PhD Research Progress Report (2016-2020)

TITLE: Genetic Analysis of Resistance against *Fusarium oxysporum* f. sp. *cubense* (Foc) in Selected Banana Populations

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Timeline of study: 2016-2020

University: University of Malaya

Research Objectives

List the individual topics of study – objectives or study areas

1. To identify contrasting diploid parents for use in *Foc* race 1 and race 4 genetic studies,
2. To develop and phenotype unrelated diploid mapping populations for *Foc* race 1 and race 4 resistances,
3. Assess the genetics of *Foc* race 1 and race 4 resistance in diploid bananas,
4. To identify polymorphic and heritable molecular markers for *Foc* race 1 and race 4, and
5. To perform a QTL analysis for *Foc* race 1 and race 4.

Achievements

Highlight significant achievements – e.g. in bullets

1. Sources of genetic variability to *Foc* race 1 already identified; Calcutta 4 and Monyet are resistant whereas Mshale and Kokopo are susceptible to *Foc* race 1
   - Parents for *Foc* race 4 have been rescreened at UM.
2. Two unrelated diploid mapping populations for *Foc* race 1 resistances developed. One population of Kokopo x Monyet is phenotyped (90%) and another of Mshale x

Calcutta 4 planted in the field where the suckers will be picked and multiplied in tissue. The multiplied genotypes will be screened for *Foc* race 1.

3. Different molecular markers IRAP, SSR and ISSR have been evaluated on the parents contrasting for *Foc* race 1. 2 IRAP, 14 SSR and 10 ISSR markers showing polymorphism within parents of Monyet and Kokopo and 2 IRAP, 15 SSR and 10 ISSR markers showing polymorphism within parents of Mshale and Calcutta 4 have been identified and used to screen the subsequent F_1 hybrids. Heritable markers for both populations have been identified.
4. Marker trait association using GACD software revealed two QTLs on linkage group (LG) 1 at LOD score of 2.5. When a higher LOD score of 4.0 was used, one QTL at LG 1 remained. The markers used were distributed on 7 LG within the whole genome
5. DNA for both populations sent for SNP typing.

Background/introduction

Brief background

Bananas and plantains (*Musa* spp.) are a major staple food for many millions of people in the tropics and subtropics. In Uganda, 13 million people with 66% of the country's urban population depend on the crop for food. However, banana production is constrained by low soil fertility, high perishability, pests and diseases. Among the key diseases is Fusarium wilt. Fusarium wilt is a destructive fungal disease of banana and plantain, caused by *Fusarium oxysporum* f. sp. *cubense* (Foc). Fusarium wilt is a soil-borne disease, reproduced by spores, survives in the soil for decades and has four races that are separated based on host susceptibility. *Fusarium* wilt causes banana yield loss of about 30-40 % with a yield loss of 2-90% estimated in South India alone (Mustaffa & Thangavelu, 2011; Kumar, 2006).

Efforts to manage banana Fusarium wilt using biological, chemical and cultural control measures have not been effective. Long-term survival of Foc in soil and ability to evolve into variants that can affect different varieties has made control very difficult. Host plant resistance seems to be the best alternative to control Fusarium wilt: durable, environmentally friendly, cheap for the poor resource farmers. Diploid banana segregating populations can enable the study of inheritance and understand the resistance mechanisms of Foc race 1 and 4. Also, to shorten the banana breeding cycle, there is a need to apply Markers/ (MAS, MAB) in banana improvement. Markers/ (MAS, MAB) increase the effectiveness in breeding and

significantly shorten the selection time of plants, which is useful additional tool in plant breeding (van Bueren *et al*., 2010).

Objective / Study 1. To identify contrasting diploid parents for use in *Foc* race 1 and race 4 genetic studies

Several diploids available at the banana breeding programmes of both NARO-Uganda and IITA, Sendusu-Uganda were screened for Foc race 1 resistance. Whereas, open pollinated *malaccensis* banana diploids are under screening for Foc race 4 at University of Malaya. This is to identify parent diploids contrasting for Foc race 1 and race 4 for use in generating segregating diploid populations.

Table 1. Banana diploids screened for resistance to Fusarium wilt

	Diploid parents (Race 1)	**F2 diploid banana plants (Race 1)**	**OP-*malaccensis* (Race 4)**
	1 TMB2X614-1	123 F2 diploid banana plants	45 plants from an open pollinated bunch of malaccensis
	2 Pahang		
	3 Kokopo		
	4 Long tavoy		
	5 Calcutta 4		
	6 Zebrina		
	7 Kasaska		
	8 Borneo		
	9 Pisang Lilin		
	10 Monyet		
	11 Mwitu Pemba		
	12 Huti shamba		
	13 Kahuti		
	14 Mlilembo		
	15 Muraru		
	16 Nshonowa		
	17 Njuru		
Resistant	TMB2X8075	Mpologoma	
Susceptible	Mshale	Kayinja	

Screening procedure

Three months old TC plantlets were screened for Foc resistance in a pot experiment using colonized millet grain inoculum. Yellowing was scored at 14 days interval from the date of inoculation to see if there is any leaf showing symptoms and data was used to determine Leaf symptom index (LSI). Two months after inoculation, the plants were uprooted and assessed for corm discolouration index (RDI). Experimental design was Randomised Complete Block Design (RCBD) and Data was analysed using GenStat 14th edition.

Table 2. Scale for scoring different parameters for Fusarium disease resistance (Viljoen *et al*., 2017).

Disease rating scale	Leaf symptom index	Stem splitting	Rhizome discoloration index
1	No yellowing	No cracking	No internal symptoms
2	Yellowing of < 1/3 of the leaves	Slight cracking	Few internal spots
3	Yellowing of 1/3 to 2/3 of leaves	Advanced	<1/3 discolored
4	Yellowing of > 2/3 of leaves		1/3-2/3 Discoloured
5	Plant dead		>1/3 Discoloured
6			Entire inner rhizome

Table 3. Interpretation of LSI and RDI, DSI (Sutanto *et al*, 2011).

DSI (RDI)	**DSI (LSI)**	**Translation**
1	1	Resistant
1.1-3	1.1-2	Partial resistance
3.1-5	2.1-3	Susceptible
5.1-6	3.1-4	Highly Susceptible

Results

Table 4. Analysis of variance for LSI and RDI for screened banana diploids

	Diploid parents			**F2 diploid banana plants**			**OP-*malaccensis***		
Source of variation	df	RDI	LSI	d.f	RDI	LSI	d.f	RDI	LSI
Total	131	3.17	0.56	530	4.2	0.4	77	2.6	0.3
Rep	5	2.2	0.17	4	3.4	1.1	1	19.8	0.3
Genotype	21	11.90***	1.61***	124	9.3***	0.9***	44	3.8***	0.3^{ns}
Residual	105	1.48	0.37	402	3.1	0.3	32	1.3	0.2

Table 5. Categorisation of the genotypes within germplasm using DSI (RDI)

