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Molecular screening and allele identification of Thai banana genotypes for resistance to *Fusarium* wilt using validated SCAR markers

THANITA BOONSRANGSOM[1,2,♥], WERAPAT CHANSONGKRAM[1,2], JUTAMAS SAENGJANCHAY[1,2], JUANGJUN JUMPATHONG[1,2], PONGSANAT PONGCHAROEN[1,2], WANWARANG PATHAICHINDACHOTE[1,2], KUMROP RATANASUT[1,2], KAWEE SUJIPULI[1,2]

[1]Department of Agricultural Science, Faculty of Agriculture, Natural Resources and Environment, Naresuan University. Phitsanulok 65000, Thailand. Tel.: +66-559-62736, Fax.: +66-559-62704, ♥email: thanitab@nu.ac.th

[2]Center of Excellence in Research for Agricultural Biotechnology, Faculty of Agriculture, Natural Resources and Environment, Naresuan University. Phitsanulok 65000, Thailand

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Abstract. *Boonsrangsom T, Chansongkram W, Saengjanchay J, Jumpathong J, Pongcharoen P, Pathaichindachote W, Ratanasut K, Sujipuli K. 2024. Molecular screening and allele identification of Thai banana genotypes for resistance to* Fusarium *wilt using validated SCAR markers. Biodiversitas 25: 2590-2601. Fusarium* wilt (FW) caused by *Fusarium oxysporum* f. sp. *cubense* (*Foc*), significantly damages commercial banana (*Musa* spp.) cultivation worldwide, including in Thailand. Cultivating resistant banana genotypes is crucial for managing FW. However, the unique biology of banana plants makes this process challenging and time-consuming. Genetic markers can enhance the efficiency and precision of traditional breeding methods for developing resistant cultivars. This study aimed to screen and identify *Fusarium* wilt-resistant alleles across six Thai banana genomes. Established sequence-characterized amplified region (SCAR) markers, strongly linked to loci associated with *Foc* susceptibility or resistance, were used in this investigation. The results revealed significant genetic differences in FW resistance loci among 106 Thai banana genotypes, comprising AA, AAA, AAB, ABB, ABBB, and BB genomes. All genotypes carried at least one FW resistance locus except *Musa* (AAA) 'Kluai Khieo Pakchong'. Cluster analysis divided the 106 genotypes into two *Foc*R1-resistant and four *Foc*TR4-resistant clusters. No allelic variation was found among *M. balbisiana* with the BB genomes. Further research is needed to understand the interaction between banana genotypes and *Foc* strains. These validated SCAR markers will assist in genotype selection for field and greenhouse assessments as part of the Thai banana genetic improvement program.

Keywords: Banana, *Fusarium oxysporum* f. sp. *cubense* (*Foc*), *Fusarium* wilt disease, *Musa* spp., resistance gene, SCAR markers

Abbreviations: FW: *Fusarium* wilt; S: susceptibility; R: resistance; UPGMA: unweighted pair group method with arithmetic average

INTRODUCTION

Bananas (*Musa* spp.), members of the family Musaceae, are among the most widely grown, traded, and consumed fruits worldwide. Originating in Southeast Asia and the Pacific region, bananas were first domesticated in Papua New Guinea approximately 7000 years ago (Li and Ge 2017; Sardos et al. 2022). In 2021, global banana production reached an estimated 125 million tons, with over 135 countries consuming a significant portion (Statista 2021). With 1.4 million tons produced in 2021, Thailand ranks among the world's leading producers of bananas. Generally, bananas primarily derive from two wild diploid species, namely *M. acuminata* (AA genome, 2n=22) and *M. balbisiana* (BB genome, 2n=22), resulting in a wide range of genomic levels and genetic diversity.

Cultivated bananas (*M. acuminata*, *M. balbisiana*, and *Musa* × *paradisiaca*) are categorized into three genomic groups based on ploidy levels: diploids (AA, AB, BB; 2n=2x=22), triploids (AAA, AAB, ABB; 2n=3x=33), and tetraploids (AAAA, AAAB, AABB, ABBB, BBBB; 2n=4x=44) (Tomaszewska 2021). Many studies have shown significant genetic variation across *Musa* accessions based on morphological (Malikongwa et al. 2022; Premjet et al. 2022; Mingmanit et al. 2023) and molecular data (El-Shahed et al. 2017; Boonsrangsom et al. 2020; Changkham and Boonsrangsom 2021; Mertens et al. 2021; Igwe et al. 2022; Boonsrangsom et al. 2023; Safhi et al. 2023; Slameto 2023).

Due to their long evolutionary history, bananas are susceptible to a wide range of diseases and pests. One of the most damaging diseases is *Fusarium* wilt (FW), caused by the soilborne fungus *Fusarium oxysporum* f. sp. *cubense* (*Foc*). This disease significantly impacts commercial banana production by infecting the plant's roots and vascular system, leading to wilting and death (Guo et al. 2014; Zheng et al. 2018; Niwas et al. 2022). *Foc* is divided into three races, namely *Foc*R1, *Foc*R2, and *Foc*R4, with *Foc*R1 and *Foc*R4 causing more severe damage than *Foc*R2 (Guo et al. 2014; Dita et al. 2018). Susceptibility varies among banana subgroups. 'Gros Michel' (AAA), 'Silk' (AAB), 'Pome' (AAB), and 'Pisang Awak' (ABB) are susceptible to *Foc*R1, while these subgroups, along with 'Cavendish' (AAA) and 'Bluggoe' (ABB), are susceptible to *Foc*R4 (Ploetz 2015; Chen et al. 2019). *Foc*R4 is further divided into tropical race 4 (*Foc*TR4) and subtropical race 4 (*Foc*STR4), with *Foc*TR4 being more virulent (Ploetz 2015; Magdama et al. 2019).

Advances in DNA technologies have accelerated the identification of disease-resistant sources in various plants. One particularly effective tool is the sequence-characterized amplified region (SCAR) marker, derived from sequencing specific DNA fragments and designing longer primers (16-30 bp). This marker is associated with a range of traits, including disease resistance, yield, quality, and stress tolerance (Nadeem et al. 2018; Qv et al. 2024). Consequently, these markers offer more precise data than other DNA markers. Sanyong et al. (2020) assessed FW resistance in 23 Thai banana cultivars using SCAR markers and greenhouse tests. Two cultivars of *M. acuminata* with a *Foc*-resistant DNA fragment were found to be resistant to *Fusarium* wilt. Therefore, genetic markers linked to FW resistance are highly valuable.

SCAR markers, such as ScaU1001 and ScaS0901, are effective in identifying *Foc*TR4-resistant banana genotypes (Wang et al. 2012), while SuscPD and SC1/SC2 can detect susceptibility to *Foc*R1 and *Foc*TR4 (Cunha et al. 2015; Wang et al. 2018). Despite their use in banana screening, the resistance of Thai banana germplasm to FW remains poorly documented. This study aimed to screen Thai banana genotypes and identify *Fusarium* wilt-resistant alleles using SCAR markers linked to susceptibility or resistance. The results will support commercial banana breeding, identify new *Fusarium* wilt-resistant genotypes, and advance the understanding of host-pathogen interactions.

