their putative banana cultivars were evaluated for FWD resistance under greenhouse conditions after artificial infection by *Foc* race 1 (*Foc*1). The *Foc*1 genotype was characterized from six *Fusarium* fungi isolated from the pseudostem of banana cv. 'Kluai Namwa' (ABB genome) using specific primer (*Foc*1), resulting in two *Foc* isolates (PL3 and PL6) with an expected 354 bp PCR product. Both ScaU1001 and ScaS0901 markers were amplified as a unique PCR product (1694 bp and 1429 bp, respectively) in two banana cultivars 'Kluai Khai Kasetsart 2' (AA genome) and 'Kluai Hom Khiew' (AAA genome), indicating that both cultivars probably carried a *Foc*-resistant DNA fragment. These markers could not amplify an expected PCR product in twenty-one other banana cultivars, suggesting that the cultivars were *Foc*-susceptible banana genotypes. To evaluate FWD symptoms among six tested banana cultivars infected by *Foc*1, only 'Kluai Khai Kasetsart 2' and 'Kluai Hom Khiew' were considered as resistant to *Foc*1. They exhibited a few yellowed lower leaves with a darkish-brown discoloration inside the pseudostem at 21 days post-inoculation. Results suggest that these markers show great promise to identify new *Foc*-resistant genotypes in banana germplasm and this will be beneficial for further genetic study to improve banana resistance to FWD in breeding programs.

Keywords: Fusarium wilt disease, Fusarium oxysporum f. sp. *cubense, Molecular marker, Musa* spp.

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INTRODUCTION

Banana (*Musa* spp.) are popular, economically viable fruits with high demand in global markets. In 2019, over 100 million tons of fruit were cultivated in more than 130 countries (FAO, 2019). Banana belongs to the family *Musaceae* and is believed to have originated from tropical and subtropical regions in South East Asia and East and Central West Africa (Jooste et al., 2016). Most of the edible banana cultivars are derived from two wild species as hybridizations of *M. acuminata* (carrying AA genome) and *M. balbisiana* (carrying BB genome). Inter and intraspecific hybridization have resulted in the generation of many genome groups such as AA, AB, AAA, AAB, ABB, AABB, AAAB, and ABBB (Ploetz, 2015). Of these groups, the triploids, including genomes AAA and AAB are the most significant cultivars (Ploetz, 2015). In Thailand, banana (particularly genome ABB) is one of the most important fruit crops. Bananas are sold in local markets and shops as various dessert items and food productions such as banana chips, banana cookies and solar- dried banana (Rotchanapreeda et al., 2016).

Fusarium wilt disease (FWD) is caused by the soil-borne fungal *Fusarium oxysporum* f. sp. *cubense* (*Foc*) and results in major devastations worldwide in commercial banana production (Ploetz, 2015; Zheng et al., 2018). Recently, *Foc* triggered a serious epidemic throughout the banana-growing regions of Australia, Taiwan, China, Philippines (Dita et al., 2018 and Zheng et al., 2018), India, Jordan, Lebanon, Mozambique, Pakistan (Thangavelu et al., 2019), Laos and Vietnam (Chittarath et al., 2017; Hung et al., 2018). Fungal spores in the soil infect the banana root tip after the mycelia directly penetrate the epidermal cell wall and then proliferate into the vascular tissue causing leaf wilt and ultimately death of the whole plant (Li et al., 2011).

Four *Foc* races (1, 2, 3, and 4) have been classified as causing FWD in different banana cultivars. Among these, *Foc*1 and *Foc*4 cause major devastation in banana crops while the roles of *Foc*2 and *Foc*3 are still unclear in banana infection (Chittarath et al., 2017; Hung et al., 2017; Zheng et al., 2018). *Foc*1 causes disease in 'Gros-Michel' (AAA), 'Sukali Ndizi' (AAB), 'Kisubi' (AB) and 'Pisang Awak' (ABB) cultivars, while *Foc*4 attacks the Cavendish cultivar (AAA) (Chen et al., 2019). In general, outbreaks of FWD in susceptible banana cultivars are very difficult to manage using chemical controls (soil fumigants and fungicides), biological controls (plant growth promoting rhizobacteria; PGPR, and arbuscular mycorrhizal fungi; AMF), and agricultural practice method (crop rotation) (Bidabadi et al., 2018). Chemicals are environmentally unfriendly and can accumulate in the soil for decades (Bidabadi et al., 2018).