	Diploid parents				**F2 diploid banana plants**				**OP-*malaccensis***			
	R	PR	S	HS	R	PR	S	HS	R	PR	S	HS
	Long tavoy	TMB2X614-1, Mwitu Pemba, Monyet, Pisang Lilin, Borneo, Kasaska, Zebrina, Pahang	Kokopo (3.5) Hutishima Kahuti Mililembo Muraru Nsonowa Njuru		55, 62, 80, 82, 120, 109, 234	2, 3, 4, 7, 8, 11, 13, 14, 16, 17, 19, 25, 26 , 30, 35, 37, 39, 41, 42, 49, 51, 52, 54, 59, 61, 63, 64, 65, 67, 69, 74, 77, 79, 81, 83, 84, 85, 87, 91, 94, 96, 110, 113, 117, 120, 128, 131, 132, 135,137, 138, 141, 142, 143, 144, 146, 151, 153, 159, 160, 161, 165, 171, 174, 178, 184, 196, 204, 205, 215, 216, 217, 218, 219, 221, 222, 227, 229	1, 5,10, 15,18, 20, 33, 38, 43, 51, 56, 66, 90, 102, 112, 114, 121, 125, 134 135, 139, 143,169 179, 205, 211, 223, 230	68, 162, 164	LJ56	LJ37 LJ41 LJ26 LJ49 LJ52 LJ1 LJ38 LJ61	LJ2 LJ32 LJ16 LJ28 LJ29 LJ13 LJ33 LJ31 LJ17	LJ50 LJ39 LJ75 LJ40 LJ47 LJ74 LJ34 LJ51 LJ62 LJ48 LJ3 LJ5 LJ14 LJ9 LJ15 LJ19 LJ45 LJ20 LJ25 LJ42
TOTAL	1	9	7		7	81	32	3				
Resistant	**TMB2X8075 (DSI=1)**				**Mpologoma (DSI=1.2)**							
Susceptible	**Mshale (DSI=4.2)**				**Kayinja (DSI=5.4)**							

Conclusion

- Germplasm screened showed variability to Foc race 1 and 4 and grouping into resistant and susceptible.

- Identified contrasting diploid banana parents can be used for crossing to generate a Foc segregating population for studying genetics of resistance to Foc race 1 and 4 and identifying markers for Foc race 1 and 4 resistance and Linkage map construction and identifying QTL for Foc race 1 and 4.

Objective / Study 2. To develop and phenotype unrelated diploid mapping populations for *Foc* race 1 and race 4 resistances

Developing two populations for Foc race 1.

1. A resistant Monyet was crossed with a susceptible Kokopo banana plant to generate an F_1 population.

The 142 F_1 genotypes were screened with Foc race 1 in a pot experiment as described in objective one.

Results

Analysis of variance for the F_1 diploid population revealed a significant difference among the genotypes at P<0.001 for RDI, LSI and PS, Table 6. This is evidence that the population is segregating to Foc race 1 resistance. Most of the F_1's were triploids about 87.1%, 8.2% were tetraploids and 4.7% were diploids.

Table 6. Mean square of an F1 population derived from a cross between Monyet and Kokopo screened for Foc race 1

Source of variation	d.f	RDI	LSI	PS
Total	851	2.37	0.48	0.21
Rep	5	2.36	0.42	0.32
Genotype	141	5.66***	0.81***	0.34***
Residual	705	1.71	0.41	0.17

*** P<0.001

1. A resistant Calcutta 4 was crossed with a susceptible Mshale banana plant to generate an F_1 population of 105 genotypes.

2. The Mshale x Calcutta F_1 population of 105 genotypes was planted in the field in January 2018.

Table 7. Timelines for screening the remaining genotypes and populations

Mshale x Calcutta	**OP-malacensis**
1. 105 genotypes planted in field in January 2018 2. To be screened in 2020	1. Parents at UM screened with Foc 4 2. Selected parents to be multiplied and planted for crossing

Objective / Study 3. Assessing the genetics of Foc race 1 and race 4 resistance in diploid bananas

i. Nature of inheritance

Nature of inheritance was determined using frequency histograms.

ii. Genetic ratios

Chi-square test of goodness of fit to determine was used to determine number of genes involved in each trait

iii. Broad sense heritability (H) was computed with formula below

$H = VG/VP$

Results (Foc race 1 in Monyet x Kokopo population)

Frequency histograms revealed continuous variation with skewness to the right for RDI and LSI (Figure 1 and 2). For pseudostem splitting, the histogram revealed two categories that is discrete variation, Figure 3.

i. Nature of inheritance

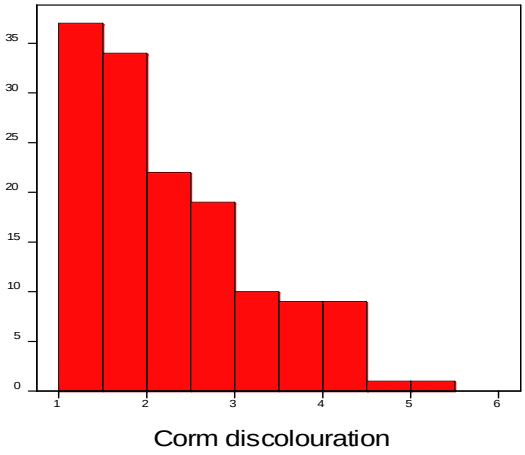


Figure 1. Cumulative histogram showing nature of inheritance for corm discoloration: Continuous variation (Quantitative/polygenic trait(s)

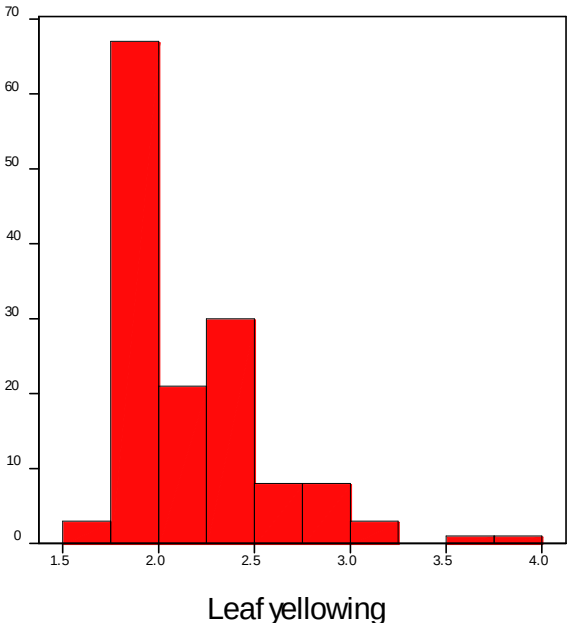


Figure 2. Cumulative histogram showing nature of inheritance for leaf yellowing: continuous variation (Quantitative/polygenic trait(s)

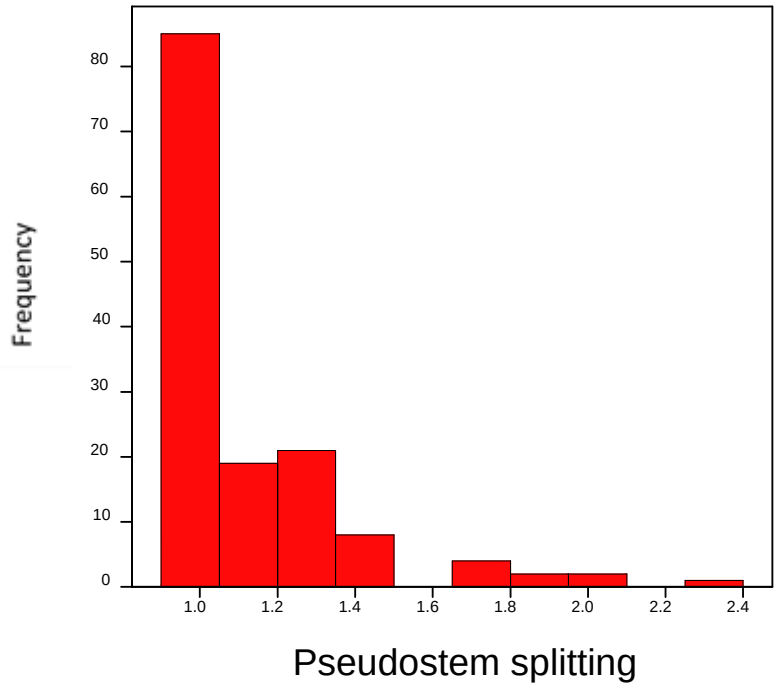


Figure 3. Cumulative histogram showing nature of inheritance for stem splitting: Qualitative (monogenic) traits