MATERIALS AND METHODS

Research materials

A total of 106 Thai banana (*M. acuminata, M. balbisiana,* and *Musa* × *paradisiaca*) genotypes, consisting of six combinations of the A and B *Musa* genomes, were gathered from three conservation sites of *Musa* germplasm in Thailand: (i) the Plant Propagation Center No. 6 (PPC), Mueang Phitsanulok District, Phitsanulok Province; (ii) the Pakchong Research Station of Kasetsart University (PRSKU), Pakchong District, Nakhon Ratchasima Province; and (iii) the Sukhothai Horticultural Research Center (SHRC), Si Satchanalai District, Sukhothai Province, Thailand (Table 1). The 106 genotypes of Thai *Musa* are classified as follows: 22 of *M. acuminata* (AAA group), 20 of *M. acuminata* (AA group), 17 of triploid hybrids (AAB group), 35 of triploid hybrids (ABB group), one of tetraploid hybrids (ABBB group), and 11 of *M. balbisiana* (BB group). In this study, two banana cultivars, 'Grande Naine' (AAA) and 'Williams' (AAA) were employed as reference genotypes.

Procedures

DNA extraction

To detect the presence of resistance or susceptibility loci using a PCR assay, the young, cigar-shaped banana leaves that emerge or develop early on a banana plant were harvested and ground into a fine powder with liquid nitrogen. The fine powder was then used for genomic DNA (gDNA) extraction using the Genomic DNA Isolation Kit (Plant) (PureDireX, Taiwan) according to the standard manufacturer's directions. The extracted gDNA was approximated on 1.0% (w/v) agarose gel electrophoresis for quality and measured by a UV-spectrophotometer at OD_{260} and OD_{280} nm (Microplate Reader, Synergy H1 Biotek, USA) for quantify. The gDNA was diluted to 25 ng/µL with TE buffer and stored at -20°C until required for use.

PCR amplification using validated SCAR markers

All banana genotypes were examined for the presence of FW susceptibility (S) loci (*+SuscPD* and *+SC1/SC2*) susceptible to *Foc*R1 (SCAR_1), and *Foc*TR4 (SCAR_2), as well as FW resistance (R) loci (*+ScaU1001* and *+ScaS0901*) resistant to *Foc*TR4 (SCAR_3 and SCAR_4) using a set of SCAR markers (Table 2). Furthermore, the *Musa* Actin housekeeping gene was amplified with *Actine1* gene primers (F: 5'-TCC TTT CGC TCT ATG CCA GT-3' and R: 5'-GCC CAT CGG GAA GTT CAT AG-3') with the expected size of DNA fragment 420 bp (Gayral et al. 2008) to confirm the amplificability of the DNA from 106 banana genotypes. The duplex PCR reaction was performed in a total volume of 20 µL, containing 50 ng of *Musa* gDNA, 10 µL of 2x OnePCR master mix (PureDireX, Bio-Helix, Co., Ltd., Taiwan) which includes Taq DNA polymerase, PCR buffer, dNTPs, gel loading dyes, and fluorescence dye, and 3.0 µM of each primer. The reactions were carried out in a thermal cycler (Bio-Rad T100™, USA) with the following program: an initial denaturation for 5 min at 95°C, followed by 35 cycles of 40 seconds of denaturation at 95ºC, annealing for 40 seconds at 55-58ºC, and extension for 1 min at 72ºC. The final extension step was carried out at 72ºC for 10 min. All reactions were carried out with three replicates.

Electrophoresis

The PCR products were separated on 1.5% agarose gels in 1x TAE buffer, then stained with RedSafe™ Nucleic Acid Staining Solution (iNtRON Biotechnology, USA), visualized under an ultraviolet (UV) light and photo-documented in a Gel Documentation System (Thermo Fisher Scientific, Waltham, MA, USA).

Data analysis

The presence or absence of DNA bands in the resistant or susceptible banana genotypes was recorded. The DNA bands on the gels were converted to binary data by being scored as either '1' or '0' for their occurrence or absence of each PCR product for each SCAR marker, accordingly. Based on a DNA ladder of 100 bp (OneMARK 100 DNA ladder, Bio-Helix), the sizes of the DNA fragments that correspond to the allelic sizes were precisely scored. Using DNA profiling data, a Jaccard coefficient of similarity-based cluster analysis was constructed to investigate the genetic variations among Thai banana genotypes resistant to *Foc*R1 and/or *Foc*TR4 (Jaccard 1908). This matrix was then utilized to generate a dendrogram using the unweighted pair group method with arithmetic average (UPGMA) using the FreeTree and TreeView software (Hampl et al. 2001).

Table 1. List of 106 Thai banana genotypes including their genome groups and collection sites, across six *Musa* genomes were used in this study

