

Communication

Toward Marker-Assisted Selection in Breeding for *Fusarium* Wilt Tropical Race-4 Type Resistant Bananas

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Abstract: *Fusarium* wilt is a soil borne fungal disease that has devastated banana production in plantations around the world. Most Cavendish-type bananas are susceptible to strains of *Fusarium oxysporum* f. sp. *cubense* (Foc) belonging to the Subtropical Race 4 (STR4) and Tropical Race 4 (TR4). The wild banana diploid *Musa acuminata* ssp. *malaccensis* (AA, 2n = 22) carries resistance to Foc TR4. A previous study using segregating populations derived from *M. acuminata* ssp. *malaccensis* identified a quantitative trait locus (QTL) (12.9 cM) on the distal part of the long arm of chromosome 3, conferring resistance to both Foc TR4 and STR4. An SNP marker, based on the gene Macma4_03_g32560 of the reference genome ‘DH-Pahang’ v4, detected the segregation of resistance to Foc STR4 and TR4 at this locus. Using this marker, we assessed putative TR4 resistance sources in 123 accessions from the breeding program in Brazil, which houses one of the largest germplasm collections of *Musa* spp. in the world. The resistance marker allele was detected in a number of accessions, including improved diploids and commercial cultivars. Sequencing further confirmed the identity of the SNP at this locus. Results from the marker screening will assist in developing strategies for pre-breeding Foc TR4-resistant bananas. This study represents the first-ever report of marker-assisted screening in a comprehensive collection of banana accessions in South America. Accessions carrying the resistance marker allele will be validated in the field to confirm Foc TR4 resistance.

Keywords: breeding; cleaved amplified polymorphic sequences markers; *Fusarium oxysporum* sp. *cubense*; *Musa acuminata* ssp. *malaccensis*



Citation: Ferreira, C.F.; Chen, A.; Aitken, E.A.B.; Swennen, R.; Uwimana, B.; Rocha, A.d.J.; Soares, J.M.d.S.; Ramos, A.P.d.S.; Amorim, E.P. Toward Marker-Assisted Selection in Breeding for *Fusarium* Wilt Tropical Race-4 Type Resistant Bananas. *J. Fungi* **2024**, *10*, 839. <https://doi.org/10.3390/jof10120839>

Academic Editor: Zonghua Wang

Received: 22 October 2024

Revised: 28 November 2024

Accepted: 30 November 2024

Published: 4 December 2024



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1. Introduction

Bananas are among the leading fruits that provide economic sustainability for both small- and large-scale growers, with a worldwide trade value surpassing USD 30 billion per annum [1]. However, banana production has been severely affected by major pests and diseases caused by viruses, bacteria, and fungi. Amongst the fungal diseases affecting bananas, *Fusarium* wilt, caused by *Fusarium oxysporum* f. sp. *cubense* (Foc), is the most economically important.

Fusarium wilt has been an issue in banana monoculture plantation production systems since the 1900s. However, it is the virulent Foc strain known as Tropical Race 4 (TR4) that

threatens commercial banana production dominated by Cavendish-type clones, as well as a broad range of other banana varieties [2–4]. TR4 was first reported in the regions of southeast Asia and Australia in the 1990s [5]. In recent years, it has made its way into other parts of the world, such as Latin America, reaching the northern region of La Guarija, Colombia, in 2019 [3], the Querecotillo district of Sullana province in the Piura department of Peru in 2021 [2], and more recently, Venezuela [6]. These countries could provide proxies in the potential spread of *Foc* TR4 across the border to neighboring countries. Thus, pre-emptive breeding to contain and stop the spread of the disease is of utmost importance. Latin America and the Caribbean, which is the world's largest banana exporting region, is thus endangered [7]. Understanding the epidemiology of *Fusarium* wilt in bananas is critical for developing control measures, breeding resistant varieties, and formulating effective quarantine strategies to prevent further spread of *Foc* TR4 in banana-producing regions of the world [8,9]. *Foc* TR4 remains a critical threat to banana plantations worldwide, partly due to the lack of effective control measures and limited sources of *Fusarium* wilt resistance within *Musa* spp. [8,10,11].

Disease symptoms are manifested as vascular discoloration in the corm, while the disease is visually identified on plants displaying leaf yellowing and vascular-induced wilt. *Foc*, a soilborne pathogen, enters the host plant through the roots and moves through the plant's vascular system to colonize the whole plant. Its colonization and proliferation in the xylem vessels block water and nutrient supplies to the aerial parts of the plant, leading to wilting of leaves and eventual plant death [8,10]. Internally, *Foc*-infected corms and pseudo-stems show a reddish-brown discoloration. So far, there are no effective means to control the spread of this disease. *Foc* can survive in the soil for decades as chlamydospores and cannot be completely eradicated [12]. Therefore, the use of resistant cultivars not only offers the most sustainable and environmentally friendly means of controlling *Foc* but is perhaps one of the few viable options [13]. This highlights the importance of identifying resistance in wild relatives of cultivated bananas and introducing resistance alleles into commercial cultivars to enhance their resilience against diseases [4,9].

Embrapa Mandioca e Fruticultura (Embrapa Cassava and Fruits, <https://www.embrapa.br/mandioca-e-fruticultura>, accessed on 21 October 2024) conducts research to improve crop productivity and has an active program for the genetic improvement in bananas since 1976 [14]. It maintains one of the largest banana germplasm collections in South America. This collection includes accessions gathered from various regions worldwide, established through comprehensive collecting expeditions. These expeditions were conducted in the banana center of origin in Southeast Asia and further extended to include accessions from Africa, Central America, and South America. This germplasm collection now has over 400 accessions, aimed at preserving genetic diversity and developing new cultivars suited to both local and global agricultural needs.