A possible intensive option to control FWD as sustainably effective disease management is the use of *Foc*-resistant banana cultivars (Ploetz, 2015 and Dita et al., 2018). Therefore, reliable genetic identification of *Foc* resistance among existing cultivated and wild banana germplasm is necessary to characterize the genes (Zheng et al., 2018). Isolation of *Foc*-resistant genes has been applied to develop new *Foc*-resistant banana cultivars via conventional breeding methods using gene technology (Dita et al., 2018) as selected markers for *Foc*-resistant phenotypes. However, currently, scant information is available regarding associated *Foc*-resistant genes in worldwide banana species (Zheng et al., 2018). Molecular markers linked to disease

resistance may enable improved screening to identify *Foc*-resistant genes in banana germplasm.

In *Musa* spp., two SCAR (sequence characterized amplified region) markers, called ScaU1001 and ScaS0901 show potential as a powerful tool to effectively identify putative *Foc*-resistant banana cultivars containing resistant genes to *Foc* (Wang et al., 2012). Both markers were able to consistently amplify the PCR product in resistant genotypes, with absence in susceptible genotypes, (Wang et al., 2018). Therefore, here, we assessed the *Foc*-resistance response of 23 *Musa* cultivars using ScaU1001 and ScaS0901 markers. Furthermore, their putative cultivars were subsequently evaluated for FWD resistance under greenhouse conditions after artificial infection by *Foc*. Our findings will enable the identification of new *Foc*-resistant banana cultivars, and promote further study of the genetic regulation of resistance mechanisms to benefit and improve commercial breeding programs.

METHODOLOGY

1) Fungal isolation and purification

The occurrence of Panama disease or FWD was surveyed at banana plantations in the lower northern region of Thailand. The infected pseudostem from banana plant cultivar 'Kluai Namwa' (ABB genome) was collected from three different plantations in Bang Krathum district, Phitsanulok Province (Figure 1) and one plantation at Mueang Phichit district, Phichit Province. Each infected banana pseudostem was cut into small pieces and the surface was sterilized by soaking in 10% sodium hypochlorite for 10 minutes. Sterilized tissue was cultured on sterilized water agar medium for 4 days. Mycelium with agar was cut, subsequently transferred on Potato Dextrose Agar (PDA) medium and incubated at room temperature. After 14 days, the colony produced white fluffy mycelium while color of the culture media turned to darkish purple. *Fusarium* species was identified under a compound microscope by checking two types of spore production as macroconidia and microconidia. *Fusarium* mycelium was kept under 80% glycerol and stored at -80°C until required for further use.

Figure 1 Characterization of symptomatic pseudostem of FWD in banana cv. 'Kluai Namwa' (*Musa* spp. ABB genome) collected from a plantation in Phitsanulok Province, Thailand

Note: Cross section of non-symptomatic pseudostem (left), cross (middle) and longitudinal (right) section of symptomatic pseudostem showing reddish-brown internal vascular discoloration of *Foc* in naturally infected banana.

2) Plant material

Twenty-three Thai banana cultivars, consisting of seven different genomes (5 AA, 3 BB, 3 AAB, 3 AAA, 7 ABB, 1 BBB, 1 ABBB) (Table 1), were used in this

study. All the cultivars were grown in germplasm at the Phitsanulok Agricultural Extension and Development Center, Phitsanulok Province Thailand.

Table 1 *Musa* accessions used in this study

No	Botanical name 'Cultivar'	Genome	No	Botanical name 'Cultivar'	Genome
1	*Musa* 'Kluai Tani Sukothai'	BB	13	*Musa* 'Kluai Nam Fad'	AAB
2	*Musa* 'Kluai Tani Hin'	BB	14	*Musa* 'Kluai Langka'	AAB
3	*Musa* 'Kluai Tani Esan'	BB	15	*Musa* 'Kluai Tip Yai'	ABB
4	*Musa* 'Kluai Leb Muer Nang'	AA	16	*Musa* 'Kluai Hak Muk'	ABB
5	*Musa* 'Kluai Nam Thai'	AA	17	*Musa* 'Kluai Namwa Maliong'	ABB
6	*Musa* 'Kluai Nam Thai'	AA	18	*Musa* 'Khuai Namwa Pakchong 50'	ABB
7	*Musa* 'Kluai Khai Kamphaengphet'	AA	19	*Musa* 'Kluai Namwa Kap Khao'	ABB
8	*Musa* 'Kluai Khai Kasetsar 2'	AA	20	*Musa* 'Kluai Namwa Khom'	ABB
9	*Musa* 'Kluai Khai Pra Tabong'	AAA	21	*Musa* 'Kluai Namwa Dam'	ABB
10	*Musa* 'Kluai Hom Khiew'	AAA	22	*Musa* 'Kluai Lep Chang Kut'	BBB
11	*Musa* 'Kluai Hom Thong'	AAA	23	*Musa* 'Kluai Theparod'	ABBB
12	*Musa* 'Kluai Nang Phaya'	AAB			

3) DNA extraction

For fungal DNA extraction, *Foc* isolates were grown individually on PDA medium for 7 days. The medium (approximately 1 g) was then collected, ground to a fine powder in liquid nitrogen, and applied for DNA extraction using the Genomic DNA Isolation Kit (Plant) (PureDireX, Taiwan) according to the manufacturer's instructions.