No.	Local name	Genome group [a]	Collection site [b]	No.	Local name	Genome group [a]	Collection site [b]
M. acuminata				54	'Kluai Tanot Phatthalung'	AAB	SHRC
1	'Grande Naine'	AAA	PPC	55	'Kluai Thong Kampan'	AAB	SHRC
2	'Williams'	AAA	PPC	56	'Kluai Nam Kab Dam'	AAB	SHRC
3	'Tumok'	AAA	PPC	57	'Kluai Nam Phatthalung'	AAB	SHRC
4	'Hochuchu'	AAA	PPC	58	'Kluai Niu Chorakhe'	AAB	SHRC
5	'Mahoi'	AAA	PPC	59	'Kluai Khai Boran'	AAB	SHRC
6	'Kluai Nak'	AAA	PPC	***Musa × paradisiaca***			
7	'Kluai Man'	AAA	PPC	60	'Kluai Tip'	ABB	PPC
8	'Kluai Hom Kariang'	AAA	PPC	61	'Kluai Thepanom'	ABB	PPC
9	'Kluai Hom Khieo'	AAA	PPC	62	'Kluai Namwa Kab Khao'	ABB	PPC
10	'Kluai Hom Thong'	AAA	PPC	63	'Kluai Namwa Khieo'	ABB	PPC
11	'Kluai Hom Thong Ayutthaya'	AAA	PPC	64	'Kluai Namwa Khom'	ABB	PPC
12	'Kluai Hom Eisan'	AAA	PPC	65	'Kluai Namwa Ngoen'	ABB	PPC
13	'Kluai Khieo Pakchong'	AAA	PRSKU	66	'Kluai Namwa Tha Yang'	ABB	PPC
14	'Kluai Hom Thong Pa'	AAA	PRSKU	67	'Kluai Namwa Nuan Chan'	ABB	PPC
15	'Kluai Hom Si Sa Ket'	AAA	PRSKU	68	'Kluai Namwa Mali-Ong'	ABB	PPC
16	'Kluai Kung Khieo Khom'	AAA	SHRC	69	'Kluai Namwa Luk Sai Dam'	ABB	PPC
17	'Kluai Nak Khom'	AAA	SHRC	70	'Kluai Namwa Sai Lueng'	ABB	PPC
18	'Kluai Nak Muk'	AAA	SHRC	71	'Kluai Namwa Ubon'	ABB	PPC
19	'Kluai Nak Yak'	AAA	SHRC	72	'Kluai Landy'	ABB	PPC
20	'Kluai Hom Khom'	AAA	SHRC	73	'Kluai Som'	ABB	PPC
21	'Kluai Hom Phama'	AAA	SHRC	74	'Kluai Huk Muk Nuan'	ABB	PPC
22	'Kluai Khai Phra Tabong'	AAA	SHRC	75	'Kluai Huk Muk Phama'	ABB	PPC
M. acuminata				76	'Kluai Huk Muk Thong'	ABB	PPC
23	'Kluai Khai Kasetsart 2'	AA	PPC	77	'Kluai Tip 269'	ABB	PRSKU
24	'Kluai Khai Kamphaeng Phet'	AA	PPC	78	'Kluai Tip Yai'	ABB	PRSKU
25	'Kluai Khai Chin'	AA	PPC	79	'Kluai Nom Mi'	ABB	PRSKU
26	'Kluai Khai Thong Ngoei'	AA	PPC	80	'Kluai Namwa Tanow Sri'	ABB	PRSKU
27	'Kluai Khai Thong Ruang'	AA	PPC	81	'Kluai Namwa Thong Ma Eng'	ABB	PRSKU
28	'Kluai Thong Ki Maew'	AA	PPC	82	'Kluai Namwa Luk Sai Daeng'	ABB	PRSKU
29	'Kluai Nam Nom'	AA	PPC	83	'Kluai Nam Wo Pakchong'	ABB	PRSKU
30	'Kluai Niu Nang Ram'	AA	PPC	84	'Kluai Huk Muk Khao'	ABB	PRSKU
31	'Kluai Sa'	AA	PPC	85	'Kluai Huk Muk Khieo'	ABB	PRSKU
32	'Kluai Khai 159'	AA	PRSKU	86	'Kluai Huk Muk Som'	ABB	PRSKU
33	'Kluai Khai Dam'	AA	PRSKU	87	'Kluai Hin'	ABB	PRSKU
34	'Kluai Takui'	AA	PRSKU	88	'Kluai Tip 1'	ABB	SHRC
35	'Kluai Thong Dok Mak'	AA	PRSKU	89	'Kluai Tip Kam'	ABB	SHRC
36	'Kluai Nam Thai'	AA	PRSKU	90	'Kluai Namwa Dam'	ABB	SHRC
37	'Kluai Kok Mak'	AA	SHRC	91	'Kluai Namwa Pakchong 50'	ABB	SHRC
38	'Kluai Thong Oei'	AA	SHRC	92	'Kluai Namwa Yak'	ABB	SHRC
39	'Kluai Thong Ho'	AA	SHRC	93	'Kluai Phama Haek Kuk'	ABB	SHRC
40	'Kluai Leb Mue Nang'	AA	SHRC	94	'Kluai Theparod'	ABB	PRSKU
41	'Kluai Hom Chan'	AA	SHRC	***Musa × paradisiaca***			
42	'Kluai Hom Champa'	AA	SHRC	95	'Kluai Tin Tao'	ABBB	PRSKU
Musa × paradisiaca				***M. balbisiana***			
43	'Kluai Khanun'	AAB	PPC	96	'Kluai Kib Ma'	BB	PPC
44	'Kluai Khai Chum Phae'	AAB	PPC	97	'Kluai Lep Chang Kut'	BB	PRSKU
45	'Kluai Nga Chang'	AAB	PPC	98	'Kluai Tani 67'	BB	PPC
46	'Kluai Tam Nuan'	AAB	PPC	99	'Kluai Tani A15'	BB	PPC
47	'Kluai Thong Som'	AAB	PPC	100	'Kluai Tani Kib Ma'	BB	PPC
48	'Kluai Nom Sawan'	AAB	PPC	101	'Kluai Tani Dam'	BB	PPC
49	'Kluai Nom Sao'	AAB	PPC	102	'Kluai Tani Mo'	BB	PRSKU
50	'Kluai Roi Wi'	AAB	PPC	103	'Kluai Tani Nuea'	BB	PRSKU
51	'Kluai Sae Ma'	AAB	PPC	104	'Kluai Tani 167'	BB	SHRC
52	'Kluai Niu Mue Nang'	AAB	PRSKU	105	'Kluai Tani Roiplee'	BB	SHRC
53	'Kluai Sam Duan Phichit'	AAB	PRSKU	106	'Kluai Tani Sirikit'	BB	SHRC

Note: [a]: genome classification based on Valmayor et al. (2000), Silayoi (2015) and Tomaszewska (2021); [b]: collection site: PPC: the Plant Propagation Center No. 6, PRSKU: the Pakchong Research Station of Kasetsart University, SHRC: the Sukhothai Horticultural Research Center

Table 2. Four SCAR markers tightly linked to the susceptibility and resistance loci for *Fusarium* wilt in bananas were used in this study

Marker name	Locus	Primer sequences (5' - 3'(	Expected size)bp(	Susceptibility/resistance to *Foc*R1 or *Foc*TR4	References
SCAR_1	*SuscPD*	F: GAACCAGAGCCAGGGCATAG R: TCTATGCGCCTACCCTCCTT	842	Susceptibility to *Foc*R1	Cunha et al. (2015)
SCAR_2	*SC1/SC2*	F: CTCGCCGACACCTTACTTGA R: AGGAGAGTCCAGCTCCAGTT	324	Susceptibility to *Foc*TR4	Wang et al. (2018)
SCAR_3	*ScaU1001*	F: ACCTCGGCACTCGAAGACACAT R: ACCTCGGCACTATTACCCATCAT	1694	Resistance to *Foc*TR4	Wang et al. (2012)
SCAR_4	*ScaS0901*	F: TCCTGGTCCCAGTACAAATAC R: TCCTGGTCCCTCTGAATTTTC	1429	Resistance to *Foc*TR4	Wang et al. (2012)