Molecular markers based on Single Nucleotide Polymorphisms (SNPs) are used successfully in many crops in the identification of a plant's resistance to diseases [15–18]. Previous studies show that lines belonging to the wild diploid banana *Musa acuminata* subsp. *malaccensis* (Ma) (AA, $2n = 22$) carry resistance to *Foc* TR4 and STR4 strains [8,10,17,18].

Recently, a detailed study of two *Musa acuminata* ssp. *malaccensis* populations segregating for *Foc* TR4 and STR4 resistance was carried out [18]. Marker loci and trait association using 11 SNP-based PCR markers allowed the candidate region conferring *Foc* STR4/TR4 resistance to be delimited to a 12.9 cM genetic interval corresponding to a 959 kb region on chromosome 3 of the 'DH-Pahang' reference assembly v4 [18].

Within this region, multiple pattern recognition receptors, such as leucine-rich repeats containing receptor-like protein kinases, cysteine-rich cell wall-associated protein kinases, and other genes related to resistance, were identified. To confirm the segregation of single-gene resistance, an inter-cross between the resistant parent 'Ma850' and a susceptible line 'Ma848' was generated [18]. An informative SNP marker, 29730, based on a single nucleotide change (T544C) in the first intron of a Nuclear transcription factor Y subunit (Macma4_03_g32560, 'DH-Pahang' assembly v4.3) was reported as associated with *Foc* TR4

resistance [18] and allowed the resistance locus to be assessed in a collection of diploid and polyploid banana accessions at Embrapa. The results provide crucial insights into *Foc* TR4 resistance sources within Embrapa's banana breeding program, supporting the design of a breeding pipeline geared toward the development of *Foc* TR4-resistant bananas and as such, mitigate TR4-caused yield losses once TR4 arrives in Brazil. These findings will undoubtedly contribute to the global initiatives aimed at developing *Foc* TR4-resistant banana varieties.

2. Materials and Methods

M. acuminata-specific primers for the 29730 marker were developed previously, 29730-A-SNP1-F2 5'-GCAATGAGTACCTCTAAGCA-3' and 29730-A-SNP1-R2 5'-TAAGTTCTAG TATCAAGTACAA-3', and used to amplify an A-genome specific product of 795 bp. This product was then digested with BcoDI to produce the bi-allelic forms, an undigested dominant band that is putatively associated with resistance, and digested products of 429 bp and 366 bp, linked to susceptibility [18]. Then, 123 accessions from the Embrapa banana germplasm collection (Table 1) were genotyped with this SNP marker, 29730-A-SNP1-F2 and 29730-A-SNP1-R2.

Table 1. Sample number, accession name and number (Embrapa/ITC), type, genealogy, BcoDI digest, and allele nucleotide of 123 Embrapa accessions genotyped with SNP markers 29730-A-SNP1-F2 and 29730-A-SNP1-R2.

Sample Number	Accession Name	Accession Number	Type	Pedigree	Marker-Allele Defined by BcoDI Digest	SNP and Allele Zygosity	Predicted <i>Foc</i> TR4 Responses
1	042023-03	042023-03	Improved diploid (AA)	M53 × Cultivar sem nome N° 2	B	C/C	Sus
2	091087-02	091087-02	Improved diploid (AA)	001016–01 (Borneo × Guyod) × 003038–01 (Calcutta 4 × Heva)	B	C/C	Sus
3	SH3362 ^R	-	Improved diploid (AA)	-	H	T/T	Res
4	003004-02	003004-02	Improved diploid (AA)	Calcutta 4 × Madang	B	C/C	Sus
5	013019-01 ^R	013019-01	Improved diploid (AA)	Malaccensis × Tjau Lagada	H	T/C	Res
6	041054-04	041054-04	Improved diploid (AA)	003004-01 (Calcutta 4 × Madang) × 001004-01 (Borneo × Madang)	B	-	Sus
7	050012-02 ^{HR}	050012-02	Improved diploid (AA)	M61 × Lidi	B	-	Sus
8	086094-15	086094-15	Improved diploid (AA)	03037-02 (Calcutta 4 × Galeo) × SH3263	H	T/C	Res
9	042015-02	042015-02	Improved diploid (AA)	M53 × Madu	B	C/C	Sus
10	SH3263 ^R	-	Improved diploid (AA)	-	A	T/T	Res
11	042085-02	042085-02	Improved diploid (AA)	M53 × 015003-01 (Madu × Calcutta 4)	B	-	Sus
12	042079-06	042079-06	Improved diploid (AA)	M53 × 028003-01 (Tuu Gia × Calcutta 4)	B	C/C	Sus
13	017041-01	017041-01	Improved diploid (AA)	Jari Buaya × 003004–01 (Calcutta 4 × Madang)	B	C/C	Sus
14	042052-03	042052-03	Improved diploid (AA)	M53 × Kumburgh	B	-	Sus
15	042049-04	042049-04	Improved diploid (AA)	M53 × M48	B	C/C	Sus
16	003023-03	003023-03	Improved diploid (AA)	Calcutta 4 × S/N° 2	B	T/C	Res
17	086094-20	086094-20	Improved diploid (AA)	03037-02 (Calcutta 4 × Galeo) × SH3263	H	C/C	Sus
18	001016-01 ^{HR}	001016-01	Improved diploid (AA)	Borneo × Guyod	H	C/C	Sus
19	091094-04	091094-04	Improved diploid (AA)	001016–01 (Borneo × Guyod) × SH3263	B	C/C	Sus

Table 1. Cont.