For plant DNA extraction, individual young banana leaf samples (approximately 2 g) were ground to a fine powder in liquid nitrogen and applied for isolating genomic DNA (gDNA) using the InnuPrep Plant DNA Kit (Analytik Jena, Germany) according to the manufacturer's instructions.

The gDNA was quantified and qualified by a UV-spectrophotometer at OD_{260} and OD_{280} nm (Microplate Reader, Synergy H1 Biotek, USA). Its integrity was examined by 1.0% (w/v) agarose gel electrophoresis assay and stained with 0.5 µg/ml ethidium bromide (EtBr) (Invitrogen™ UltraPure™, USA). The gel image was visualized by the gel documentation system (Thermo Fisher Scientific, USA) and the gDNA was stored at -20°C until required for use.

4) PCR amplification

The PCR amplifications were performed using OnePCR master mix (GeneDireX, USA) and then added with individual marker pairs (Table 2) and 1.0 µl of DNA template (25 ng/µl) according to the manufacturer's instructions. The PCR reaction was carried out under the following conditions: pre-denaturation 1 cycle of 95°C for 5 minutes, followed by 35 cycles of denaturation at 95°C for 30 seconds, annealing at either 42°C (28s rRNA), 52°C (Fum1, Foc1 and Foc4), or 55°C (ScaU1001 and ScaS0901) for 40 seconds, extension at 72°C for 1 minute, and final extension for 1 cycle of 72°C for 5 minutes in a thermal cycler (Bio-Rad T100™, USA). The PCR products were separated on 1.5% (w/v) agarose gel, stained with EtBr (Invitrogen™ UltraPure™, USA) and visualized and photographed under ultraviolet light.

Table 2 PCR amplification primers used in this study

Primer set	Base sequence (5´→3´)	Tm (°C)	Product size (bp)	Specificity
28s rRNA	LROR: ACCCGCTGAACTTAAGC LR7: TACTACCACCAAGATCT	53 42	1223	Eukaryotic (*28S rRNA* gene)
Fum1	F: GCAGTCGTACGTCATCGACC R: CCATGGCAGATGGCGAGTCA	58 60	729	*F. oxysporum* (*fum1* gene)
Foc1	F: GTTGAGTCTCGATAAACAGCAAT R: GACGAGGGGAGATATGGTC	58 48	354	*Foc1*
Foc4	F: ACGTTTAAGGTGCCATGAGAG R: GCCAGGACTGCCTCGTGA	48 67	455	*Foc4*
ScaU1001	F: ACCTCGGCACTCGAAGACACAT R: ACCTCGGCACTATTACCCATCA	55 48	1694	*Musa* spp. HQ613949
ScaS0901	F: TCCTGGTCCCAGTACAAATAC R: TCCTGGTCCCTCTGAATTTTC	48 48	1429	*Musa* spp. HQ613950

5) DNA cloning and sequencing of PCR product

According to the manufacturer's instructions, the expected size of PCR product was excised from 1.5% agarose gel, and the DNA was purified using the PureLink Quick Gel Extraction Kit (Invitrogen, USA). The purified DNA was then cloned into the TA Cloning Vector Kit (RBC BioScience, Taiwan). This inserted vector was further transformed into *Escherichia coli*, strain DH5α using the Subcloning Efficiency DH5α Competent Cell Kit (Invitrogen, USA). The bacterial transform was cultured on SOC medium, and its plasmid DNA was isolated using the Plasmid Miniprep Kit (GeneDireX, Taiwan). Plasmid DNA was quantified as at least 100-150 ng/µl by a UV spectrophotometer (Microplate Reader, Synergy H1 BioTek, USA). Three biological replicates in each clone were submitted for sequencing at Macrogen (Korea) through

Gibthai Co., Ltd. (Thailand). Sequencing data were analyzed to find locus similarity of reference sequences and published on genome databases using GeneDoc software.

6) *Foc* inoculation experiment

To access the FWD symptom among putative resistant or susceptible banana cultivars, a *Foc* inoculation experiment was performed by preparing fungal mycelia and banana plantlets. The *Foc* was cultured on a plate containing PDA medium and incubated at 25-28°C for 14 days. The mycelial tip with agar was then excised into small piece (0.5 cm diameter) using a cork borer. Meanwhile, six-week-old banana plantlets, grown in pots (8 inches diameter), were wounded with a 1 cm long cut at its basal stem using a scalpel blade. Subsequently, the mycelial piece was placed on the wound region of the banana plantlets and kept under greenhouse conditions. After 21 days post-fungal inoculation, plants were monitored for external and internal FWD symptoms. As expected, the *Foc*-resistant cultivars did not show any detectable disease symptoms during the whole experimental period. By contrast, *Foc*-susceptible plants showed FWD symptoms and exhibited yellowed upper leaves and wilted basal leaves, while a longitudinal section of the pseudostem showed darkish-brown discoloration of the vascular bundles that appeared as tiny brownish streaks surrounding the core. Two-four biological replicates were experimentally performed.