RESULTS AND DISCUSSION

Detection of FW resistance loci with validated SCAR markers

DNA samples from 106 Thai banana genotypes with various combinations of the A and B *Musa* genomes were screened with a selection of SCAR markers to determine whether or not FW resistance loci were present. The results of detecting SCAR primers that are tightly linked to the loci responsible for FW resistance in Thai banana genotypes are shown in Figure 1. The sizes of the generated PCR products were compared to the expected allele sizes. The results showed that the SCAR_1 marker was amplified to a unique PCR product with a length of approximately 842 bp in 54 banana genotypes (Figure 1.A), while the SCAR_2 marker was amplified to a distinct PCR product with a length of about 324 bp in 59 banana genotypes (Figure 1.B). The two remaining markers, SCAR_3 and SCAR_4, were amplified in 47 and 79 banana genotypes, respectively, yielding a unique PCR product with a length of approximately 1694 bp (Figure 1.C) and 1429 bp (Figure 1.D). No fragment was found for any of the SCAR markers employed in 22 banana genotypes, namely 'Kluai Sa' (AA) (31), 'Kluai Huk Muk' (ABB, Bluggoe subgroup) (74, 75, 76, 84, 85, 86), 'Kluai Tip 269' (77), 'Kluai Nom Mi' (ABB, Monthan subgroup) (79), 'Kluai Nam Wo' (ABB) (83), 'Kluai Tin Tao' (ABBB) (95), and all *M. balbisiana* genotypes (BB) (96-106) (Table 3). Depending on the presence or absence of the DNA bands derived from the four SCAR markers, each genotype had a total of 0 to 3 resistance loci.

Allele diversity of the FW resistance loci

The results of the four SCAR markers employed in the genetic analysis across six *Musa* genomes for FW resistance loci are displayed in Figure 2 and Table 3. Among the 42 *M. acuminata* genotypes (AAA and AA groups), 11 genotypes showed the absence of the *SuscPD* locus, which is associated with resistance to *Foc*R1 (26.2%). Of these, 33 genotypes possessed two FW resistance loci (+*ScaU1001* and +*ScaS0901*), which confer resistance to *Foc*TR4 (78.6%). The two check genotypes in this study were 'Grande Naine' (AAA) and 'Williams' (AAA), with the former carrying two FW resistance loci (+*ScaU1001* and +*ScaS0901*) and the latter having three (-*SuscPD*, +*ScaU100*1, and +*ScaS0901*). These three FW resistance loci (-*SuscPD*, +*ScaU1001*, and +*ScaS0901*) were also found in four of the nine genotypes of the 'Kluai Hom' (AAA) subgroup (9, 10, 11, and 20) in this investigation.

Among 53 *Musa x paradisiaca* hybrids (AAB, ABB, and ABBB groups), most of the AAB genotypes carried the *ScaS0901* locus for FW resistance to *Foc*TR4 (88.2%). The 'Kluai Sam Duan Phichit' (AAB) (53) and 'Kluai Tanot Phatthalung' (AAB) (54) genotypes lacked bands for the SCAR_1 and SCAR_2 markers, which are associated with resistance to *Foc*R1 and *Foc*TR4, respectively. Of the 35 ABB genotypes, 27 (77.1%) had negative results for the SCAR_1 marker, indicating resistance to *Foc*R1, whereas four genotypes (11.4%) and 25 genotypes (71.4%) had positive results for the SCAR_3 and SCAR_4 markers, indicating resistance to *Foc*TR4. Within the ABB genomic group, all 16 genotypes of *Musa* 'Kluai Namwa' or 'Pisang Awak' (62-71, 80-82, 90-92) carried three FW resistance loci (-*SuscPD*, -*SC1/SC2* and +*ScaS0901*), whereas six genotypes of *Musa* 'Kluai Huk Muk' or 'Bluggoe' (74-76, 84-86) contained only two FW resistance loci (-*SuscPD* and -*SC1/SC2*). Additionally, three genotypes, 'Kluai Thepanom' (ABB) (61), 'Kluai Som' (73) and 'Kluai Hin' (ABB, Saba subgroup) (87) possessed two FW resistance loci (-*SC1/SC2* and +*ScaS0901*). The two FW resistance loci (-*SuscPD* and -*SC1/SC2*) were present in 'Kluai Tin Tao' (ABBB) (95) tetraploid hybrids.

In *M. balbisiana* genotypes (BB and BBB groups), no allelic variation was found. All 11 genotypes bearing the B genome lacked the FW resistance loci (-*ScaU1001* and -*ScaS0901*) and FW susceptibility (S) loci (+*SuscPD* and +*SC1/SC2*).

Cluster analysis for identifying *Fusarium* wilt resistance in six *Musa* genomes

A cluster analysis was performed on 106 Thai banana genotypes utilizing DNA fingerprint data from four validated SCAR markers. These markers were found to be closely associated with FW resistance loci. Jaccard similarity coefficients were then employed to generate a UPGMA dendrogram. Based on the results, all banana genotypes could be classified into two main clusters associated with the *Foc*R1 resistance loci (Figure 3.A). The two reference genotypes, 'Grande Naine' (AAA) (K1) and 'Williams' (AAA) (K2), were assigned to different clusters, with 'Grande Naine' in Cluster I and 'Williams' in Cluster II. Cluster I included 54 banana genotypes from four *Musa* genomes: 15 AAA, 16 AA, 15 AAB, and 8 ABB, all

carrying the *Foc*R1 susceptibility loci (+*SuscPD*). The remaining 52 genotypes in Cluster II displayed six different genomes: 7 AAA, 4 AA, 2 AAB, 27 ABB, 1 ABBB, and 11 BB, all lacking the *Foc*R1 susceptibility loci (-*SuscPD*).

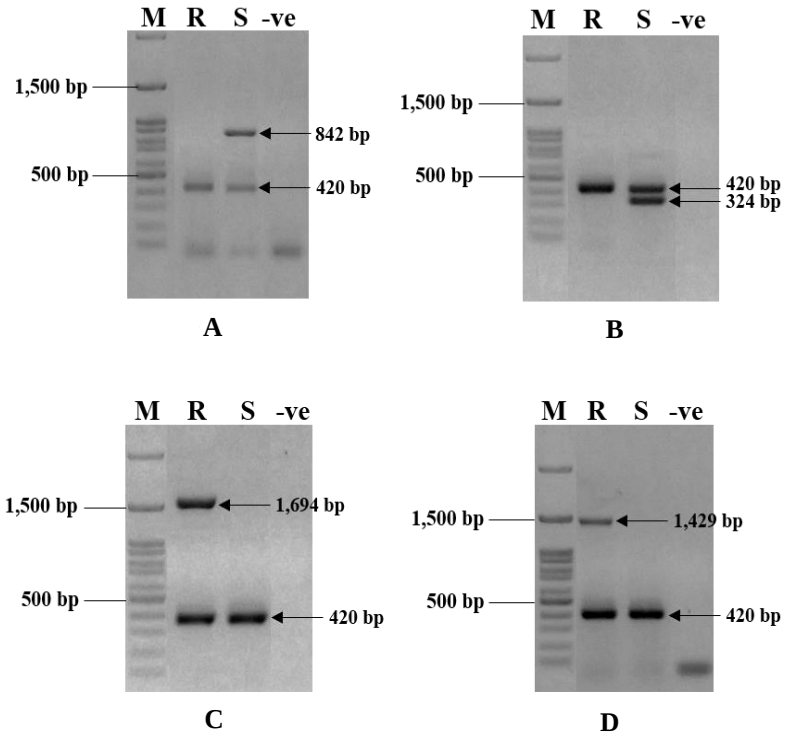

Figure 1. Detection of four SCAR primers linked to *Fusarium* wilt resistance in Thai banana genotypes: A. SCAR_1; B. SCAR_2; C. SCAR_3; D. SCAR_4. Results are shown for two genotypes: 'Kluai Khai Kasetsart 2' (AA) (23) and 'Kluai Sa' (AA) (31). The *Actine1* primer pair was used as a positive control (420 bp). M: 100 bp DNA ladder (OneMark 100 RTU, Bio-Helix); R: resistance loci; S: susceptibility loci; -ve: negative control