Sample Number	Accession Name	Accession Number	Type	Pedigree	Marker-Allele Defined by BcoDI Digest	SNP and Allele Zygosity	Predicted <i>Foc</i> TR4 Responses
20	TH-0301	TH-0301	Improved diploid (AA)	Terrinha × Calcutta 4	B	-	Sus
21	041054-08	041054-08	Improved diploid (AA)	003004-01 (Calcutta 4 × Madang) × 001004-01 (Borneo × Madang)	B	-	Sus
22	058054-03	058054-03	Improved diploid (AA)	003005-01 (Calcutta 4 × Pahang) × 001004-01 (Borneo × Madang)	B	-	Sus
23	089087-01	089087-01	Improved diploid (AA)	013018-01 (Malaccensis × Sinwobogi) × 003038-01 (Calcutta 4 × Heva)	B	-	Sus
24	003037-02	003037-02	Improved diploid (AA)	Calcutta 4 × Galeo	B	C/C	Sus
25	086079-09	086079-09	Improved diploid (AA)	003037-02 (Calcutta 4 × Galeo) × 028003-01 (Tuu Gia × Calcutta 4)	B	-	Sus
26	073041-01	073041-01	Improved diploid (AA)	Khai × 003004-01 (Calcutta 4 × Madang)	B	-	Sus
27	013004-04	013004-04	Improved diploid (AA)	Malaccensis × Madang	H	C/C	Sus
28	013018-01	013018-01	Improved diploid (AA)	Malaccensis × Sinwobogi	B	-	Sus
29	028003-01	028003-01	Improved diploid (AA)	Tuu Gia × Calcutta 4	B	-	Sus
30	091079-03	091079-03	Improved diploid (AA)	01016-01 (Borneo × Guyod) × 028003 (Tuu Gia × Calcutta 4)	B	C/C	Sus
31	Tropical	-	Silk hybrid (AAAB)	Yangambi no.2 × M53	B	-	Sus
32	Belluna	-	Cultivar (AAA)	-	B	-	Sus
33	Caipira	-	Cultivar (AAA)	-	H	T/C	Res
34	Prata Graúda	-	Prata hybrid (AAB)	SH3642	H	T/C	Res
35	Thap-Maeo	ITC1301	Cultivar (AAB)		B	-	Sus
36	Prata-Anã	ITC0962	Cultivar (AAB)		B	-	Sus
37	BRS Vitória	-	Prata hybrid (AAAB)	Pacovan × M53	B	-	Sus
38	BRS Preciosa	-	Prata hybrid (AAAB)	Pacovan × M53	B	C/C	Sus
39	BRS Japira	-	Prata hybrid (AAAB)	Pacovan × M53	B	-	Sus
40	BRS Garantida	-	Prata hybrid (AAAB)	Pacovan × M53	H	C/C	Sus
41	BRS Pacovan-Ken	-	Prata hybrid (AAAB)	Pacovan × M53	B	-	Sus
42	Pacovan	-	Prata Type (AAB)	Prata Type triploid	B	-	Sus
43	Platina	ITC0262	Prata hybrid (AAAB)	Prata Anã × M53	B	-	Sus
44	BRS Caprichosa	-	Prata hybrid (AAAB)	Prata Comum × M53	B	-	Sus
45	BRS Pioneira	-	Prata hybrid (AAAB)	Prata São Tomé × M53	B	-	Sus
46	BRS Princesa	-	Silk Hybrid (AAAB)	Yangambi no.2 × M53	B	-	Sus
47	Pelipita	ITC0095	Cultivar (ABB)	-	B	-	Sus
48	Mongolo	-	Plantain (AAB)	-	B	-	Sus
49	Red Yade	ITC1140	Plantain (AAB)	-	B	-	Sus
50	Comprida	-	Plantain (AAB)	-	B	-	Sus
51	Tipo Velhaca	-	Plantain (AAB)	-	B	-	Sus
52	Terra Ponta	-	Plantain (AAB)	-	B	-	Sus
53	Aparada	-	Plantain (AAB)	-	B	-	Sus
54	Pinha	-	Plantain (AAB)	-	B	-	Sus
	Samura B	-	Plantain (AAB)	-	B	C/C	Sus

Table 1. Cont.

