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Dissemination of Fusarium wilt of banana in Mozambique caused by *Fusarium odoratissimum* Tropical Race 4

Plant Disease

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1 **Dissemination of Fusarium wilt of banana in Mozambique caused by *Fusarium odoratissimum***

2 **Tropical Race 4**

3

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15

16 Abstract

17 *Fusarium* wilt of banana (FWB) is a serious soil-borne fungal disease. In the previous century,
18 FWB already destroyed Gros Michel-based banana cultures in Central America, and currently,
19 the disease threatens all major banana-producing regions of the world. The causal agents of
20 these epidemics, however, are diverse. Gros Michel was infected by a wide range of *Fusarium*
21 species, the so-called Race 1 strains, whereas the contemporary Cavendish-based cultures are
22 affected by *Fusarium odoratissimum*, colloquially called Tropical Race 4 (TR4). TR4 was
23 reported in Mozambique on two commercial banana farms in 2013, but no incursions were
24 found outside the farm boundaries in 2015, suggesting that the disease was under control. Here
25 we report the presence of TR4 outside of these farm boundaries. We obtained fungal samples
26 from 13 banana plants in smallholder and roadside plantings at various locations throughout
27 northern Mozambique. These samples tested positive for TR4 by molecular diagnostics and in
28 greenhouse pathogenicity assays. The results were confirmed with re-isolations, thereby
29 completing Koch's postulate. To study the diversity of TR4 isolates in Mozambique, we
30 selected five samples for whole-genome sequencing. Comparison with a global collection of
31 TR4 samples revealed very little genetic variation, indicating that the fungus is clonally
32 spreading in Mozambique. Furthermore, isolates from Mozambique are clearly genetically

33 separated from other geographic incursions, and thus we cannot trace the origin of TR4 in
34 Mozambique. Nevertheless, our data demonstrate the dissemination of TR4 in Mozambique,
35 underscoring the failure of disease management strategies. This threatens African banana
36 production.

37

38

39 Main

40 *Fusarium* wilt of banana (FWB) is threatening global banana cultivation. In the previous
41 century, FWB devastated the Gros Michel-based banana cultivation in Central America (Ploetz,
42 2005). The resistant Cavendish bananas eventually saved the industry and the cultivation of
43 Cavendish expanded quickly since its resistance withstands FWB, even on heavily infested
44 soils. Currently, Cavendish dominates global production (>47%) and export trade (>98%)
45 (Arias et al., 2003). The causal agent of FWB is a fungal plant pathogen belonging to the
46 *Fusarium oxysporum* species complex. Previously, it was known as *Fusarium oxysporum* f.sp.
47 *cubense* (Foc), but recently Maryani et al. (2019) showed that Foc contains multiple genetically
48 diverse lineages which should be divided into species. The Race 1 strains, causing FWB on
49 Gros Michel, belong to several species, and a new species, *Fusarium odoratissimum*, known as
50 Tropical Race 4 (TR4), currently causes FWB in Cavendish and many local banana varieties
51 (Garcia-Bastidas, 2019). Hence, where Cavendish was once the solution to FWB, it is now
52 become a vehicle for the worldwide dissemination of TR4, due to its dominance in global
53 banana cultivation (Westerhoven et al., 2022). Since the first incursion of TR4 outside
54 Southeast Asia in Jordan (García-Bastidas et al., 2014), TR4 has now spread to twelve other
55 countries in all major banana-producing regions of the world (Westerhoven et al., 2022). This

56 rapid international and intercontinental spread, as exemplified by recent incursions in Latin
57 America (Acuña et al., 2021; Garcia-Bastidas et al., 2020), underscores that successfully
58 quarantining TR4 is apparently impossible.

59

60 In East Africa banana is an important cash and staple crop for millions of people
61 (Karamura & Frison, 1998). In 2013, TR4 was reported in Mozambique (Butler, 2013), but a
62 formal confirmation was only published in 2020 (Viljoen et al., 2020) and genome sequences
63 are not available. Recently, TR4 was also reported in Mayotte, likely originating from
64 Mozambique (Aguayo et al., 2021), but this cannot be verified since the isolates or genome
65 sequences are not released. The report of Viljoen et al. (2020) describes the first African TR4
66 incursion in a commercial farm (Matanuska farms) in 2013 (Fig. 1a), and subsequent
67 management measures, including the cultivation of Giant Cavendish Tissue Culture Variants
68 or so-called GCTCV soma-clones that are less susceptible to TR4. These management and
69 biosecurity measures were seemingly successful as no TR4-affected plants were observed
70 outside the farms during a national survey in 2015 (Viljoen et al., 2020). However, we obtained
71 13 pseudostem samples from banana plants that showed FWB symptoms (Figure 1a, Table 1)
72 in 2020 and 2021 in various smallholder plantations (50-200 mats maintained and managed by
73 local communities) and at roadsides (10-20 mats belonging to local people in Netia and

74 Monapo localities), up to 210 km away from the Matanuska farms (Figure 1b). We report the
75 isolation, characterization, and genome sequences of TR4 strains from symptomatic Cavendish
76 plants in Mozambique.