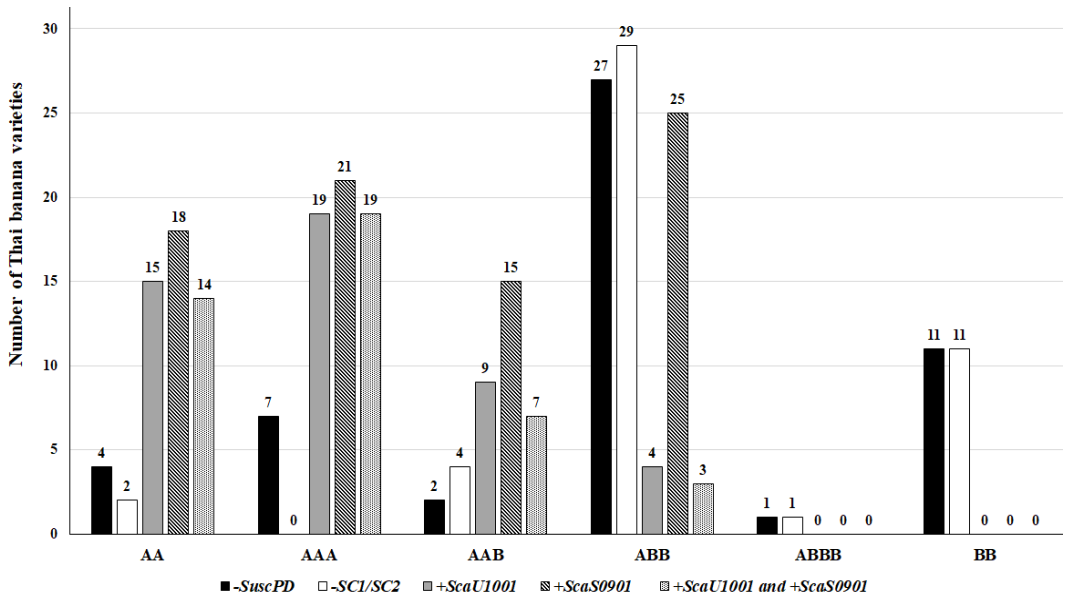

Figure 2. Frequency distribution of six *Musa* genomes (AA, AAA, AAB, ABB, ABBB, and BB) in Thai banana germplasm, analyzed using four SCAR primers. The analysis identifies the absence of *Fusarium* wilt susceptibility loci (-*SuscPD*, -*SC1/SC2*) and the presence of resistance loci (+*ScaU1001*, +*ScaS0901*). The bars represent the presence of FW resistance loci, with black, white, grey, diagonal stripe, and dotted patterns indicating -*SuscPD*, -*SC1/SC2*, +*ScaU1001*, +*ScaS0901*, and a combination of +*ScaU1001* and +*ScaS0901*, respectively

Table 3. Results of the genetic analysis of 106 Thai banana genotypes, showing the presence or absence of *Fusarium* wilt susceptibility or resistance loci as determined using four validated SCAR markers

Sample no.	Detection of FW resistance loci by PCR-based DNA markers					Total R loci
	Control	*Foc*1	*Foc*TR4			
	Actin1	*SuscPD* (S)	*SC1/SC2* (S)	*ScaU1001* (R)	*ScaS0901* (R)	
***M. acuminata* (AAA genome)**						
1	+	+	+	+	+	2
2	+	-	+	+	+	3
3	+	-	+	+	+	3
4	+	+	+	+	+	2
5	+	+	+	+	+	2
6	+	+	+	+	+	2
7	+	+	+	+	+	2
8	+	+	+	-	+	1
9	+	-	+	+	+	3
10	+	-	+	+	+	3
11	+	-	+	+	+	3
12	+	+	+	+	+	2
13	+	+	+	-	-	0
14	+	+	+	+	+	2
15	+	+	+	-	+	1
16	+	-	+	+	+	3
17	+	+	+	+	+	2
18	+	+	+	+	+	2
19	+	+	+	+	+	2
20	+	-	+	+	+	3
21	+	+	+	+	+	2
22	+	+	+	+	+	2
***M. acuminata* (AA genome)**						
23	+	+	+	+	+	2
24	+	+	+	+	+	2
25	+	-	+	+	+	3
26	+	+	+	+	+	2
27	+	+	+	+	+	2
28	+	+	+	+	+	2
29	+	+	+	+	+	2
30	+	+	+	+	+	2
31	+	-	-	-	-	2
32	+	+	+	-	+	1
33	+	+	+	-	+	1
34	+	-	+	-	+	2
35	+	+	+	+	+	2
36	+	+	+	+	+	2
37	+	+	+	+	+	2
38	+	+	+	+	+	2
39	+	-	+	+	-	2
40	+	+	+	+	+	2
41	+	+	-	-	+	2
42	+	+	+	+	+	2
***Musa x paradisiaca* (AAB genome)**						
43	+	+	+	+	+	2
44	+	+	+	-	+	1
45	+	+	+	+	+	2
46	+	+	+	+	+	2
47	+	+	-	-	+	2
48	+	+	+	+	+	2
49	+	+	+	+	+	2
50	+	+	+	-	+	1
51	+	+	+	-	+	1
52	+	+	-	-	+	2
53	+	-	-	+	-	3
54	+	-	-	-	+	3
55	+	+	+	+	+	2
56	+	+	+	-	+	1
57	+	+	+	-	+	1
58	+	+	+	+	+	2
59	+	+	+	+	-	1