Sample Number	Accession Name	Accession Number	Type	Pedigree	Marker-Allele Defined by BcoDI Digest	SNP and Allele Zygosity	Predicted <i>Foc</i> TR4 Responses
55	Trois Vert	ITC1127	Plantain (AAB)	-	B	-	Sus
56	Terra Sem Nome	-	Plantain (AAB)	-	B	-	Sus
57	FHIA 21	ITC1306	Hybrid (AAAB)	-	B	C/C	Sus
58	Terrinha	-	Plantain (AAB)	-	B	C/C	Sus
59	Njock Kon	ITC1133	Plantain (AAB)	-	B	-	Sus
60	Curare Enano	ITC0559	Plantain (AAB)	-	B	C/C	Sus
61	Chifre De Vaca	-	Plantain (AAB)	-	B	-	Sus
62	Terra Maranhão	-	Plantain (AAB)	-	B	-	Sus
63	D'Angola	-	Plantain (AAB)	-	B	-	Sus
64	CNPMF 0557 ^R	111090-07	Improved diploid (AA)	[(M61 × 'Pisang Lilin')] × [(Malaccensis × Tjau Lagada)]	H	-	Res
65	CNPMF 0496 ^R	111040-03	Improved diploid (AA)	[(M61 × 'Pisang Lilin')] × [(Terrinha × Calcutta 4)]	H	-	Res
66	CNPMF 0513 ^R	111102-01	Improved diploid (AA)	[(M61 × 'Pisang Lilin')] × [(M53 × Kumburgh)	B	-	Sus
67	CNPMF 0519	116116-01	Improved diploid (AA)	Self-fertilization (wild diploid Tambi)	B	-	Sus
68	CNPMF 0536 ^{HR}	9041090-02	Improved diploid (AA)	[(Calcutta 4 × Madang)] × [(Borneo × Guyod)]	H	T/C	Res
69	CNPMF 0534	041090-20	Improved diploid (AA)	[(Calcutta 4 × Madang)] × [(Borneo × Guyod)]	B	C/C	Sus
70	CNPMF 0542 ^R	094089-01	Improved diploid (AA)	[(SH3263)] × [(Malaccensis × Sinwobogi)]	H	T/C	Res
71	CNPMF 0565	096077-01	Improved diploid (AA)	[(Calcutta 4 × Pahang) × (Borneo × Madang)] × Khae	B	C/C	Sus
72	CNPMF 0572	106090-01	Improved diploid (AA)	[(Khai × (Calcutta 4 × Madang)] × [(Calcutta 4 × Madang)]	B	C/C	Sus
73	CNPMF 0612	098094-01	Improved diploid (AA)	[(M53 × Madu) × Madu] × SH3263	H	C/C	Sus
74	CNPMF 0731 ^R	088079	Improved diploid (AA)	[(Malaccensis × Madang)] × [(Tuu Gia × Calcutta 4)]	H	T/C	Res
75	CNPMF 0767	088100	Improved diploid (AA)	[(Malaccensis × Madang)] × [(Khai × (Calcutta 4 × Madang)]	B	C/C	Sus
76	CNPMF 0811	106096	Improved diploid (AA)	[(Khai × (Calcutta 4 × Madang)] × [(Calcutta 4 × Pahang) × (Borneo × Madang)]	B	-	Sus
77	CNPMF 0037	098090-01	Improved diploid (AA)	[(M53 × Madu)] × [(Malaccensis × Tjau Lagada)]	B	-	Sus
78	CNPMF 0898	PA × 119	Improved diploid (AA)	[(Prata Anā)] × [(Malaccensis × Sinwobogi) × (Calcutta 4 × Galeo)]	B	-	Sus
79	CNPMF 0038	098090-01	Improved diploid (AA)	[(M53 × Madu)] × [(Malaccensis × Tjau Lagada)]	B	-	Sus
80	CNPMF 1102	093117	Improved diploid (AA)	[(Jari Buaya × (Calcutta 4 × Madang)] × [(Borneo × Guyod) × (Tuu Gia × Calcutta 4)]	B	-	Sus
81	CNPMF 1171	088108	Improved diploid (AA)	[(Malaccensis × Madang)] × [(M53 × (Tuu Gia × Calcutta 4)]	B	-	Sus
82	CNPMF 0993 ^R	117100	Improved diploid (AA)	[(Borneo × Guyod) × (Tuu Gia × Calcutta 4)] × [(Khai × (Calcutta 4 × Madang)]	B	-	Sus
83	CNPMF 1323	089087	Improved diploid (AA)	[(Malaccensis × Sinwobogi)] × [(Calcutta 4 × Heva)]	B	-	Sus
84	CNPMF 1105	123097	Improved diploid (AA)	[(Borneo × Guyod) × (Calcutta 4 × Heva)] × [(Calcutta 4 × Madang)]	B	-	Sus
85	CNPMF 0998 ^R	091124	Improved diploid (AA)	[(Borneo × Guyod)] × [(Borneo × Guyod) × SH3263]	B	-	Sus
86	CNPMF 0978	041040	Improved diploid (AA)	[(Calcutta 4 × Madang)] × [(Terrinha × Calcutta 4)]	B	C/C	Sus

Table 1. Cont.

Sample Number	Accession Name	Accession Number	Type	Pedigree	Marker-Allele Defined by BcoDI Digest	SNP and Allele Zygosity	Predicted <i>Foc</i> TR4 Responses
87	CNPMF 1272	123079	Improved diploid (AA)	[(Borneo × Guyod) × (Calcutta 4 × Heva)] × [(Tuu gia × Calcutta 4)]	B	C/C	Sus
88	CNPMF 1286	041040	Improved diploid (AA)	[(Calcutta 4 × Madang)] × [(Terrinha × Calcutta 4)]	B	C/C	Sus
89	M53 ^R	-	Landrace cultivar (AA)	Malaccensis–Kedah × banksii-Samoa) × (Paka × Banksii-Samoa)	B	C/C	Sus
90	Pisang Jaran	ITC0678	Wild diploid (AA)	-	B	C/C	Sus
91	Malbut	-	Wild diploid (AA)	-	B	C/C	Sus
92	Calcutta4	ITC0249	Wild diploid (AA)	-	B	C/C	Sus
93	Birmanie	-	Wild diploid (AA)	-	B	C/C	Sus
94	Akondro Mainty	ITC0281	Mchare landrace (AA)	-	B	-	Sus
95	NBA-14	ITC0267	Wild diploid (AA)	-	B	C/C	Sus
96	Khai Nai On	ITC0663	Wild diploid (AA)	-	B	C/C	Sus
97	Khai	ITC0532	Wild diploid (AA)	-	B	C/C	Sus
98	Pisang Berlin	ITC0611	Edible diploid (AA)	-	B	T/C	Res
99	Khi Maeo	-	Wild diploid (AA)	-	B	-	Sus
100	Borneo	ITC0253	Wild diploid (AA)	-	B	C/C	Sus
101	Mambee Thu	ITC0612	Edible diploid (AA)	-	B	C/C	Sus
102	Niyarma Yik	ITC0269	Edible diploid (AA)	-	B	C/C	Sus
103	<i>M. a. spp. malaccensis</i>	ITC0399	Wild diploid(AA)	-	H	-	Res
104	Pisang Tongat	ITC0063	Edible diploid (AA)	-	B	C/C	Sus
105	Pa Mysore2	ITC0668	Wild diploid (AA)	-	H	C/C	Sus
106	Pisang Jari Buaya	ITC0690	Edible diploid (AA)	-	B	C/C	Sus
107	Tuu Gia	ITC0610	Edible diploid (AA)	-	B	C/C	Sus
108	Pisang Nangka	ITC0004	Edible triploid (AAB)	-	B	C/C	Sus
109	Tjau Lagada	ITC0090	Wild diploid (AA)	-	B	C/C	Sus
110	Tong Dok Mak	ITC0411	Wild diploid (AA)	-	H	C/C	Sus
111	M-61	-	Improved diploid (AA)	-	H	C/C	Sus
112	Pisang Mas	ITC1403	Edible diploid (AA)	-	H	T/C	Res
113	Pisang Cici	ITC0681	Wild diploid (AA)	-	B	C/C	Sus
114	Saney	-	Wild diploid (AA)	-	B	C/C	Sus
115	Yangambi no.2	ITC1275	Cultivar (AAB)	-	B	C/C	Sus
116	Yangambi Km5	ITC1123	Cultivar (AAB)	-	H	T/C	Res
117	Highgate	ITC0263	Cultivar (AAA)	-	B	-	Sus
118	BRS Pacoua PV 03-76	-	Prata hybrid (AAAB)	Pacovan × Calcutta 4	B	C/C	Sus
119	PA 42-38	PA 42-38	Prata hybrid (AAAB)	Prata Anã × M53	B	C/C	Sus