i. Genetic ratios

The DSI for RDI grouped the F_1 progenies as 117 resistant (scale of 1.0-3.0) and 25 as susceptible (Scale 3.1-6.0), DSI for LSI grouped the F_1 progenies as 73 resistant (Scale 1.0-2.0) and 69 susceptible (Scale 2.1-5.0) whereas for PSS, 88 F_1 progenies were resistant (Scale 1.0) and 54 susceptible (1.1-3.0). The segregation ratio for RDI fitted the two gene model ratio of 13:3 while PSS fitted two gene model ratio of (11:5) using chi square goodness of fit test (Table 8). LSI segregation did not fit either of the one gene model ratios nor the two gene model ratios tested. The 13:3 ratio is described as, complete dominance at both gene pairs; however, when either gene is dominant, it hides the effects of the other gene while 11:5 is complete dominance for both gene pairs only if both kinds of dominant alleles are present; otherwise, the recessive phenotype appears.

Table 8 The goodness of fit χ^2 test for the response of 142 F_1 banana progenies from Monyet x Kokopo following inoculation with *Fusarium oxysporum* f. sp. *cubense* race 1.

Parameter	Genetic ratio	Resistant	Susceptible	$\chi 2$	$\chi 2$ (Probability)
RDI	13:3	117	25	0.12	0.73
PSS	11:5	88	54	3.04	0.08

χ^2 = Chi-square test statistic

ii. Broad sense heritability

Computing the broad sense heritability variance components indicated that parameters for resistance to Foc race 1 had a relatively low heritability. Corm discoloration which is the main parameter for estimating fusarium wilt resistance in banana had a heritability of 27.8%. Leaf yellowing and pseudostem splitting had broad sense heritabilities of 13.9% and 14.7% respectively, Table 9.

Table 9. Broad sense heritability for fusarium resistance parameters

Source of variation	d.f.	Corm discoloration	Leaf yellowing	Pseudostem splitting
Rep	5	2.4	0.4	0.3
Gen	141	5.7	0.8	0.4
Residual	705	1.7	0.4	0.2
VE		1.7	0.4	0.2
VG		0.7	0.1	0.03
VP		2.4	0.5	0.2
Heritability (%)		27.8	13.9	14.7

Objective / Study 4. To identify polymorphic and heritable molecular markers for *Foc* race 1 and race 4

- DNA were extracted from cigar leaves of *Foc* segregating populations + parents (Min CTAB)
    - DNA qualification by electrophoresis using a 1% agarose gel
    - DNA quantification using Nanodrop

- PCR was run for parent DNA (Monyet + Kokopo and Calcutta 4 + Mshale) against **4 IRAP and 40 ISSR** markers to identify markers showing polymorphism for the contrasting parents.
- Gradient PCR was run for **37 SSR** markers against parent DNA (Monyet + Kokopo and Calcutta 4 + Mshale) to determine the best annealing temperature at which the primers amplify the DNA. Then the primers that showed polymorphism at those temperatures were selected.
- Data was analysed by scoring presence or absence of bands

Results

A. Identifying markers showing polymorphism within parents contrasting for Foc race 1 parents.

1. Monyet and Kokopo

Some of the markers screened with the Kokopo and Monyet parents showed polymorphism among the two parents. 2 IRAPS, 10 ISSRs (Table 10) and 15 SSRs (Table 11) showed polymorphism between Kokopo and Monyet and were subsequently used to screen their F_1 hybrids to identify markers that are heritable.

Table 10. IRAP and ISSR markers that showed polymorphism between the contrasting parents of Monyet and Kokopo

Category		Primer Name	Annealing Temps.	Sequence
IRAP			62^{o}C	
	1	GyLTRev		5'CTTAGGCAAAACCAGCTAAGTCCG 3'
	2	Sukkula		5' GATAGGGTCGCATCTTGGGCGTGAC 3'
ISSR			50^{o}C	
	1	CTC6T		5'CTCCTC CTCCTC CTCCTCT3'
	2	AC10T		5'ACACACAC ACACACACACACT3'
	3	CA10G		5'CACA CACA CACACACA CACAG3'
	4	AC10G		5'ACAC ACAC ACACACAC ACACG3'
	5	CTC6G		5'CTCCTC CTCCTC CTCCTCG3'
	6	TG10G		5'TGTG TGTG TGTGTGTG TGTGG3'
	7	GTG6T		5'GTGGTG GTGGTG GTGGTGT3'
	8	TC10A		5'TCTC TCTC TCTCTCTC TCTCA'
	9	GTG6A		5'GTGGTG GTGGTG GTGGTGA3'
	10	CAC6T		5'CACCAC CACCAC CACCACA3'

Table 11. SSR markers that showed polymorphism between the contrasting parents of Monyet and Kokopo

Category		Primer Name		Sequence	Annealing Temp
SSR	1	AGMI189	Forward	5'AACACCGTACAGGGAGTCAC3'	49.9
		AGMI190	Reverse	5'GTGAGATAAACAATTACTAGGG3'	
	2	AGMI129	Forward	5'GGAGGCCCAACATAGGAAGAGGAAT3'	54
		AGMI130	Reverse	5'CACAACCACACACAGCCAATCTTTC3'	
	3	AGMI197	Forward	5'CTTTTGGAGATTATTGCCTACA3'	55
		AGMI198	Reverse	5’AGTAATCTTTTGTCCTTCAGCT3'	
	4	AGMI199	Forward	5'TATCCATCGACGTGATCCC3'	55
		AGMI200	Reverse	5'TACGATATTGGAATCTCCG3'	
	5	AGMI127	Forward	5'AAGTTAGGTCAAGATAGTGGGATTT3'	55
		AGMI128	Reverse	5'GTCCCTCGATTGGTTCCAAGC3'	
	6	AGMI187	Forward	5'GCAACTTTGGCAGCATTTT3'	55
		AGMI188	Reverse	5'TGAGATATAGAGGAAAATAATGTTA3'	
	7	AGMI131	Forward	5'ATCTTTTCTTATCCTTCTAACG3'	55
		AGMI132	Reverse	5'CGCTTTAGATTCTGTTTAAG3'	
	8	AGMI145	Forward	5'AGCTATTACTTGTTTTTATCTTGAA3'	55
		AGMI146	Reverse	5'AAGGACANAAAAGACAGGA3'	
	9	AGMI139	Forward	5'GGGGAACAGCACGGTCACAT3'	55
		AGMI140	Reverse	5'ACGATGACAACCATTACTAC3'	
	10	AGMI141	Forward	5'TACAAAGAGAAAGTGCAGGGGAATA3'	55
		AGMI142	Reverse	5'CNGCTATAAAGACCACCAGCTTCAT3'	
	11	AGMI137	Forward	5'CTTCCTTTCTGTCTTTTTGATTGTA3'	56
		AGMI138	Reverse	5'GCAAGTCCTTCTGAATCTTAT3'	
	12	AGMI159	Forward	5'GTTTGGTTGATCCTCCCTTTA3'	56
		AGMI160	Reverse	5'GAAAACAAGAGAGAGAGAGAGAG3'	
	13	AGMI203	Forward	5'TGCTGCCTTCATCGCTACTA3'	56
		AGMI204	Reverse	5'GGAACATCGCCCCCGCCAC3'	
	14	AGMI147	Forward	5'CTGCAGCAACCCAAATTTATTTC3'	56
		AGMI148	Reverse	5'AAATAAGCTCATATGGGTACAGTCA3'	
	15	AGMI143	Forward	5'TCAAGAGCAATGAAGACCTCAAA3'	56
		AGMI144	Reverse	5'TTTTACATGTACAAGGTCAAGCAAT3'	

2. Mshale and Calcutta 4

Some of the markers screened with the Mshale and Calcutta 4 parents showed polymorphism among the two parents. 2 IRAPS, 10 ISSRs (Table 12) and 15 SSRs (Table 13) showed polymorphism between Mshale and Calcutta 4 and were subsequently used to screen their F_1 hybrids to identify markers that are heritable.