RESULTS AND DISCUSSION

1) Identification of *Fusarium* species through *28sRNA* and *fum1* genes

Fusarium fungi were isolated from the pseudostem of 'Kluai Namwa' (ABB genome) that showed FWD symptoms (Figure 1). Ten putative *Fusarium* fungi were selected to extract DNA and characterized for *Fusarium* species using PCR assay with the *28S rRNA* and *fum1* genes.

Individual fungal DNA from putative *Fusarium* fungi was amplified and sequenced using LR0R and LR7 primers (specific to the *28SrRNA* gene) by Macrogen (Korea). Each sequence read (approximately 1395-1493 bp long) was aligned and then compared to the known 28S rRNA sequence of *Fusarium* species published from the NCBI database using ClustralW program (EMBL-EBI, UK) and nucleotide BLAST sequences (NCBI, US). Results revealed that nucleotide sequence reads from nine isolates gave 100% identity as *Fusarium oxysporum* species, while only one (PL7) gave 100% identity as *Glomerella cingulate* species (Table 3), indicating that the nine *Fusarium oxysporum* species did not have genetic distance at the 28S rRNA region. In agreement with previous findings, nucleotide sequencing analysis at the 28S rRNA region precisely identified nineteen putative *Fusarium* strains isolated from banana rhizosphere in China (Kai-li et al., 2019).

Table 3 Analysis of nucleotide-sequence reads at the 28S rRNA region of ten putative fungal *Fusarium* species compared to references using the BLAST program

Isolate[w]	Sample ID[x]	Length (nt)[y]	Species	Identity (%)[z]
PL1	D1-LR0R	1453	*Fusarium oxysporum* (partial 28S rRNA gene, strain Fod008)	100
	D1_LR7	1434	*Fusarium oxysporum* (partial 28S rRNA gene, strain Fod008)	100
PL2	D2_LR0R	1448	*Fusarium oxysporum* (partial 28S rRNA gene, strain Fod008)	100
	D2_LR7	1451	*Fusarium oxysporum* (partial 28S rRNA gene, strain Fod008)	100
PL3	E1_LR0R	1444	*Fusarium oxysporum* (partial 28S rRNA gene, strain Fod008)	100
	E1_LR7	1437	*Fusarium oxysporum* (partial 28S rRNA gene, strain Fod008)	100
PL4	E2_LR0R	1441	*Fusarium oxysporum* (partial 28S rRNA gene, strain Fod008)	100
	E2_LR7	1444	*Fusarium oxysporum* (partial 28S rRNA gene, strain Fod008)	100
PL5	F1_LR0R	1451	*Fusarium oxysporum* (partial 28S rRNA gene, strain Fod008)	100
	F1_LR7	1436	*Fusarium oxysporum* (partial 28S rRNA gene, strain Fod008)	100
PL6	F2_LR0R	1453	*Fusarium oxysporum* (partial 28S rRNA gene, strain Fod008)	100
	F2_LR7	1449	*Fusarium oxysporum* (partial 28S rRNA gene, strain Fod008)	100
PL7	J_LR0R	1432	*Glomerella cingulate* (partial 5.8S rRNA strain AR2801)	100
	J_LR7	1395	*Glomerella cingulate* (partial 5.8S rRNA strain AR2801)	100
PL8	H1_LR0R	1452	*Fusarium oxysporum* (partial 28S rRNA gene, strain Fod008)	100
	H1_LR7	1444	*Fusarium oxysporum* (partial 28S rRNA gene, strain Fod008)	100
PL9	H2_LR0R	1459	*Fusarium oxysporum* (partial 28S rRNA gene, strain Fod008)	100
	H2_LR7	1438	*Fusarium oxysporum* (partial 28S rRNA gene, strain Fod008)	100
PL10	I_LR0R	1412	*Fusarium oxysporum* (partial 28S rRNA gene, strain Fod008)	100
	I_LR7	1441	*Fusarium oxysporum* (partial 28S rRNA gene, strain Fod008)	100

Note: [w] indicates *Fusarium* fungi isolated from the pseudostem of *Musa* cv. 'Kluai Namwa' (ABB genome).

[x] indicates sample ID results provided by Macrogen (Korea).

[y] indicates PCR-product length (approximately 1395-1493 bp long) amplified by the 28S rRNA primer pair of LROR (forward) and LR7 (reverse).