Table 1. Cont.

Sample Number	Accession Name	Accession Number	Type	Pedigree	Marker-Allele Defined by BcoDI Digest	SNP and Allele Zygosity	Predicted <i>Foc</i> TR4 Responses
120	PA 42-28	PA 42-28	Prata hybrid (AAAB)	Prata Anã × M53	B	C/C	Sus
121	PA 42-19	PA 42-19	Prata hybrid (AAAB)	Prata Anã × M53	-	C/C	Sus
122	M48	-	Improved diploid (AA)	-	B	C/C	Sus
123	Ouro	-	Cultivar (AA)	-	H	T/T	Res

B = Marker homozygous for the susceptible allele, predicted to be *Foc* TR4-susceptible; H = Marker heterozygous, predicted to be *Foc* TR4-resistant; A = Marker homozygous for the resistant allele, predicted to be *Foc* TR4-resistant; Nucleotide 'T' corresponds to the resistant allele and nucleotide 'C' the susceptible allele; 'T/C' and 'T/T' are predicted to be resistant whereas 'C/C' = is predicted to be susceptible. "HR" and "R" within the "Accession name" column indicate accessions that were "Highly Resistant" and "Resistant", respectively, to *Foc* STR4, as determined in a previous study [19]. Accessions listed in bold within the "Pedigree" column denote parental accessions previously shown to be resistant to *Foc* TR4. Sanger sequencing was used to determine the SNP identity at this locus. "-" within the "SNP and allele zygosity" column indicates that Sanger sequencing was not performed for these particular accessions.

3. Results and Discussion

The overall results of the marker-assisted screening for all 123 accessions are summarized in Table 1. A restriction digest was performed using BcoDI on PCR amplicons of all 123 Embrapa accessions. A BcoDI cut site within the PCR amplicon allowed bi-allelic forms to be discriminated on an agarose gel (Figure 1). Accessions displaying a dominant resistant band were subsequently analyzed through Sanger sequencing. The sequencing results confirmed the presence of the informative SNP, as identified in the alignment of chromatograms at the expected position (Figure S1).