Table 12. IRAP and ISSR markers that showed polymorphism between the contrasting parents of Calcutta 4 and Mshale

Category		Primer Name	Annealing Temps.	Sequence
IRAP				
	1	GyLTRev	62^{o}C	5'CTTAGGCAAAACCAGCTAAGTCCG 3'
	2	Sukkula		5' GATAGGGTCGCATCTTGGGCGTGAC 3'
ISSR				
	1	CTC6T	50^{o}C	5'CTCCTC CTCCTC CTCCTCT3'
	2	AC10T		5'ACACACAC ACACACACACACT3'
	3	AC10G		5'ACAC ACAC ACACACAC ACACG3'
	4	CTC6G		5'CTCCTC CTCCTC CTCCTCG3'
	5	TC10A		5'TCTC TCTC TCTCTCTC TCTCA'
	6	GTG6A		5'GTGGTG GTGGTG GTGGTGA3'
	7	CAC6T		5'CACCAC CACCAC CACCACA3'
	8	CT10G		5'CTCT CTCT CTCTCTCT CTCTG3'
	9	TCG6G		5'TCGTCG TCGTCG TCGTCGG3'
	10	TCG6A		5'TCGTCG TCGTCG TCGTCGA3'
	11	CTC6A		5'CTCCTC CTCCTC CTCCTCA3'
	12	ACC6T		5'ACCACC ACCACC ACCACCT3'
	13	AC10C		5'ACAC ACAC ACACACAC ACACC3'
	14	ACC6G		5'ACCACC ACCACC ACCACCG3'

Table 13. SSR markers that showed polymorphism between the contrasting parents of Calcutta 4 and Mshale

Category		Primer Name		Sequence	Annealing Temp
SSR	1	AGMI189	Forward	5'AACACCGTACAGGGAGTCAC3'	47
		AGMI190	Reverse	5'GTGAGATAAACAATTACTAGGG3'	
	2	AGMI133	Forward	5'GTGGTTTGGCAGTGGAATGGAA3'	47
		AGMI134	Reverse	5'GTATGGCTCAGCTGTATCCATC3'	
	3	AGMI155	Forward	5'CGAAACCTGCTGGACGAGT3'	50
		AGMI156	Reverse	5'CGGGACCCAAGGAGGAGG3'	
	4	AGMI187	Forward	5'GCAACTTTGGCAGCATTTT3'	52
		AGMI188	Reverse	5'TGAGATATAGAGGAAAATAATGTTA3'	
	5	AGMI131	Forward	5'ATCTTTTCTTATCCTTCTAACG3'	52
		AGMI132	Reverse	5'CGCTTTAGATTCTGTTTAAG3'	
	6	AGMI201	Forward	TGGTTGAGTAGATCTTCTTGTGTTC	52
		AGMI202	Reverse	CAAGAAAATGATAATACCATAATGA	
	7	AGMI145	Forward	5'AGCTATTACTTGTTTTTATCTTGAA3'	54
		AGMI146	Reverse	5'AAGGACANAAAAGACAGGA3'	
	8	AGMI129	Forward	5'GGAGGCCCAACATAGGAAGAGGAAT3'	54
		AGMI130	Reverse	5'CACAACCACACACAGCCAATCTTTC3'	
	9	AGMI147	Forward	5'CTGCAGCAACCCAAATTTATTTC3'	55.2
		AGMI148	Reverse	5'AAATAAGCTCATATGGGTACAGTCA3'	
	10	AGMI139	Forward	5'GGGGAACAGCACGGTCACAT3'	56
		AGMI140	Reverse	5'ACGATGACAACCATTACTAC3'	
	11	AGMI137	Forward	5'CTTCCTTTCTGTCTTTTTGATTGTA3'	56
		AGMI138	Reverse	5'GCAAGTCCTTCTGAATCTTAT3'	
	12	MusaBAG1_SSR1_F	Forward	5'GACTCTGGAGCATCTTGTCCAT3'	56
		MusaBAG1_SSR1_R	Reverse	5'CTTTATCTTCGCCAACCCTAACGG3'	
	13	AGMI203	Forward	5'TGCTGCCTTCATCGCTACTA3'	58
		AGMI204	Reverse	5'GGAACATCGCCCCCGCCAC3'	
	14	AGMI143	Forward	5'TCAAGAGCAATGAAGACCTCAAA3'	58
		AGMI144	Reverse	5'TTTTACATGTACAAGGTCAAGCAAT3'	
	15	MusaBAG1_SSR3_F	Forward	5'GGATGGAATTCTCCTCCATCTC3'	58
		MusaBAG1_SSR3_R	Reverse	5'GGAAGGAGAAGGATGCATGAAACAGG3'	

B. Polymorphic and heritable markers within Kokopo x Monyet F1 hybrid population

1. Kokopo x Monyet

Screening the markers that revealed polymorphism with Kokopo and Monyet parents with their F_1 hybrids revealed important polymorphism within the population. 2 IRAP, 5 ISSR and 10 SSR markers showed heritable bands from the parents to the F_1 offspring (Table 14). The inherited bands will be used to determine the recombination frequency and subsequently determine if they are linked to Fusarium wilt.

Table 14. Polymorphic and heritable markers within Kokopo x Monyet F1 hybrid population

	IRAP	**% Polymorphism**
1	GyLTRev	35.7
2	Sukkula	
	ISSR	
1	GTG6A	20
2	AC10T	11.8
3	CTC6T	9.1
4	CA10G	8.3
5	GTG6T	5.6
	SSR	
1	AGMI 131-132	
2	AGMI 137-138	
3	AGMI 139-140	
4	AGMI 145-146	
5	AGMI 147-148	
6	AGMI 187-188	
7	AGMI 141-142	
8	AGMI 189-190	
9	AGMI 197-198	
10	AGMI 199-200	

2. Mshale x Calcutta 4

Screening the markers that revealed polymorphism with Mshale x Calcutta 4 parents with their F_1 hybrids revealed important polymorphism within the population. 2 IRAP, 5 ISSR and 13 SSR markers showed heritable bands from the parents to the F_1 offspring (Table 15). The inherited bands will be used to determine the recombination frequency and subsequently determine if they are linked to Fusarium wilt.