77

78 Initially, five small vascular strands from different locations (Figure 1b, Table 1) were
79 diagnosed for the presence of TR4. Later, a second series of samples from eight symptomatic
80 bananaplants in Mozambique was obtained and processed (Figure 1b, Table 1). All samples
81 were surface sterilized and placed on Komada medium, an agar-based selective medium used
82 to isolate *Fusarium* spp. from infested plant materials or soil (Leslie & Summerell, 2008).
83 Subsequently, two monosporic isolates were obtained from two independent colonies per
84 vascular strand and used for multiplex diagnostic PCR for TR4 (Dita et al., 2010). All isolates
85 tested positive for TR4, and all controls showed the expected outcomes, thereby confirming
86 that TR4 disseminates across Mozambique (Figure 2a).

87

88 We then conducted greenhouse pathogenicity assays on ten-week-old Cavendish
89 (Grand Naine variety) banana plants using two independent monospores per location (Table
90 1). Conidia for the pathogenicity assays were obtained as described by Garcia-Bastidas et al.
91 (2019). The plants were uprooted and dipped for 30 min in a conidial spore suspension of 10^6
92 conidia/mL and maintained in a greenhouse with a constant day temperature of 25°C, a night
93 temperature of 23°C and 16 h ambient light, and relative humidity of >75%. Strain TR4-II5
94 was used as a positive control and a Race 1 strain (CNPMF.R1) was used as a negative control,
95 along with a water mock treatment. All plants inoculated with monosporic isolates from Moz
96 1-5 showed typical FWB symptoms four weeks after inoculation, whereas the water and Race

97 1 controls remained healthy (Figure 2c). Re-isolations from all symptomatic plants were
98 confirmed as TR4 by diagnostic PCRs (Dita et al., 2010, Figure 2b), thereby establishing
99 Koch's postulate.

100

101 To uncover the genetic variation and potential origin of TR4 in Mozambique, we

102 performed whole-genome sequencing on one monospore isolate from locations Moz 1-5 (Table

103 1) using BGI sequencing, yielding on average 49 million reads per isolate with a read length

104 of 150bp. To analyse the genetic diversity of *F. odoratissimum* in Mozambique in relation to

105 previous incursions in other geographical regions, we included 23 previously sampled short-

106 read sequences of TR4 isolates from different geographic locations, consisting of

107 approximately 27 million reads of 150bp per isolate. We determined single nucleotide

108 polymorphisms (SNPs) among the five isolates - as well as the 23 isolates from different

109 incursions worldwide – by comparing with the TR4 (II5) reference genome assembly using the

110 GATK Best Practice (see Supplementing Materials for details). The whole-genome sequence

111 further corroborated TR4 as the isolated organism, and we subsequently identified 1,164 high-

112 quality single-nucleotide variants in the set of 28 samples. We observed little genetic diversity

113 across the sequenced isolates, with only 53 polymorphic sites in a genome of approximately

114 46 Mb, suggesting that local transmission likely occurs through a single clonal lineage that

115 most probably results from a single incursion event. To further elucidate the relationship among

116 the entire panel of TR4 isolates, we utilized the high-quality single-nucleotide variants to
117 perform principle component analysis (PCA) and to reconstruct a maximum-likelihood
118 phylogeny. The Mozambique isolates clearly group together, separate from the isolates
119 originating from incursions in other geographic regions (Figure 3). These data therefore suggest
120 that the incursion of TR4 in Mozambique accumulated SNPs independent from previously
121 reported incursions. Therefore, we cannot unambiguously link TR4 in Mozambique with the
122 occurrence of TR4 in other geographic regions. At this moment, the data cannot resolve
123 whether the identified and sequenced TR4 isolates are related to the two initially infested farms
124 or represent novel incursions, because no isolates from these farms are publicly available. Local
125 transport of bananas from these farms to various locations as well as the proximity of our
126 sampling sites to the originally infested farms, however, strongly suggest that TR4 was not
127 successfully contained. This underscores the failure of the implemented management strategies,
128 which threatens food security in East Africa.