[z] indicates identity percentage of nucleotide comparison of sequence read matched to sequence reference from the NCBI database, while nt is an abbreviation for nucleotide.

Findings showed that six out of ten fungal isolates were characterized as *Fusarium* species using PCR assay. The primer pair of Fum1-F and Fum1-R targeting the *fum1* gene played a key role in fumonisin biosynthesis (Ramana et al., 2011). The PCR amplicon sizes were confirmed by 1.5% gel electrophoresis. This primer pair amplified a unique DNA fragment corresponding to 729 bp long in six fungal isolates (Figure 2A) that were confirmed as *Fusarium* species. This finding was supported by Das et al. (2012) and Kai-li et al. (2019). They reported the Fum1 primer set as a powerful tool for identifying members of *Fusarium* species. Moreover, Ji et al. (2019) reported that almost all fungal *Fusarium* species naturally produced toxin fumonisins.

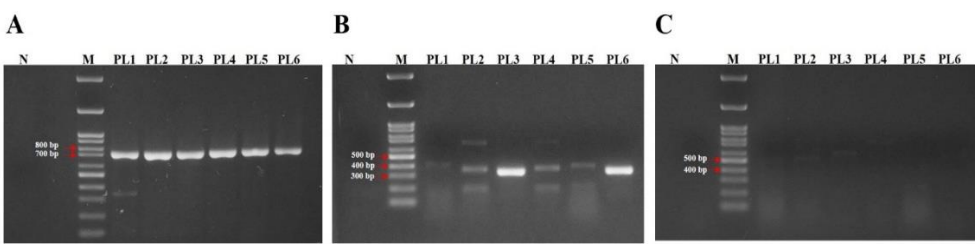

Figure 2 Identification of *fum1* gene (A), *Foc*1 (B) and *Foc*4 (C) among six unknown fungal *Fusarium* isolates through PCR assay

Note: The amplified PCR product profile from six unknown fungal *Fusarium* isolates (PL1, PL2, PL3, PL4, PL5, and PL6) were fractionated on 1.5% agarose gel and then DNA bands were visualized under UV light with expected specific size of approximately 729 bp, 354 bp, or 455 bp long amplified by specific primers of Fum (Ramana et al., 2011), Foc1 or Foc4 (Kai-li et al., 2019), respectively.

2) Identification of *Foc* race using PCR assay with specific primer

Race-specific identification for the six *Fusarium* isolates was performed using standard PCR assay with the specific primer set for *Foc* race 1 (Foc1-F/Foc1-R) and *Foc* race 4 (Foc4-F/Foc4-R). Subsequently, the expected PCR-product size was separated on 1.5% agarose gel electrophoresis, corresponding to 354 bp and 455 bp long (Li et al., 2011; Kai-li et al., 2019) to identify *Foc*1 and *Foc*4, respectively. Results revealed that the Foc1 primer pair was specifically amplified to a unique PCR product in two *Foc* isolates (PL3 and PL6), corresponding to 354 bp long expected size which belonged to *Foc*1 species, and showed distinct target banding patterns from the other *Foc* isolates (Figure 2B). Meanwhile, the other four tested *Foc* isolates were not identified to both *Foc*1 and *Foc*4 fungi because the primer pair of Foc1 or Foc4 did not yield the amplified PCR product as expected size, corresponding to either 354 bp or

455 bp long, respectively (Figure 2C). This result illustrated that both *Foc* isolates of PL3 and PL6 were classified as *Foc*1 and experimentally induced typical symptoms of FWD. Recently, several published studies have successively used the Foc1 gene primer set for rapid detection of the *Foc*1 gene in *Fusarium* species isolated in bananas. For example, Das et al. (2012) reported that this primer was able to characterize 24 *Foc*1 isolates from two regions of India, while Kai-li et al. (2019) found that this primer characterized 11 *Foc*1 isolates from four regions of China.

3) Evaluation of *Foc*-resistant banana cultivars using SCAR markers

Here, two SCAR marker sets of ScaU1001 and ScaS0901 (Wang et al., 2012) were used to identify DNA fragments linked to 23 *Foc*-resistant banana cultivars in germplasm kept at the Phitsanulok Agricultural Extension and Development Center, Thailand. Results revealed that ScaU1001 and ScaS0901 markers were amplified to a unique PCR product, corresponding to a length of approximately 1694 bp (Figure 3A) and 1429 bp long (Figure 3B) respectively, in two banana cultivars of 'Kluai Khai Kasetsart 2' (AA genome) and 'Kluai Hom Khiew' (AAA genome). However, ScaU1001 and ScaS0901 DNA could not be amplified to a single PCR product of the expected size in the other twenty-one banana cultivars (Figure 3A and Figure 3B, respectively), suggesting that these cultivars might be *Foc*- susceptible banana genotypes.