***Musa x paradisiaca* (ABB genome)**						
60	+	+	+	+	+	2
61	+	+	-	-	+	2
62	+	-	-	-	+	3
63	+	-	-	-	+	3
64	+	-	-	-	+	3
65	+	-	-	-	+	3
66	+	-	-	-	+	3
67	+	-	-	-	+	3
68	+	-	-	-	+	3
69	+	-	-	-	+	3
70	+	-	-	-	+	3
71	+	-	-	-	+	3
72	+	+	+	-	+	1
73	+	+	-	-	+	2
74	+	-	-	-	-	2
75	+	-	-	-	-	2
76	+	-	-	-	-	2
77	+	-	-	-	-	2
78	+	+	+	+	+	2
79	+	-	-	-	-	2
80	+	-	-	-	+	3
81	+	-	-	-	+	3
82	+	-	-	-	+	3
83	+	-	-	-	-	2
84	+	-	-	-	-	2
85	+	-	-	-	-	2
86	+	-	-	-	-	2
87	+	+	-	-	+	2
88	+	-	-	+	-	3
89	+	+	+	+	+	2
90	+	-	-	-	+	3
91	+	-	-	-	+	3
92	+	-	-	-	+	3
93	+	+	+	-	+	1
94	+	-	+	-	+	2
***Musa x paradisiaca* (ABBB genome)**						
95	+	-	-	-	-	2
***M. balbisiana* (BB genome)**						
96	+	-	-	-	-	2
97	+	-	-	-	-	2
98	+	-	-	-	-	2
99	+	-	-	-	-	2
100	+	-	-	-	-	2
101	+	-	-	-	-	2
102	+	-	-	-	-	2
103	+	-	-	-	-	2
104	+	-	-	-	-	2
105	+	-	-	-	-	2
106	+	-	-	-	-	2
R loci frequency (%)		**52 (49.1)**	**47 (44.3)**	**47 (44.3)**	**79 (74.5)**	

Note: The amplicon is either present or absent, indicated by the characters ' + ' and ' - '; *Fusarium* wilt resistance (R) loci are identified in shaded matrices, while susceptibility (S) loci are located in unshaded matrices

In addition, based on this genetic information, four major clusters were identified among the 106 banana genotypes associated with the *Foc*TR4 resistance loci (Figure 3.B). The two reference genotypes, 'Grande Naine' (AAA) (K1) and 'Williams' (AAA) (K2), were grouped in Cluster I-A. Cluster I consisted of 59 banana genotypes. Subcluster I-A contained 43 genotypes (19 AAA, 14 AA, 7 AAB, and 3 ABB) carrying the *+ScaU1001* and *+ScaS0901* *Foc*TR4 resistance loci. Subcluster I-B included 2 genotypes (1 AA and 1 AAB) that carried one *Foc*TR4 resistance locus (*+ScaU1001*). Subcluster I-C comprised 13 genotypes (2 AAA, 3 AA, 5 AAB, and 3 ABB) containing another *Foc*TR4 resistance locus (*+ScaS0901*). One specific genotype, 'Kluai Khieo Pakchong' (AAA) (K13), in Subcluster I-D was found to lack the *Foc*TR4 resistance gene. Cluster II consisted of 23 banana genotypes with two *Foc*TR4 resistance loci (*-SC1/SC2* and *+ScaS0901*). Cluster III comprised 22 genotypes with only one *Foc*TR4 resistance locus (*-SC1/SC2*). In Cluster IV, two banana genotypes, 'Kluai Sam Duan Phichit' (AAB) (K53) and 'Kluai Tip 1'

(ABB) (K88), were found to possess two *Foc*TR4 resistance loci *(-SC1/SC2* and *+ScaU1001*).

Moreover, a cluster analysis was performed on 106 Thai banana genotypes associated with resistance to *Foc*R1 and *Foc*TR4, as illustrated in Figure 3.C. These genotypes were grouped into four primary clusters: I-IV. Cluster I, consisting of 58 banana genotypes, was further subdivided into six subclusters (A-F) based on the presence of the *Foc*R1 susceptibility locus (*+SuscPD*) and/or the *Foc*TR4 resistance loci (*+ScaU1001* and *+ScaS0901*). Cluster II consisted of 3 genotypes, each possessing 2-3 *Foc* resistance loci (*-SuscPD*, *+ScaU1001*, and/or *-SC1/SC2*). Cluster III was divided into two subclusters: subcluster III-A, which comprised 17 genotypes with three *Foc* resistance loci (*-SuscPD*, *-SC1/SC2*, and *+ScaS0901*), and subcluster III-B, which included 6 genotypes with two *Foc*TR4 resistance loci (*-SC1/SC2* and *+ScaS0901*). In Cluster IV, *Foc* resistance loci (*-SuscPD* and *-SC1/SC2*) were identified in 22 Thai banana genotypes.

Discussion

The primary focus of banana breeding efforts has been the development of cultivars resistant to *Fusarium* wilt, particularly against the harmful race 1 (*Foc*R1) and tropical race 4 (*Foc*TR4) strains of *Fusarium oxysporum* f. sp. *cubense* (*Foc*). However, banana genetic improvement is a challenging and time-consuming process due to the unique biology of banana plants and specific obstacles such as lengthy and arduous field evaluations, low genetic variation within genomic groups, long maturation periods, and vegetative reproduction via tissue culture or suckers. Hence, it becomes essential to identify resistant banana genotypes early in the development process using molecular methods. This enables the selection of banana plants with pathogen-resistant genes and facilitates the incorporation of these disease-resistant genes or traits into cultivated banana genotypes. To address *Fusarium* wilt, scientists have identified and studied several resistance genes and strategies. Two SCAR markers, SuscPD and SC1/SC2, exhibit high precision in determining whether banana genotypes are sensitive to either *Foc*R1 or *Foc*TR4, with discrimination power of 93% and 96.88%, respectively (Cunha et al. 2015; Wang et al. 2018). Likewise, two other SCAR markers, ScaU1001 and ScaS0901, have shown efficiency in identifying banana genotypes harboring *Foc*-resistance genes and demonstrating resistance to *Foc*TR4 (Wang et al. 2012).

This study investigated the genotypes of Thai bananas across six *Musa* genomes (AA, AAA, AAB, ABB, ABBB, and BB) to identify *Fusarium* wilt resistance loci using these four established SCAR markers. The results revealed that all genotypes of Thai bananas examined in this study possess at least one *Fusarium* wilt resistance locus, except for *Musa* (AAA) 'Kluai Khieo Pakchong'. In the genotyping of banana samples, the results indicated that the amplified SCAR_1 and SCAR_2 markers produced unique PCR products approximately 842 and 324 bp in length, respectively. These findings corroborated previous research, demonstrating that the SCAR_1 marker failed to amplify in banana genotypes resistant to *Foc*R1 ('Williams'), while the SCAR_2 marker successfully amplified in banana genotypes sensitive to *Foc*TR4 ('Grande Naine' and 'Williams'). Out of the 106 genotypes of Thai bananas, 54 genotypes (50.9%) corresponding to the chromosomal groups AA, AAA, AAB, and ABB were susceptible to *Foc*R1, while 59 genotypes (55.7%) corresponding to the chromosomal groups AA, AAA, AAB, and ABB were susceptible to *Foc*TR4. The predicted PCR product (842 bp) was obtained by identifying 20 out of the 28 genotypes of bananas in Brazil that were *Foc*R1-susceptible using the SuscPD marker (Cunha et al. 2015). Additionally, a 324 bp fragment was amplified from the *Foc*TR4-susceptible gene in the banana mitochondrial genome, located near the putative CcmB-like mitochondrial protein involved in cytochrome c production (Wang et al. 2018). Interestingly, no amplification was observed for any of the SCAR markers in 22 banana genotypes, particularly those with a predominant B genome (ABB, ABBB, BB groups). This absence suggests a potential gap in resistance within these groups, making them more susceptible to FW infection.