129

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Table 1: Sampling sites in Mozambique from where *Fusarium odoratissimum* isolates were obtained and characterized using a TR4 molecular diagnostic, a greenhouse phenotyping assay and genome sequencing.

Site	Location	GPS	Monosporic isolates	TR4 diagnostic	Phenotyped isolates	Sequenced isolates
Moz 1	Monapo	S 15° 05' 57"; E 40° 08' 34"	Moz 1A	+	+	+
			Moz 1B	+	+	
Moz 2	Netia	S 15° 04' 54"; E 40° 14' 40"	Moz 2A	+	+	+
			Moz 2B	+	+	
Moz 3	Monapo	S 15° 04' 58"; E 40° 17' 39"	Moz 3A	+	+	+
			Moz 3B	+	+	
Moz 4	Monapo	S 15° 05' 36"; E 39° 58' 25"	Moz 4A	+	+	+
			Moz 4B	+	+	
Moz 5	Monapo	S 15° 04' 53"; E 39° 58' 25"	Moz 5A	+	+	+
			Moz 5B	+	+	
Moz 6	Namapa	Not available		+		
Moz 7	Namapa	Not available		+		
Moz 8	Alua	S 14° 04' 34" E 39° 54' 46"		+		
Moz 9	Lumbo	Not available		+		
Moz 10	Lumbo	Not available		+		
Moz 11	Naguema	S 15° 00' 43" E 40° 31' 02"		+		
Moz 12	Ribaue	S 15°03' 01" E 38° 19' 22"		+		
Moz 13	Ribaue	S 15° 02' 59" E 38° 19' 44"		+		

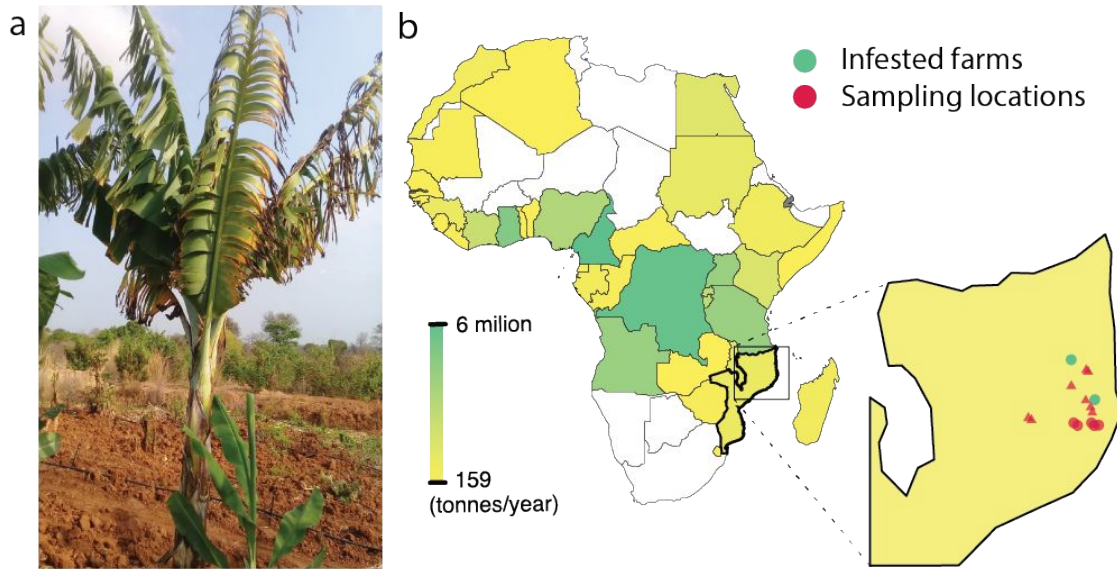


Figure 1. Pseudostem samples are obtained from Cavendish plants with FWB symptoms at different locations around the Matanuska farms. a) Cavendish banana plant in Mozambique showing external FWB symptoms, suggesting infection by TR4. b) Banana is a major food crop in Africa, especially in Sub-Saharan countries. The colors indicate banana production in tons per country. Mozambique produces 774,514 tons banana per year. TR4 has been previously detected in two farms in Metocheria and Lurio (green dots) and has been recovered from thirteen different locations in proximity and up to 210 km away from the initial incursions (red dots represent the first five sampling sites; red triangles approximate the origin of the second set of 8 sampling sites).