Furthermore, results also revealed that all banana cultivars belonging to the BB genome were absent from the target-PCR products amplified by both markers. Some banana cultivars belonging to AA ('Kluai Khai Kasetsart 2'), and AAA ('Kluai Hom Khiew') genomes contained *Foc*-resistant DNA fragments, probably associated with some key resistant genes that existed in the A genome (Zuo et al., 2018). Results for the banana cultivar 'Kluai Hom Khiew' (belonging to *Musa* Cavendish subgroup) agreed with previous findings that both markers (ScaU1001 and ScaS0901) were able to amply in *Foc*-resistant banana genotypes (Cavendish cv. Williams 8818-1) but not in *Foc*-susceptible banana genotypes (Wang et al., 2012; Silva et al., 2016). Meanwhile, both markers did not amplify the PCR product *Musa* cv. 'Kluai Hom Thong' (belonging to *Musa* AAA group, 'Gros-Michel' subgroup). Similarly, a report by Wang et al. (2012) and Silva et al. (2016) found that the PCR products amplified by these markers were absent in *Musa* AAA group, comprising three subgroups ('Gros Michel', 'Williams 8818' wild type, and 'Grande Naine'). Furthermore, Silva et al. (2016) reported that the ScaU1001 marker was highly efficient and successful in amplifying the targeted PCR product from resistant banana genotypes among 276 banana accessions collected from the Embrapa Mandioca Germplasm Bank of Brazil compared to the ScaS0901 marker. Interestingly, 'Kluai Khai Kasetsart 2' (AA group) was first reported to carry a *Foc*-resistant DNA fragment in its genomic DNA and should be further evaluated for resistance to *Foc* infection in greenhouse condition.

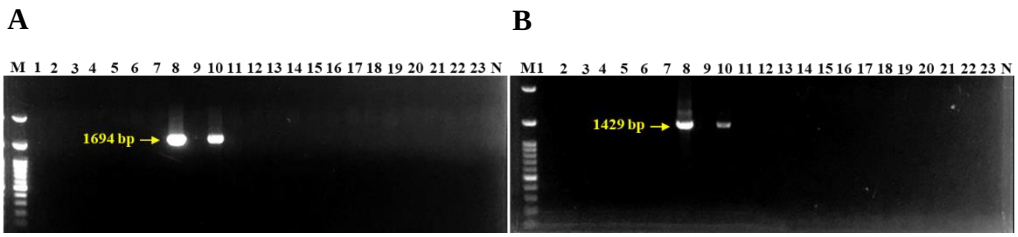

Figure 3. PCR product profiles amplified with SCAR primer set of ScaU1001 (A) and ScaS0901 (B) among twenty-three different banana cultivars

Note: The arrow represents an expected PCR amplicon, corresponding to 1694 bp (A) and 1429 bp (B) long, indicating a DNA fragment linked to *Foc* resistance. M expresses the 100 bp DNA ladder (OneMarker100, GeneDireX, Taiwan). N represents negative control without DNA template in the PCR reaction. Numbers 1-23 represent banana cultivars, related to the list shown in Table 1. SCAR abbreviates sequence characterized amplified region. bp abbreviates base pair.

4) Comparative nucleotide variation between sequence reads and reference

To rapidly identify the variation of nucleotide sequences at SCAR marker loci in two banana cultivars ('Kluai Khai Kasetsart 2' and 'Kluai Hom Khiew'), their individual sequence reads amplified by either ScaU1001 or ScaS0901 marker (Macrogen, Taiwan) were compared to sequence references of HQ613949.1 or HQ613950.1, respectively (annotated at NCBI database) using alignment of the GeneDoc program. Alignment results of the sequence reads from the ScaU1001 locus of *Musa* cv. 'Kluai Khai Kasetsart 2' and 'Kluai Hom Khiew' gave sequence identity as approximately 98.64% and 99.23%, respectively compared to HQ613949.1 (1694 nucleotides) (Figure 4). Along the nucleotide reads of Kluai Khai Kasetsart 2' or 'Kluai Hom Khiew there were 13 or 8 nucleotide substitutions and 10 or 1 nucleotide insertions, respectively compared to the nucleotide from HQ613949.1 (Figure 4).

Moreover, alignment results of the sequence reads from the ScaS0901 locus of *Musa* cv. 'Kluai Khai Kasetsart 2' and 'Kluai Hom Khiew' showed sequence identity as approximately 95.17%, and 98.74%, respectively compared to HQ613950.1 (1429 nucleotides) (Figure 5). Along the nucleotide reads of Kluai Khai Kasetsart 2' or 'Kluai Hom Khiew there were 46 or 7 nucleotide substitutions and 8 or 2 nucleotide insertions/deletions (indel), respectively compared to the nucleotide from HQ613950.1 (Figure 5).