After amplifying two additional markers, SCAR_3 and SCAR_4, in 106 Thai banana genotypes, a distinct PCR product of approximately 1694 bp and 1429 bp was observed in 47 (44.3%) and 79 (74.5%) banana genotypes, respectively, belonging to the AA, AAA, AAB, and ABB groups. Our findings revealed that two reference banana genotypes, 'Grande Naine' and 'Williams', exhibited distinct PCR products resulting from the amplification of the SCAR_3 and SCAR_4 markers. This suggests that these genotypes could be resistant to *Foc*TR4. In contrast to our results, previous studies found that the ScaU1001 (SCAR_3) and ScaS0901 (SCAR_4) markers were unable to amplify in the *Musa* AAA group, which comprises three subgroups: 'Gros Michel', 'Williams 8818', and 'Grande Naine', all susceptible to *Foc*TR4 (Wang et al. 2012; Silva et al. 2016). It is possible that somaclonal modification occurred in the 'Grande Naine' genotype used in this investigation. The ScaU1001 and ScaS0901 primers were deposited into the GenBank database with the accession numbers HQ613949 and HQ613950, respectively (Wang et al. 2012). Silva et al. (2016) reported that out of 276 banana genotypes, the ScaU1001 marker yielded the expected PCR product from 101 *Foc*TR4-resistant genotypes with significantly greater accuracy and effectiveness compared to the ScaS0901 marker. However, in this investigation, *Musa* cv. 'Kluai Hom Thong' (AAA), belonging to the Gros Michel subgroup, yielded PCR products for both SCAR_3 and SCAR_4 markers. The inability to replicate and the generation of negative results raised the possibility that the DNA fragment is not fully linked to the target gene. According to Sanyong et al. (2020), the SCAR markers (ScaU1001 and ScaS0901) linked to *Foc*TR4 resistance were employed to assess *Fusarium* wilt resistance among 23 Thai banana cultivars. The findings revealed that both SCAR markers yielded a unique PCR product in two banana cultivars, 'Kluai Khai Kasetsart 2' (AA) and 'Kluai Hom Khiew' (AAA), suggesting that both cultivars likely harbored a *Foc*-resistant DNA fragment. Furthermore, this determination was subsequently confirmed through a *Foc* inoculation experiment. The results for the banana cultivar

'Kluai Hom Khieo' (AAA), which belongs to the *Musa* Cavendish subgroup, were consistent with previous studies, demonstrating that both markers (ScaU1001 and ScaS0901) could be amplified in banana genotypes resistant to *Foc*TR4, such as the Cavendish cv. Williams 8818-1 (Wang et al. 2012; Silva et al. 2016; Sanyong et al. 2020).

Molecular marker-assisted selection (MAS) is widely employed in agriculture to enable the early selection of plants with desirable traits, expediting the breeding process. MAS uses DNA markers linked to particular traits, making it accurate, quick, and dependable in any environment. Molecular methods have been used in numerous research studies conducted on *Musa* to investigate the genetic diversity of *M. acuminata*, *M. balbisiana*, and their polyploid hybrids. Genetic markers such as RAPD, ISSR, and SRAP are often hindered by issues with validation, lack of sequence information, and reproducibility. In contrast, sequence-specific markers such as SCAR markers are preferred for their superior specificity and dependability. Because of their longer primer lengths and higher annealing temperatures, SCAR primers produce more focused, stable, and well-defined bands in the reaction (Kiran et al. 2010; Joshi and Chavan 2012; Yang et al. 2019; Zheng et al. 2021).

SCAR markers have been employed in genetic studies to identify and track specific traits or alleles in various organisms, including resistance genes in wheat, banana, apple, rice, tomato, and several other crops (Das et al. 2006; Nwauzoma et al. 2011; Karapetsi et al. 2020; Quoc et al. 2021; Zhang and Panthree 2021). Recently, two more reliable SCAR markers developed from SRAP markers demonstrated 95-100% discriminating power across 30 banana genotypes belonging to *Musa* spp. AAA Cavendish with variable degrees of *Foc* resistance (Qv et al. 2024). In this study, Thai banana genotypes showed significant genetic variation in *Fusarium* wilt resistance within and between the *Musa* genomic groups. This variation may arise from the abundance of resistance genes present in the A *Musa* genomic group, indicating that different genes responsible for *Fusarium* wilt resistance impart varying degrees of resistance.