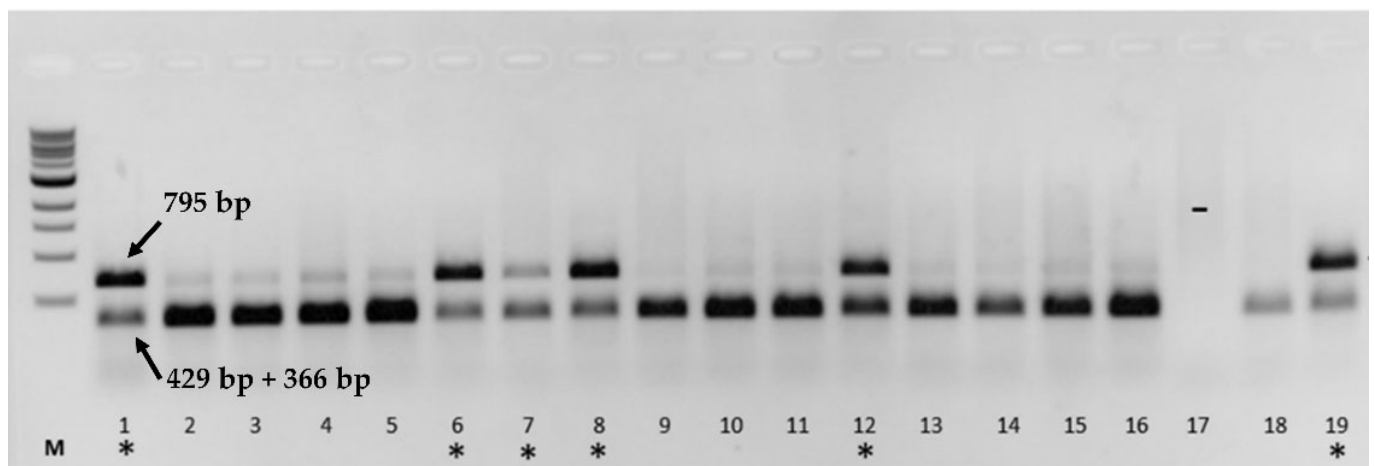


Figure 1. BcoDI digest profile of 19 Embrapa accessions PCR-amplified using the SNP marker 29730-A-SNP1-F2 and 29730-A-SNP1-R2. The 795 bp fragment is putatively associated with resistance. Samples are (1) 'PA Mysore 2', (2) 'Pisang Jari Buaya', (3) 'Tuau Gia', (4) 'Pisang Nangka', (5) 'Tjau Lagada', (6) 'Tong Dok Mak', (7) 'M-61', (8) 'Pisang Mas', (9) 'Pisang Cici', (10) 'Saney', (11) 'Yangambi no.2', (12) 'Yangambi Km5', (13) 'Highgate', (14) 'Pacoua PV03-76', (15) 'PA 42-38', (16) 'Pa 42-28', (17) 'PA 42-19', (18) 'M48', and (19) 'Ouro'. M = molecular ladder 1 kb (N3232S, New England Biolabs, VIC, Australia), (-): Non-amplified sample. The alternatively cut allele (429 bp and 366 bp) may indicate the presence of the *Foc* TR4-susceptible allele. Accessions heterozygous for the marker locus are predicted for resistance to *Foc* STR4 and *Foc* TR4 given the complete dominance of resistance over susceptibility at this resistance locus. Asterisk = putative *Foc* TR4/STR4-resistant genotypes.

The SNP marker was screened in all putative genotypes for resistance. PCR products of selected accessions were Sanger-sequenced in both directions by ACTGene Company,

using the AB3500 sequencing platform (ACTGene, Porto Alegre, RS, Brazil). Of the retrieved sequences, these were analyzed using the multiple-sequence alignment software MAFFT v 7.490 [20] in Geneious Prime v 2024.0.7 (Biomatter Pty. Ltd., Auckland, New Zealand). Overall, 14 genotypes, out of 15, showed the presence of the resistance allele (Supplementary Figure S1). Of these, three improved diploids have *Malaccensis* in their genetic background, namely ‘013019’, ‘CNPMF 0542’, and ‘CNPMF 0731’. Given that the breeding program at Embrapa focuses on a preventive breeding strategy to combat the disease, these improved diploids will play a key role in the development of *Foc* TR4-resistant cultivars aimed at enhancing resistance to *Fusarium* wilt in the field.

Among the 15 accessions previously identified as highly resistant or resistant to *Foc* STR4 [19], 10 were associated with the resistance marker band. These included the improved diploids CNPMF- ‘0496’, ‘0534’, ‘0536’, ‘0542’, ‘0557’, ‘0731’, ‘013019-01’, ‘050012-02’, ‘SH3263’, and ‘SH3362’ (Table 1). However, five accessions, including CNPMF- ‘1323’, ‘0513’, ‘0993’, ‘0998’, ‘001016-01’, and ‘M53’, did not exhibit the resistance marker allele despite being resistant to *Foc* STR4 [19] (Table 1). These findings indicate a 62.5% detection rate of resistant genotypes using the marker, suggesting that while the marker is a useful tool for identifying resistance, it may not capture all resistant genotypes due to linkage disequilibrium and resistance sources controlled by other unlinked loci.

Improved diploids are developed by crossing wild diploids with the objective of obtaining hybrids with important agronomical characteristics such as disease resistance. There are three types of improved diploids: (1) first generation – wild diploid × wild diploid, (2) second generation – Improved diploid × wild diploid, and (3) third generation – improved diploid × improved diploid. The third generation may have many wild diploids in its genealogy, whereas the hybrid combines important agronomical characteristics that were distributed among the wild diploids [14].

The screening results suggest that the marker did not detect the resistant allele in diploids including ‘Calcutta 4’, ‘Pisang Lilin’, ‘Tuu Gia’, and ‘Borneo’ despite having good or intermediate levels of resistance/tolerance to *Foc* race 1 and *Foc* TR4 [11,21]. The negative results in marker screening highlight the limitations of this marker in detecting resistance traits at loci other than the target or in genotypes lacking *Musa acuminata* ssp. *malaccensis* origin. This suggests that additional markers may be needed to identify resistance in a broader range of banana genotypes and as well in *Musa acuminata* ssp. *malaccensis*-derived bananas such as ‘Pisang Lilin’. These genotypes are scheduled for field evaluation to assess their resistance to *Foc* TR4, adhering to quarantine protocols in Australia and Colombia. Additionally, we plan to use the putative *Foc* TR4-resistant improved diploids in breeding efforts aimed at introducing sources of *Foc* TR4-resistance into dessert bananas including ‘Prata’, ‘Silk’, and ‘Cavendish’.

This work highlights the importance of improved diploid genotypes, particularly ‘CNPMF 512’ and ‘CNPMF 1323’, which carry resistance to other pests and diseases. These genotypes also possess desirable agronomic traits, including short plant height, early flowering time, and good tillering ability [22]. The genotypes will be field-tested for resistance to *Foc* TR4 in Australia and Colombia, in collaboration with the Department of Agriculture, Fisheries, and Forestry and Agrosavia, to further validate the marker.

This study represents the first report of screening an SNP marker across such an extensive number of banana accessions in Latin America. Given that *Foc* TR4 is already present in Latin America, the urgent need for validation of this SNP marker in genotypes is underscored by its potential impact. Our findings provide valuable insights to expedite resistance breeding in other banana programs worldwide, such as those conducted by IITA for cooking and plantain bananas, and by CIRAD [7,23–25].