5) Disease symptoms among bananas cultivars through *Foc*1 inoculation

From the previous results, two putative banana cultivars ('Kluai Khai Kasetsart 2' and 'Kluai Hom Khiew') were identified as *Foc* resistance carrying a *Foc*-resistant DNA fragment linked to ScaU1001 and ScaO901 markers, whereas the other twenty-one banana cultivars were identified as *Foc* susceptibility. To confirm *Foc*-resistant banana cultivars, two putative *Foc*-resistant genotypes and four selected *Foc*-susceptible genotypes were evaluated for FWD symptoms of banana plantlets after artificial infection by *Foc*-1 for 21 days under greenhouse conditions. Results found that 'Kluai Khai Kasetsart 2' and 'Kluai Hom Khiew' were considered as resistant to

*Foc*1, and externally exhibited a few yellowed lower leaves at 21 days post-inoculation (dpi) (Figure 6, top panel) with absence of darkish-brown discoloration of the vascular bundles inside the pseudostem at 21 dpi (Figure 6, bottom panel). The rest of the tested accessions ('Khai Kamphaeng Phet', 'Kluai Tani Hin', 'Kluai Hom Thong', and 'Kluai Namwa Pakchong 50') displayed susceptibility to *Foc*1 and showed typical *Foc*1-disease symptoms such as yellowed upper leaves and wilted basal leaf at 21 dpi (Figure 6, top panel). Moreover, a longitudinal section of the pseudostem showed darkish-brown discoloration of the vascular bundles with tiny brownish streaks surrounding the core at 21 dpi (Figure 6, bottom panel).

These findings suggested that *Musa* cv. 'Kluai Khai Kasetsart 2' (AA genome) and 'Kluai Hom Khiew' (AAA genome) were *Foc*1-resistant genotypes but 'Kluai Hom Thong' (related to *Musa* cv. 'Gros Michel', AAA genome) was a *Foc*1-susceptible genotype. In agreement with previous reports, some Cavendish banana cultivars (AAA genome) such as 'Williams 8818-1' were reported to be resistant to *Foc*1 (Wang et al., 2012; Silva et al., 2016) but other Cavendish *Musa* cv. 'Gros Michel' belonged to the *Foc*1-susceptible genotype (Ploetz, 2015). However, the genetic mechanism of *Foc*1-resistant banana genotype remains unclear. Several previous studies have reported that its mechanism was regulated by a recessive gene in an F_2 population derived from crosses of a susceptible *Musa* cv. 'Sukali Ndizi' (AAB group) and a resistant *Musa* cv 'TMB2X80750' (AA) (Ssali et al., 2016). By contrast, Larter (1947) reported that the *Foc1*-resistant banana genotype was controlled by a single dominant gene in a study of tetraploid progenies, crossed between 'Gros Michel' (AAA genome) and a diploid (AA genome) accession. More recently, the *Foc*1-resistant banana was suggested to be controlled by at least two dominant genes with epistatic interaction studied in *M. acuminata* hybrid (AA genome) crossed between resistant genotype ('Monyet') and susceptible ('Kokopo') (Arinaitwe et al., 2019).

Figure 4 Nucleotide sequence alignment at ScaU1001 locus using GeneDoc program

Note: The ScaU1001 locus was defined as HQ613949.1 by Wang et al (2012). KKK2 abbreviates *Musa* cv. 'Kluai Khai Kasetsart 2' and KHK abbreviates *Musa* cv. 'Kluai Hom Khiew'. Capital letters shaded in black represent identical nucleotides found in each sequence; non-highlighted capital letters represent differing nucleotides (such as substitution, insertion and deletion). Capital letters shaded in green and pink represent the forward and reverse sequences of ScaU1001 primer, respectively.