Table 15. Polymorphic and heritable markers within Mshale x Calcutta 4 F1 hybrid population

	IRAP	% Polymorphism
1	GyLTRev	50
2	Sukkula	
	ISSR	
1	GTG6A	35.7
2	AC10T	14.3
3	AC10G	26.7
4	CAC6T	23.5
5	ACC6G	11.1
	SSR	
1	AGMI 131-132	
2	AGMI 137-138	
3	AGMI 139-140	
4	AGMI 145-146	
5	AGMI 147-148	
6	AGMI 187-188	
7	AGMI 133-134	
8	AGMI 155-156	
9	AGMI 143-144	
10	AGMI 201-202	
11	AGMI 203-204	
12	MusaBAG1_SSR1	
13	MusaBAG1_SSR3	

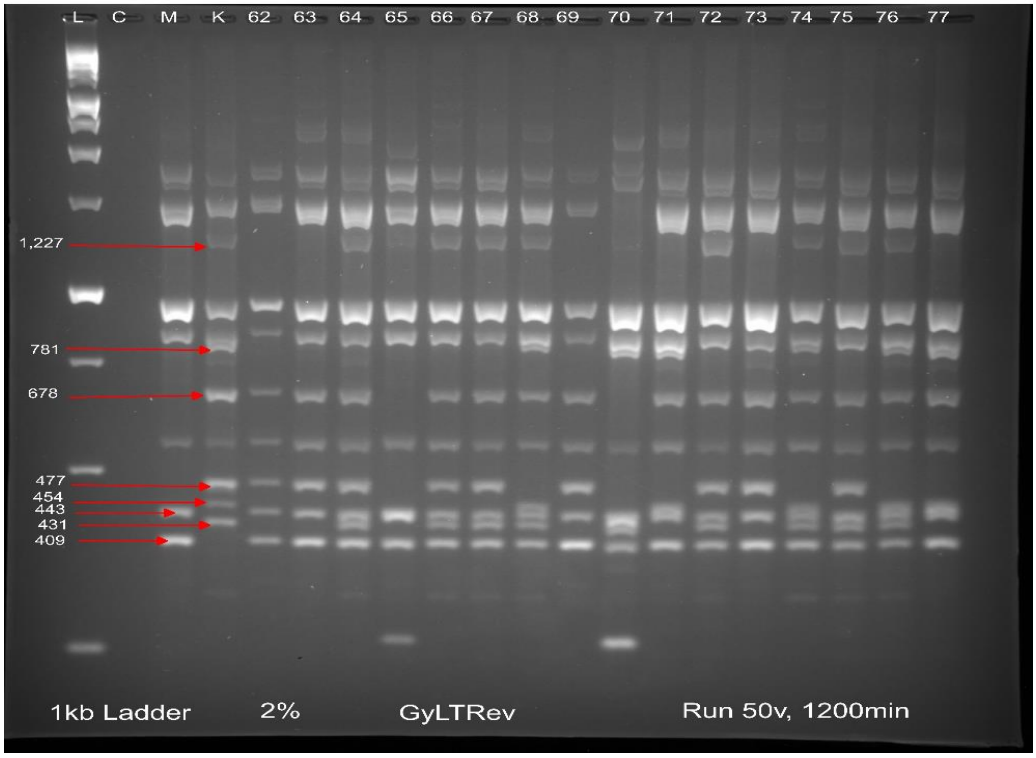


Figure 4. Gel picture showing polymorphic and heritable bands of Monyet x Kokopo using IRAP- GyLTRev

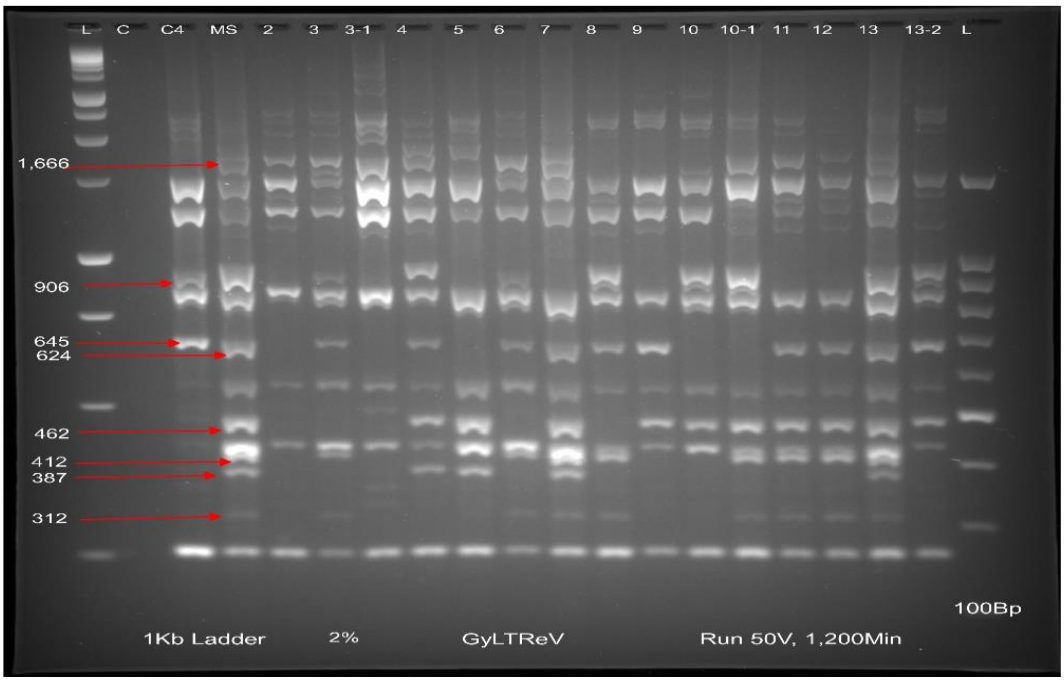


Figure 5. Gel picture showing polymorphic and heritable bands of Mshale x Calcutta 4 IRAP- GyLT Mshale x Calcutta 4

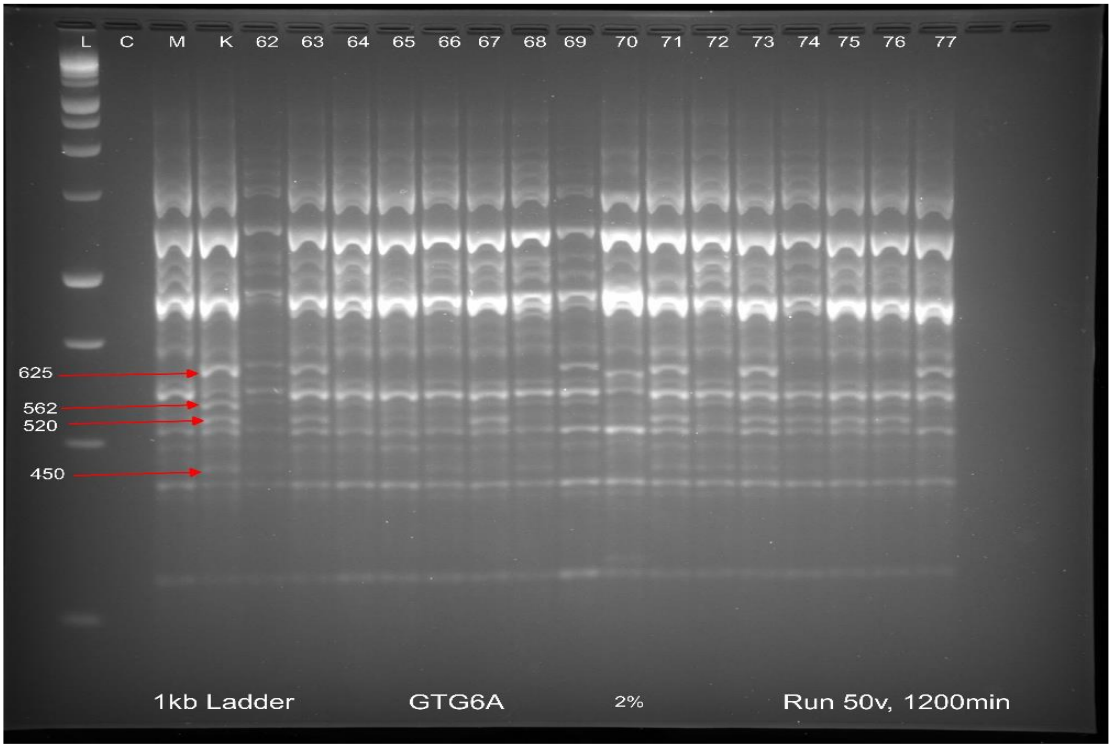


Figure 6. Gel picture showing polymorphic and heritable bands of Monyet x Kokopo ISSR- GTG6A