Supplementary Materials: The following supporting information can be downloaded at <https://www.mdpi.com/article/10.3390/jof10120839/s1>: Supplementary Figure S1: Sequencing chromatograms for the *Macma4_03_g32560* gene were generated using both forward and reverse primers.

Author Contributions: Conceptualization, C.F.F., A.C., E.A.B.A. and E.P.A.; methodology, C.F.F., A.C., E.A.B.A., A.d.J.R. and J.M.d.S.S.; software, C.F.F. and A.C.; validation, C.F.F., A.C. and A.P.d.S.R.; formal analysis, C.F.F., A.C., E.A.B.A., R.S. and B.U.; investigation, C.F.F., A.C., E.P.A., R.S. and B.U.; resources, C.F.F., A.C. and E.A.B.A.; data curation, C.F.F. and A.C.; writing—original draft preparation, C.F.F., A.C., E.A.B.A. and E.P.A.; writing—review and editing, C.F.F., A.C., E.A.B.A., E.P.A., R.S. and B.U.; visualization, C.F.F., A.C., E.A.B.A., E.P.A., R.S. and B.U.; supervision, C.F.F., A.C., E.A.B.A. and E.P.A.; project administration, A.C., E.A.B.A. and E.P.A.; funding acquisition, A.C., E.A.B.A. and E.P.A. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by The Bill and Melinda Gates Foundation (Project Grant ID: OPP1093845) through its grant to the International Institute of Tropical Agriculture (IITA) under the project Accelerated Breeding of Better Bananas, grant number IITA 20600.15/0008-8—Phase II. Andrew Chen and Elizabeth Aitken were also funded by Hort Innovation Australia through grant ‘BA17006’.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The authors will provide data upon request.

Acknowledgments: The authors would like to thank the National Council of Scientific and Technological Development—CNPq for research productivity grants of CFF and EPA and Embrapa Mandioca e Fruticultura for the technical support.

Conflicts of Interest: The authors declare no conflicts of interest.

References

- Food and Agriculture Organization. Available online: <https://www.fao.org/faostat/> (accessed on 21 October 2024).
- Acuña, R.; Rouard, M.; Leiva, A.M.; Marques, C.; Olortegui, J.A.; Ureta, C.; Cabrera-Pintado, R.M.; Rojas, J.C.; Lopez-Alvarez, D.; Cenci, A.; et al. First report of *Fusarium oxysporum* f. sp. *cubense* tropical race 4 causing *Fusarium* wilt in Cavendish bananas in Peru. *Plant Dis.* **2022**, *106*, 2268. [\[CrossRef\]](#) [\[PubMed\]](#)
- García-Bastidas, F.A.; Quintero-Vargas, J.C.; Ayala-Vasquez, M.; Schermer, T.; Seidl, M.F.; Santos-Paiva, M.; Noguera, A.M.; Aguilera-Galvez, C.; Wittenberg, A.; Hofstede, R.; et al. First report of *Fusarium* wilt tropical race 4 in Cavendish Bananas caused by *Fusarium odoratissimum* in Colombia. *Plant Dis.* **2020**, *104*, 994. [\[CrossRef\]](#)
- Zhan, N.; Kuang, M.; He, W.; Deng, G.; Liu, S.; Li, C.; Roux, N.; Dita, M.; Yi, G.; Sheng, O. Evaluation of resistance of banana genotypes with AAB genome to *Fusarium* wilt Tropical Race 4 in China. *J. Fungi* **2022**, *8*, 1274. [\[CrossRef\]](#) [\[PubMed\]](#)
- Stover, R.H. Disease management strategies and the survival of the banana industry. *Annu. Rev. Phytopathol.* **1986**, *24*, 83–91. [\[CrossRef\]](#)
- Olivares, B.O.; Rey, J.C.; Lobo, D.; Navas-Cortés, J.A.; Gómez, J.A.; Landa, B.B. *Fusarium* wilt of bananas: A review of agroenvironmental factors in the Venezuelan production system affecting its development. *Agronomy* **2021**, *11*, 986. [\[CrossRef\]](#)
- Martínez, G.; Olivares, B.O.; Rey, J.C.; Rojas, J.; Cardenas, J.; Muentes, C.; Dawson, C. The advance of *Fusarium* Wilt Tropical Race 4 in Musaceae of Latin America and the Caribbean: Current Situation. *Pathogens* **2023**, *12*, 277. [\[CrossRef\]](#)
- Dita, M.; Barquero, M.; Heck, D.; Mizubuti, E.S.; Staver, C.P. *Fusarium* wilt of banana: Current knowledge on epidemiology and research needs toward sustainable disease management. *Front. Plant Sci.* **2018**, *9*, 1468. [\[CrossRef\]](#)
- Pegg, G.K.; Coates, L.M.; O’Neill, W.T.; Turner, D.W. The epidemiology of *Fusarium* wilt of banana. *Front. Plant Sci.* **2019**, *10*, 1396. [\[CrossRef\]](#)
- Chen, A.; Sun, J.; Matthews, A.; Armars-Egas, L.; Chen, N.; Hamil, S.; Minto, S.; Tran-Nguyen, L.T.T.; Batley, J.; Aitken, E.A.B. Assessing variations in host resistance to *Fusarium oxysporum* f. sp. *cubense* Race 4 in *Musa* species with focus on the Subtropical Race 4. *Front. Microbiol.* **2019**, *10*, 1062. [\[CrossRef\]](#)
- Rocha, A.J.; Soares, J.A.M.; Nascimento, F.S.; Santos, A.S.; Amorim, V.B.O.; Ferreira, C.F.; Haddad, F.; Santos-Serejo, J.A.; Amorim, E.P. Improvements in the resistance of the banana species to *Fusarium* wilt: A systematic review of methods and perspectives. *J. Fungi* **2021**, *7*, 249. [\[CrossRef\]](#)
- Guo, L.; Wang, J.; Liang, C.; Yang, L.; Zhou, Y.; Liu, L.; Huang, J. Fosp9, a novel secreted protein, is essential for the full virulence of *Fusarium oxysporum* f. sp. *cubense* on Banana (*Musa* spp.). *Appl. Environ. Microb.* **2022**, *88*, e00604-21. [\[CrossRef\]](#) [\[PubMed\]](#)
- Ploetz, R.C. *Fusarium* wilt of Banana. *Phytopathology* **2015**, *105*, 1512–1521. [\[CrossRef\]](#) [\[PubMed\]](#)
- Amorim, E.P.; Santos-Serejo, J.A.; Amorim, V.B.O.; Silva, S.O.S. Melhoramento genético. In *O Agronegócio da Bananeira*; Ferreira, C.F., Silva, S.O., Amorim, E.P., Santos-Serejo, J.A., Eds.; Embrapa: Brasília, Brazil, 2016; 832p.
- Chagne, D.; Vanderzande, S.; Kirk, C.; Profit, N.M.; Weskett, R.; Gardiner, S.E.; Peace, C.P.; Volz, R.K.; Bassil, N.V. Validation of SNP markers for fruit quality and disease resistance loci in apple (*Malus × domestica* Borkh.) using the OpenArray® Platform. *Hortic. Res.* **2019**, *6*, 30. [\[CrossRef\]](#) [\[PubMed\]](#)