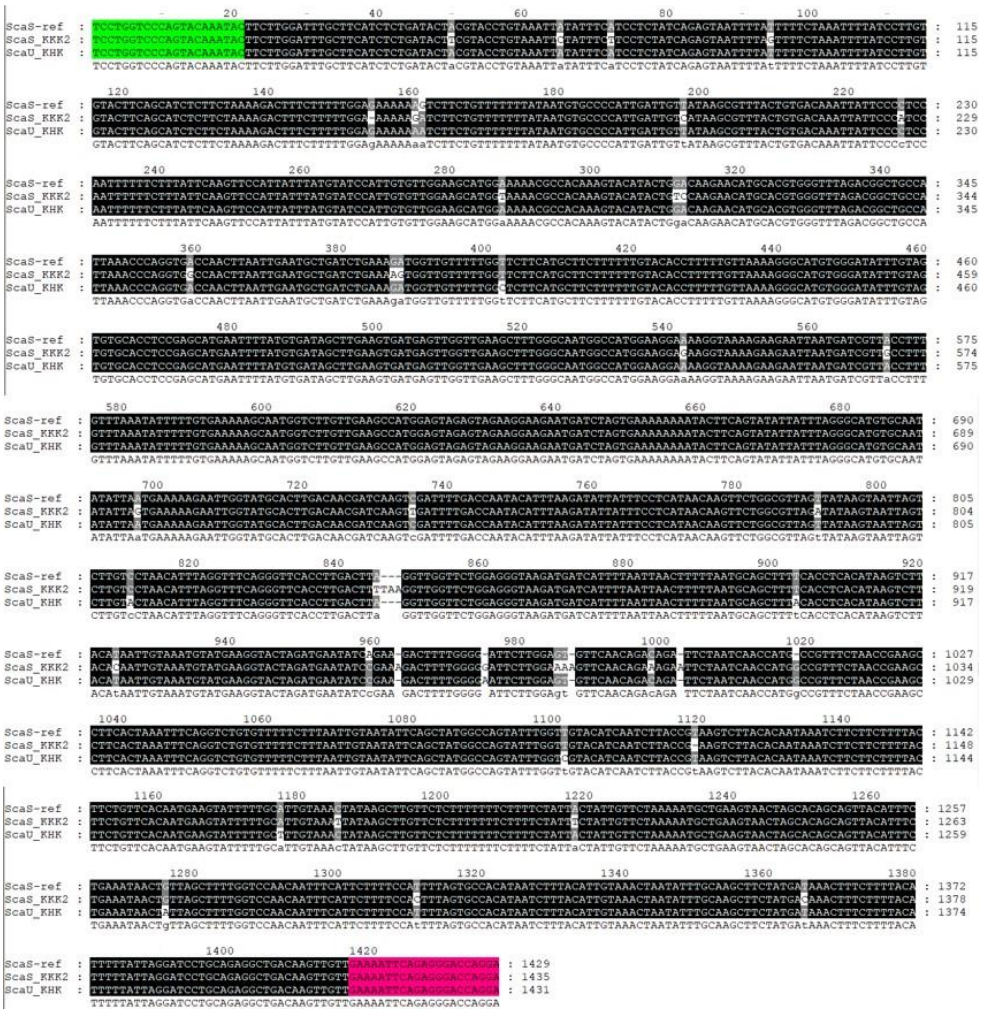

Figure 5 Nucleotide sequence alignment at ScaS0901 locus using GeneDoc program

Note: The ScaS0901 locus was defined as HQ613950.1 by Wang et al (2012). KKK2 abbreviates *Musa* cv. 'Kluai Khai Kasetsart 2' and KHK abbreviates *Musa* cv. 'Kluai Hom Khiew'. Capital letters shaded in black represent identical nucleotides found in each sequence; non-highlighted capital letters represent differing nucleotides (such as substitution, insertion and deletion). Capital letters shaded in green and pink represent the forward and reverse sequences of ScaS0901 primer, respectively.

Figure 6 External (top panel) and internal (bottom panel) FWD symptoms among six banana cultivars

Note: Six-week-old banana plantlets were inoculated with *Foc*1 and photographs were taken 21 days post-inoculation. The *Foc*1-resistant banana cultivars (‘Kluai Khai Kasetsart 2’ and ‘Kluai Hom Khiew’) show no leaf wilting symptoms as relatively healthy, while their pesudostems cut in half longitudinally show no traces of brown discoloration in the vascular bundle. The *Foc*1-susceptible banana cultivars (‘Kluai Kamphaeng Phet’, ‘Kluai Tani Hin’, ‘Kluai Hom Thong’, and ‘Kluai Namwa Pakchong 50’) display wilting of the old leaves, and their pesudostems show extensive brown discoloration inside the vascular bundles.

CONCLUSIONS

PCR amplification using specific primer (Foc1-F/Foc1-R) was able to identify two *Foc*1 strains from six unknown *Fusarum* species, isolated from FWD pseudostem of *Musa* cv. ‘Kluai Namwa’ in Phisanulok Province, Thailand. Based on screening of *Foc* resistance among 23 different banana cultivars using both molecular-marker and pathogenic assays, two new banana cultivars (‘Kluai Khai Kasetsart 2’ and ‘Kluai Hom Khiew’) were characterized as *Foc*1-resistant genotypes. Both cultivars carried DNA fragments linked by ScaU1001 and ScaS0901 markers, and internal and external FWD symptoms disappeared after artificial infection by *Foc*1 for 21 days under greenhouse conditions. Our findings will enable the identification of new *Foc*-resistant banana cultivars in Thailand. Further studies of the genetic regulation of resistance mechanisms will increase knowledge regarding the provision of genetic resources of resistant genes transferred into commercial banana cultivars to improve breeding programs.

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