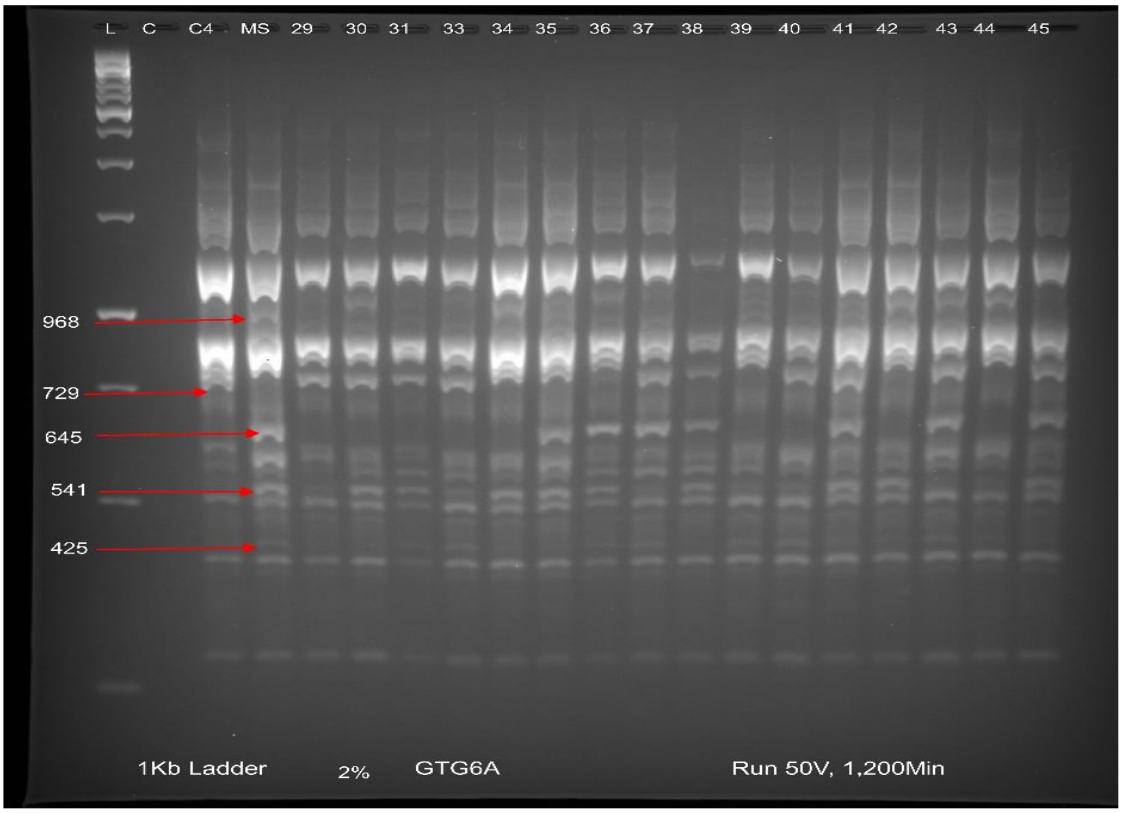


Figure 7 Gel picture showing polymorphic and heritable bands of Mshale x Calcutta4 ISSR- GTG6A

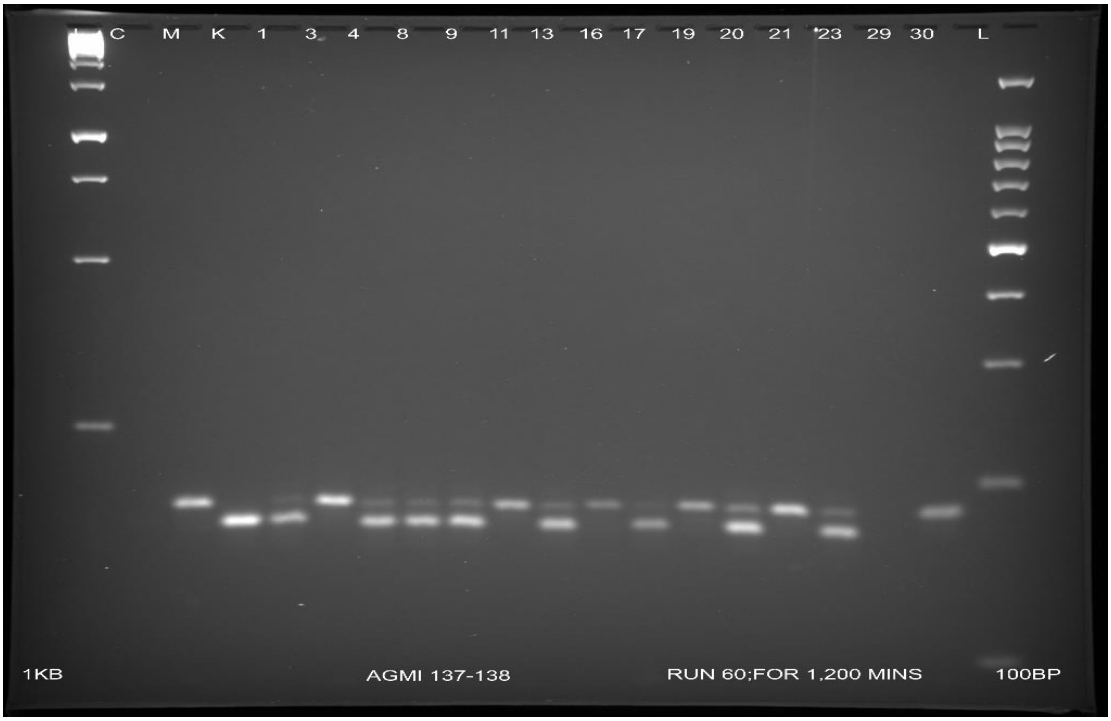


Figure 8 Gel picture showing polymorphic and heritable bands of Monyet x Kokopo SSR-AGMI 137-138