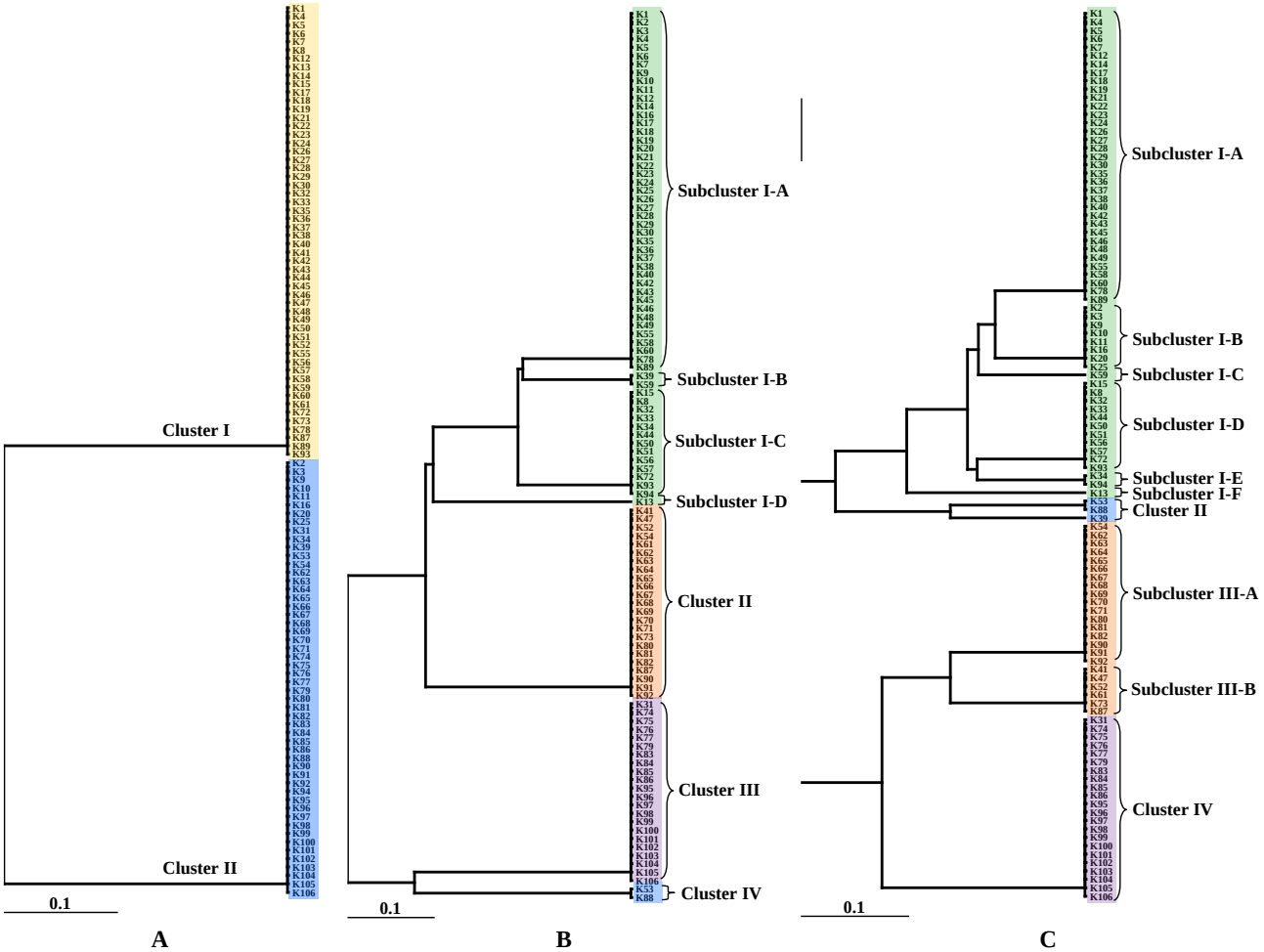

Figure 3. Based on the Jaccard coefficient of similarity, cluster analysis revealed that all 106 Thai banana genotypes could be divided into: A. Two major clusters resistant to *Foc*R1 infection; B. Four significant clusters resistant to *Foc*TR4 infection; C. Four major clusters resistant to both *Foc*R1 and *Foc*TR4 infections

Regarding the *Musa* genome in this study, Tomaszewska (2021) mentioned that there is no BBB genomic type in the *Musa* genome. Additionally, 'Kluai Theparod' was classified as an ABB type rather than ABBB. Interestingly, none of the banana genotypes belonging to the *M. balbisiana* (BB genomes) exhibited the targeted PCR products amplified by these four SCAR markers. This suggests that these SCAR markers were specifically developed using DNA sequences identified in the *Musa* A genome. Consistent with Zuo et al. (2018), the resistance characteristics may be associated with several important resistance genes located in the genome of *M. acuminata*. Arinaitwe et al. (2019) reported that banana resistance to *Foc*R1 is epistatically regulated by at least two dominant genes interacting, with limited inheritance of *Foc*R1 resistance in *Musa* spp. It was discovered that somaclonal of banana cv. 'Rasthali' (AAB, Silk subgroup) have downregulated lipoxygenase genes and enhanced resistance to *Foc*R1 infection (Ghag et al. 2014). On the other hand, Larter (1947) observed that the genotype of the *Foc*R1-resistant banana was determined by a single dominant gene. Therefore, further evaluations of the banana genotypes carrying resistant loci for *Foc*R1 and/or *Foc*TR4 are necessary to determine their resistance against different races of *Foc* infection in a greenhouse environment.

In plant breeding and crop improvement programs, it is crucial to screen for plants resistant to *Fusarium* wilt, particularly for crops like bananas that are highly susceptible. Currently, there is limited information available about banana cultivars resistant to *Foc*R1 and *Foc*TR4. Numerous studies have focused on crossbreeding and assessing the genetic inheritance of *Musa* spp. to obtain resistant hybrids. For instance, in Uganda, two *Musa* species, Monyet (*Musa acuminata* AAAA, subsp. *zebrina*, resistant to *Foc*R1) and Kokopo (*Musa acuminata* AA, subsp. *banksii*, susceptible), were crossed to develop a segregating banana population resistant to *Foc*R1 (Arinaitwe et al. 2019). Despite 'Gros Michel's' susceptibility to *Fusarium* wilt, 'Hom Thong Mokho' (AAA, Gros Michel subgroup) exhibited high resistance to *Foc*R1 in the study by Smith et al. (2018). Rebouças et al. (2018) identified banana genotypes resistant to *Foc*R1 through field and greenhouse experiments, observing that *Musa* genotypes with an ABB genome exhibited moderate resistance, whereas those with an AAB genome were found to be susceptible. Furthermore, datasets have shown that a significant portion of the *Musa* AAB genome, such as 'Pome', 'Silk', and 'Mysore', was highly susceptible to *Foc*TR4 (Huang et al. 2005; Walduck and Daly 2007; Molina et al. 2010). Several studies have suggested that employing innovative proteomic and genetic engineering techniques could enhance the potential for developing disease-resistant plants (Ramu et al. 2016; Rocha et al. 2021). Future research should focus on exploring interactions between banana genotypes and *Foc* strains. Insights gained from utilizing these polymorphic SCAR markers will be invaluable for selecting genotypes for field assessment in genetic improvement programs for Thai bananas.

In this study, cluster analysis grouped the 106 Thai banana genotypes into distinct clusters based on their resistance to *Foc*R1 and *Foc*TR4. Specifically, two major clusters were identified for *Foc*R1 resistance, while four significant clusters were associated with *Foc*TR4 resistance. This grouping highlights the broad genetic diversity among the banana genotypes concerning their resistance profiles. The presence of these distinct clusters underscores the variability in resistance mechanisms, likely driven by different loci within the *Musa* genomes. Notably, the reference genotypes 'Grande Naine' and 'Williams' were placed in different clusters, suggesting distinct resistance mechanisms or loci within the AAA genome group.

In conclusion, this study screened Thai banana genotypes across six genomes (AA, AAA, AAB, ABB, ABBB, and BB) to identify *Fusarium* wilt-resistant alleles using four validated SCAR markers. The analysis revealed significant genetic variation related to *Fusarium* wilt resistance, demonstrating that SCAR markers, with their longer primer lengths and higher annealing temperatures, are well-suited for genetic studies due to their reliability and reduced sensitivity to reaction conditions. Notably, no amplification was observed for any of the SCAR markers in 22 banana genotypes, particularly those with a predominant B genome (ABB, ABBB, BB groups), suggesting a potential gap in resistance within these groups. These findings provide a comprehensive understanding of the distribution and diversity of *Fusarium* wilt resistance loci in Thai banana genotypes and highlight the potential of these markers in breeding programs aimed at developing resistant genotypes. Further research is recommended to characterize these loci and assess their effectiveness in different banana-growing regions, contributing to the ongoing effort to combat *Fusarium* wilt.

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