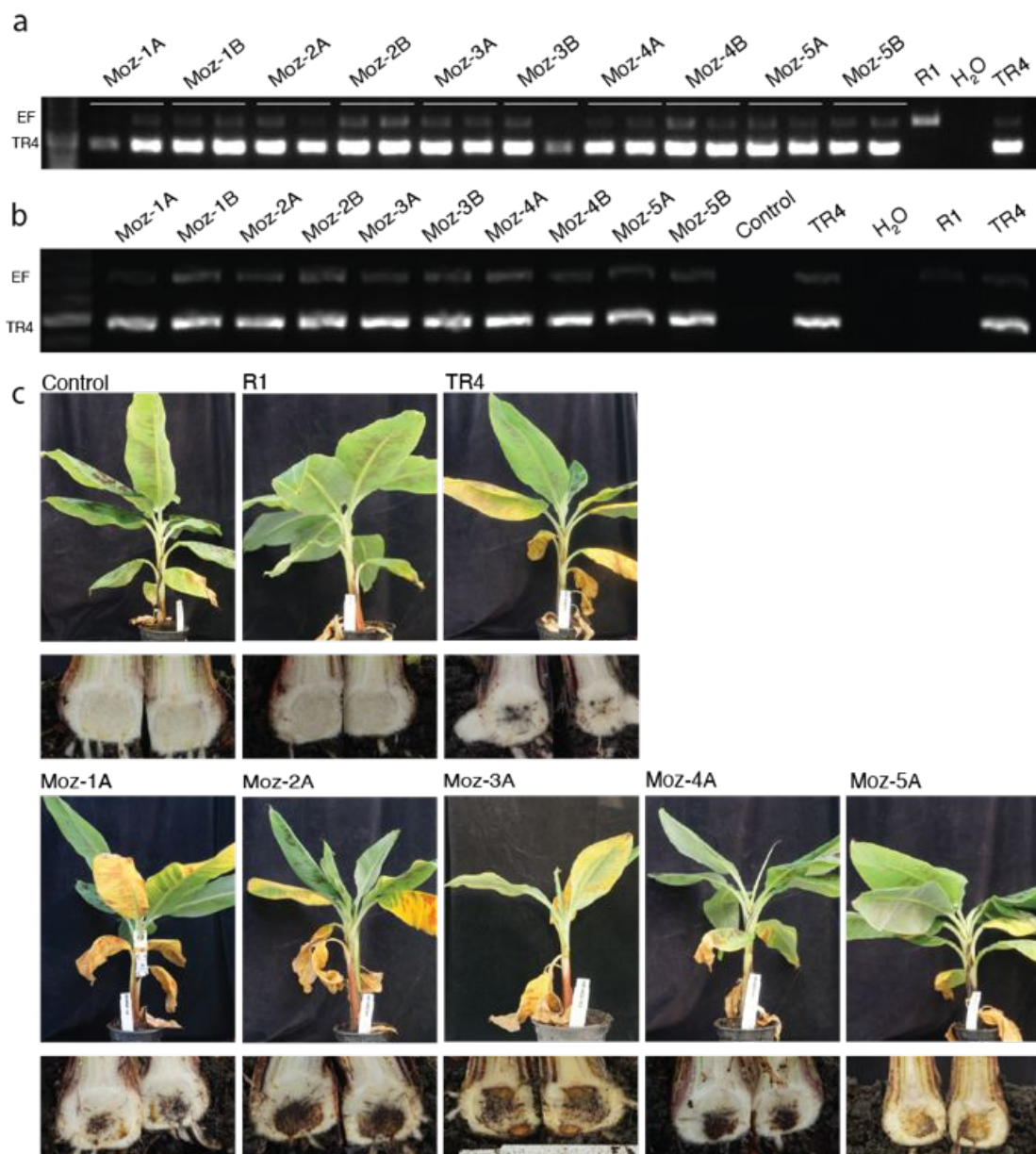


Figure 2. Fusarium wilt of banana in Cavendish bananas in Mozambique is caused by TR4. a) TR4 diagnostic PCR results are positive (TR4 specific amplicon of 463bp) on all monospore isolates, in duplicate, from five locations in Mozambique. b) TR4 diagnostic PCRs on all re-isolated strains resulted in the TR4 diagnostic 463bp amplicon. Controls in panels a, b and c include Race 1 (negative), water (negative), and TR4 isolate II-5 (positive). c) Greenhouse experiments showing Cavendish plants infected with TR4 isolates from Mozambique showing external and internal FWB symptoms.