16. Méline, V.; Caldwell, D.L.; Kim, B.S.; Khangura, R.S.; Baireddy, S.; Yang, C.; Sparks, E.E.; Brian, D.; Delp, E.J.; Iyer-Pascuzzi, A.S. Image-based assessment of plant disease progression identifies new genetic loci for resistance to *Ralstonia solanacearum* in tomato. *Plant J.* **2023**, *113*, 887–903. [[CrossRef](#)]
17. Chen, A.; Sun, J.; Martin, G.; Gray, L.-A.; Hřibová, E.; Christelová, P.; Yahiaoui, N.; Rounsley, S.; Lyons, R.; Batley, J.; et al. Identification of a Major QTL-Controlling Resistance to the Subtropical Race 4 of *Fusarium oxysporum* f. sp. *cubense* in *Musa acuminata* ssp. *malaccensis*. *Pathogens* **2023**, *12*, 289. [[CrossRef](#)]
18. Chen, A.; Sun, J.; Viljoen, A.; Mostert, D.; Xie, Y.; Mangila, L.; Bothma, S.; Lyons, R.; Hřibová, E.; Christelová, P.; et al. Genetic Mapping, Candidate Gene Identification and Marker Validation for Host Plant Resistance to the Race 4 of *Fusarium oxysporum* f. sp. *cubense* Using *Musa acuminata* ssp. *malaccensis*. *Pathogens* **2023**, *12*, 820. [[CrossRef](#)]
19. Santana, W.S.; Rocha, A.d.J.; da Silva, W.B.; Amorim, V.B.O.d.; Ramos, A.P.d.S.; Haddad, F.; Amorim, E.P. Selection of Improved Banana Diploid Resistant to *Fusarium oxysporum* f. sp. *cubense* Races 1 and Subtropical 4. *Agronomy* **2024**, *14*, 1277. [[CrossRef](#)]
20. Katoh, K.; Standley, D.M. MAFFT Multiple sequence alignment software version 7: Improvements in performance and usability. *Molecular Biol. Evol.* **2013**, *30*, 772–780. [[CrossRef](#)]
21. Arinaitwe, I.K.; Teo, C.H.; Kayat, F.; Tumuhimbise, R.; Uwimana, B.; Kubiriba, J.; Swennen, R.; Harikrishna, J.A.; Othman, R.Y. Evaluation of banana germplasm and genetic analysis of an F1 population for resistance to *Fusarium oxysporum* f. sp. *cubense* race 1. *Euphytica* **2019**, *215*, 175. [[CrossRef](#)]
22. Gonçalves, Z.S.; Rocha, A.J.; Haddad, F.; Amorim, V.B.O.; Amorim, E.P. Selection of diploid and tetraploid banana hybrids resistant to *Pseudocercospora fijiensis*. *Agronomy* **2021**, *11*, 2483. [[CrossRef](#)]
23. Batte, M.; Nyine, M.; Uwimana, B.; Swennen, R.; Akech, V.; Brown, A.; Hovmalm, H.P.; Geleta, M.; Ortiz, R. Significant progressive heterobeltiosis in banana crossbreeding. *BMC Plant Biol.* **2020**, *20*, 489. [[CrossRef](#)] [[PubMed](#)]
24. Batte, M.; Swennen, R.; Uwimana, B.; Akech, V.; Brown, A.; Tumuhimbise, R.; Hovmalm, H.P.; Geleta Dida, M.; Ortiz, R. Crossbreeding East African highland bananas: Lessons learnt relevant to the botany of the crop after 21 years of genetic enhancement. *Front. Plant Sci.* **2019**, *10*, 81. [[CrossRef](#)] [[PubMed](#)]
25. Tenkouano, A.; Lamien, N.; Agogbua, J.; Amah, D.; Swennen, R.; Traore, S.; Thiemele, D.; Aby, N.; Kobenan, K.; Gnonhouiri, G.; et al. Promising high-yielding tetraploid plantain bred-hybrids in West Africa. *Int. J. Agron.* **2019**, *2019*, 3873198. [[CrossRef](#)]

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