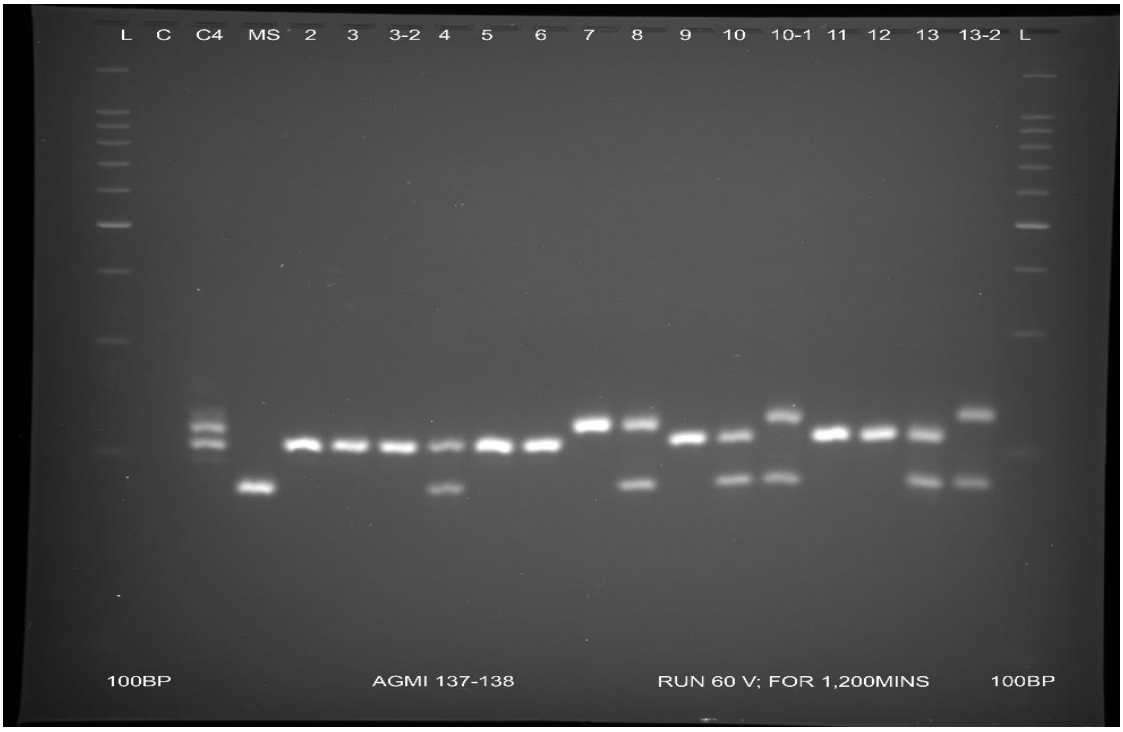


Figure 9. Gel picture showing polymorphic and heritable bands of Mshale x Calcutta4 SSR- AGMI 137-138

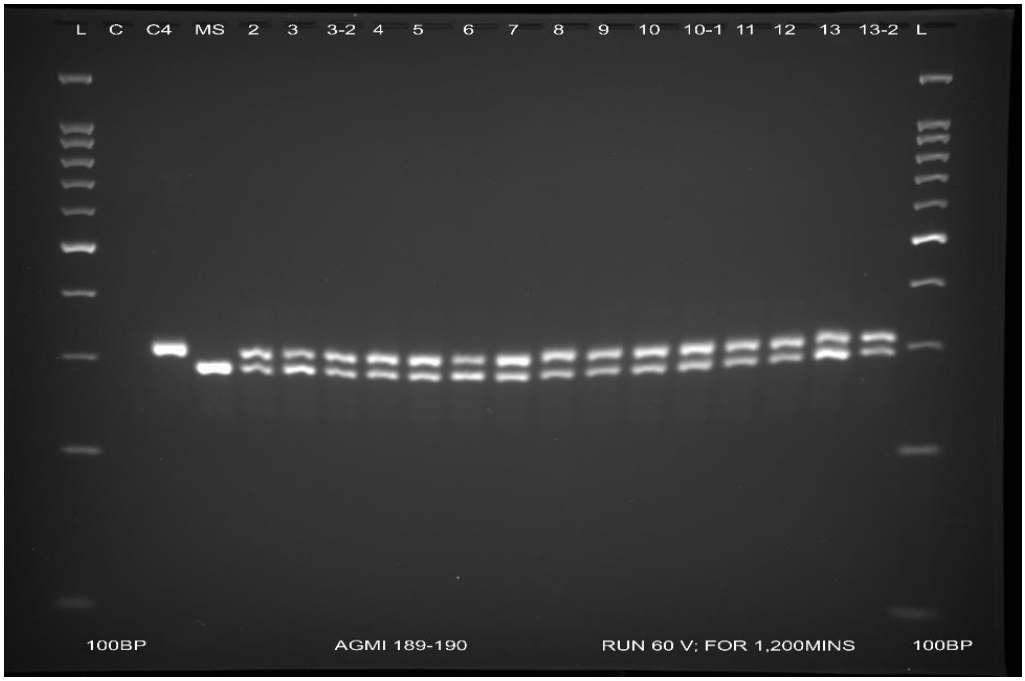


Figure 10. Gel picture showing polymorphic and heritable bands of Mshale x Calcutta4 SSR- AGMI 189-190

Neighbour joining tree for Monyet x Kokopo F1 hybrids

Most bands across the markers 12/15 generated from dominant markers that were segregating in the F_1 hybrids of Monyet X Kokopo arose from the Male parent of Kokopo (Susceptible). Only three markers (AC10T_1, AC10T_2 and Sukkula_3) had a present band in the female parent Monyet (resistant) that were segregating in the F_1 hybrids. The F_1 hybrids with a band were the most in numbers compared to hybrids without bands which arose from markers GTG6T_1, GyLTRev_2 and Sukkula_1 only. Also, most F_1 hybrids were resistant to Foc race 1 and hybrids with susceptibility were fewer (Table 16).

Table 16 Dominant markers (Bands) arising from the parents and the respective number of F_1 hybrids corresponding to those bands.

	Parental		No. of F_1 hybrids		Total bands
Marker	M (Resistant)	K (Susceptible)	R	S	
AC10T_1	1		79	25	104
		0	27	7	34
AC10T_2	1		77	26	103
		0	28	7	35
CA10G_1		1	58	18	76
	0		48	14	62
CTC6T_1		1	68	19	87
	0		41	10	51
GTG6A_1		1	65	18	83
	0		41	14	55
GTG6A_2		1	72	24	96
	0		34	8	42
GTG6A_3		1	48	12	60
	0		58	20	78
GTG6T_1		1	48	14	62
	0		59	17	76
GyLTRev_1		1	57	19	76
	0		49	12	61
GyLTRev_2		1	28	6	34
	0		78	26	104
GyLTRev_3		1	72	23	95
	0		34	9	43
GyLTRev_4		1	109	28	137
	0		1	0	1
Sukkula_1		1	40	8	48
	0		65	25	90
Sukkula_2		1	66	12	78
	0		40	20	60
Sukkula_3	1		79	16	95
		0	28	15	43

Cluster analysis using UGENE generated two cladograms using both dominant markers (ISSR and IRAP) Figure 11 (a) and codominant markers (SSR) Fig 11 (b) that were able to cluster the F_1 genotypes into various clusters.