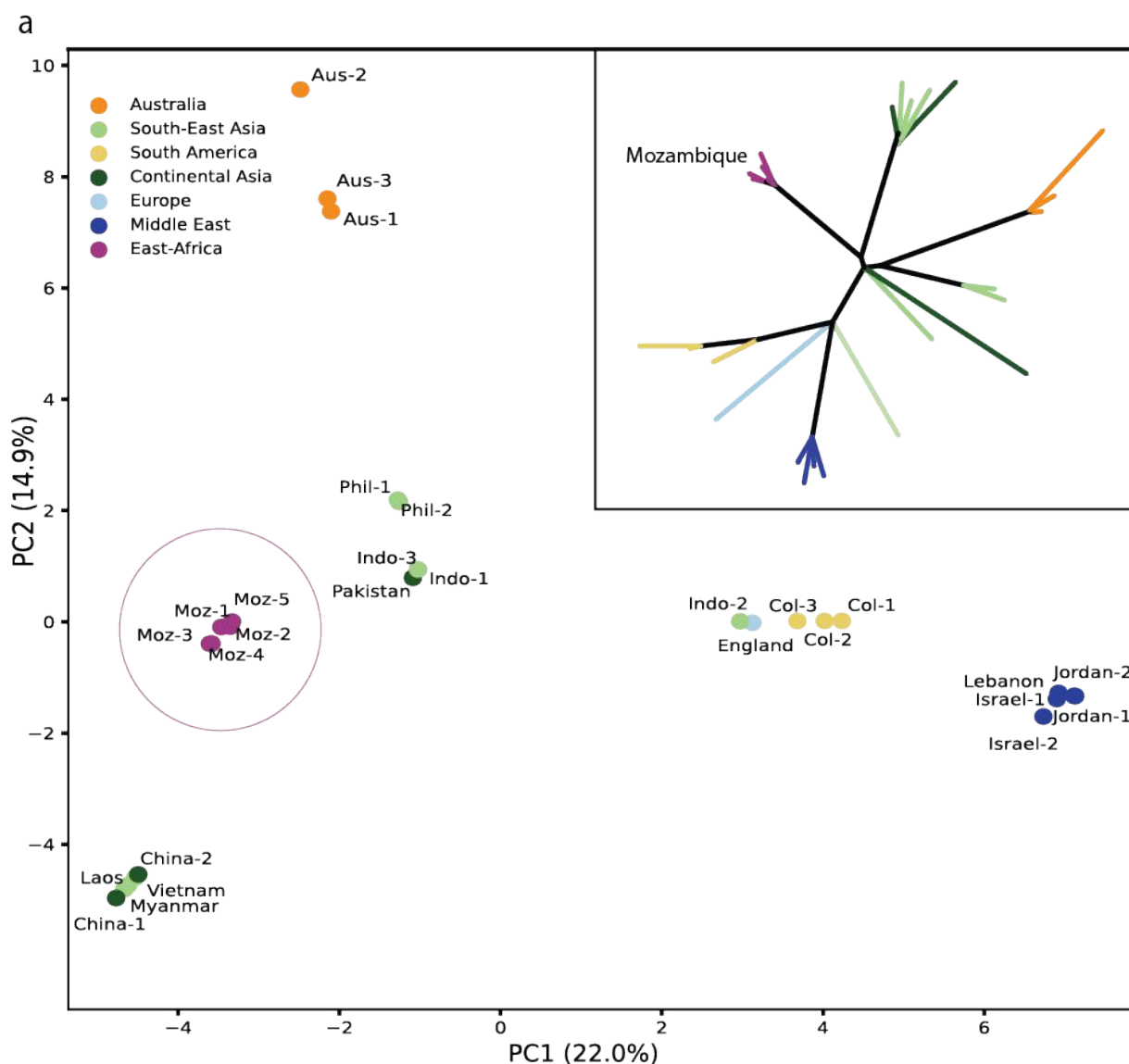


Figure 3. Whole-genome analysis of TR4 isolates from five locations in Mozambique reveals clonal dissemination and underscores failed management strategies. Whole-genome sequencing data of five isolates from five locations (Moz 1-5, Table 1) in Mozambique were compared with a global collection of 26 sequenced TR4 isolates from various locations (Dataset S1). Principle component as well as maximum-likelihood phylogenetic analyses based on 1,164 high-quality single nucleotide polymorphisms show that the Mozambique samples

are highly similar. The data suggest that these isolates originate from a single incursion and are now clonally spreading in Mozambique, but cannot be unambiguously linked with previously documented TR4 incursions in other countries.