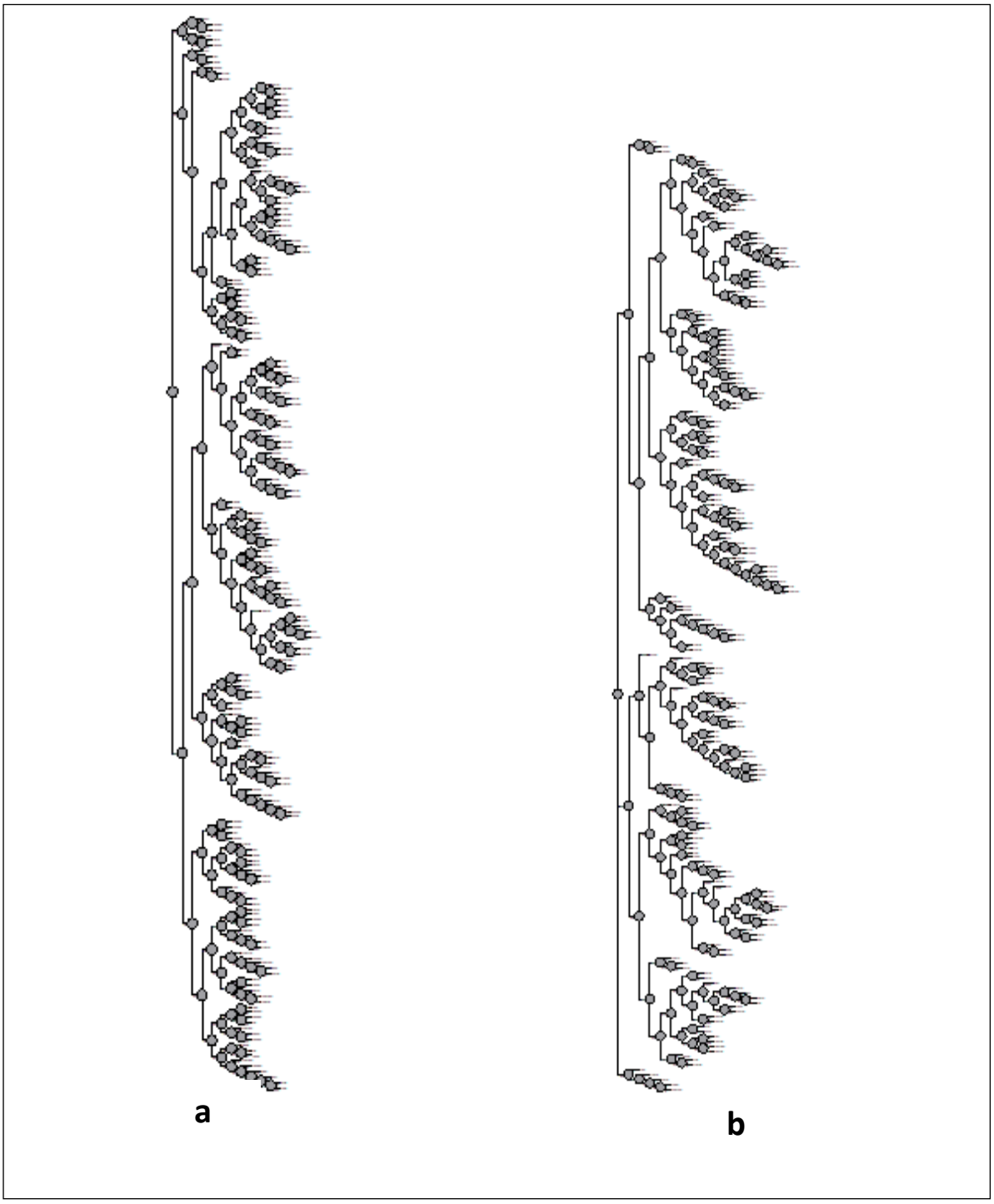


Figure 11 (a) cladogram for Kokopo x Monyet population using dominant markers (ISSR and IRAP) (b) cladogram for Kokopo x Monyet population using codominant markers (SSR).

Analysing the clusters further, revealed that there were some clusters that consisted mostly of susceptible genotypes (S) with red colour, resistant genotypes (R) with green colour and same cross (C) with yellow colour as shown in phylograms of constructed using dominant markers (ISSR and IRAP) (Figure 12) and codominant marker (SSR) (Figure 13).

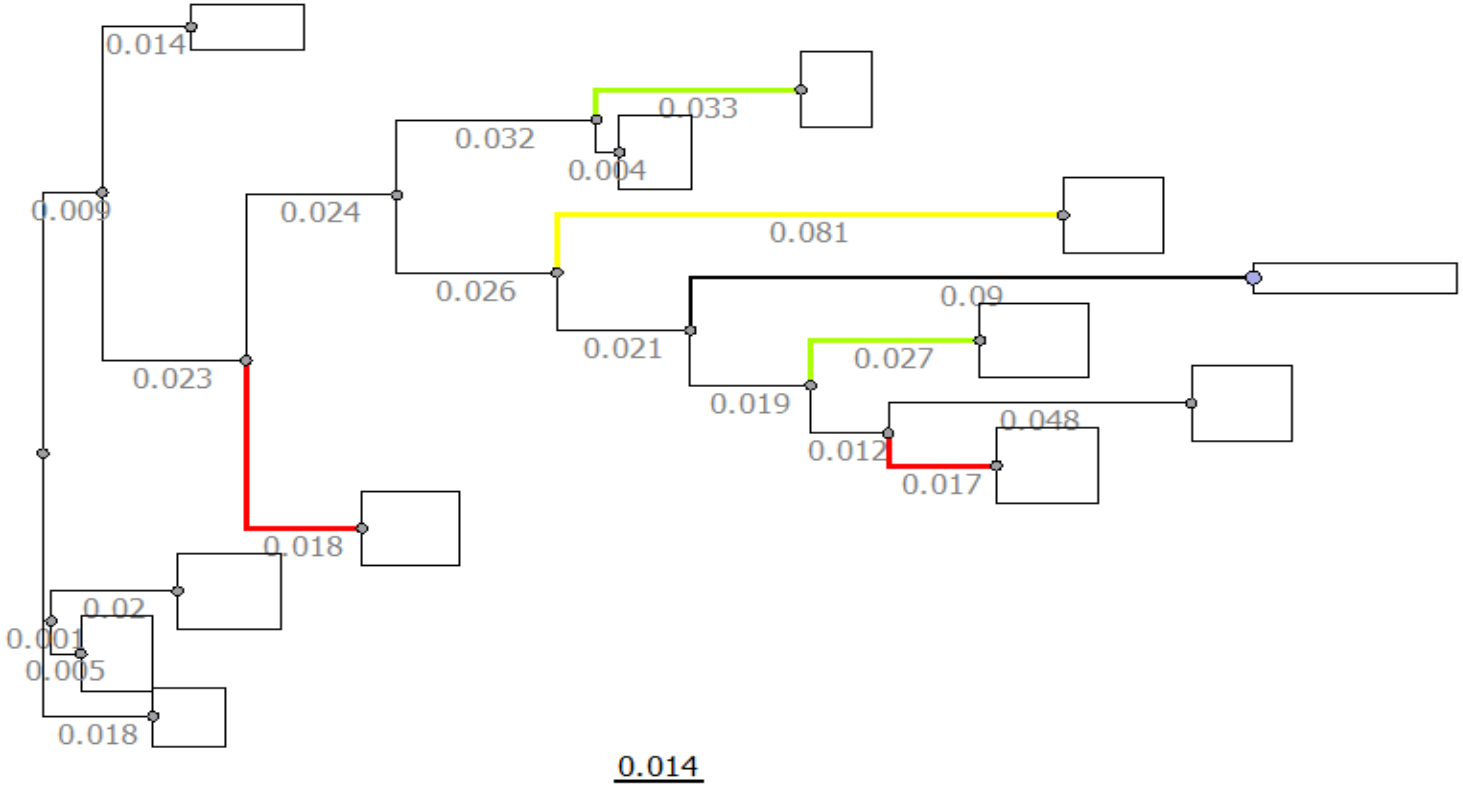


Figure 12 Phylogram showing clustering for F_1 population of Kokopo x Monyet with Dominant markers (ISSR and IRAP) with red colour indicating cluster with mostly susceptible genotypes (S), green colour showing cluster with mostly resistant genotypes (R) and yellow colour showing a cluster with genotypes obtained from mostly the same cross (C)

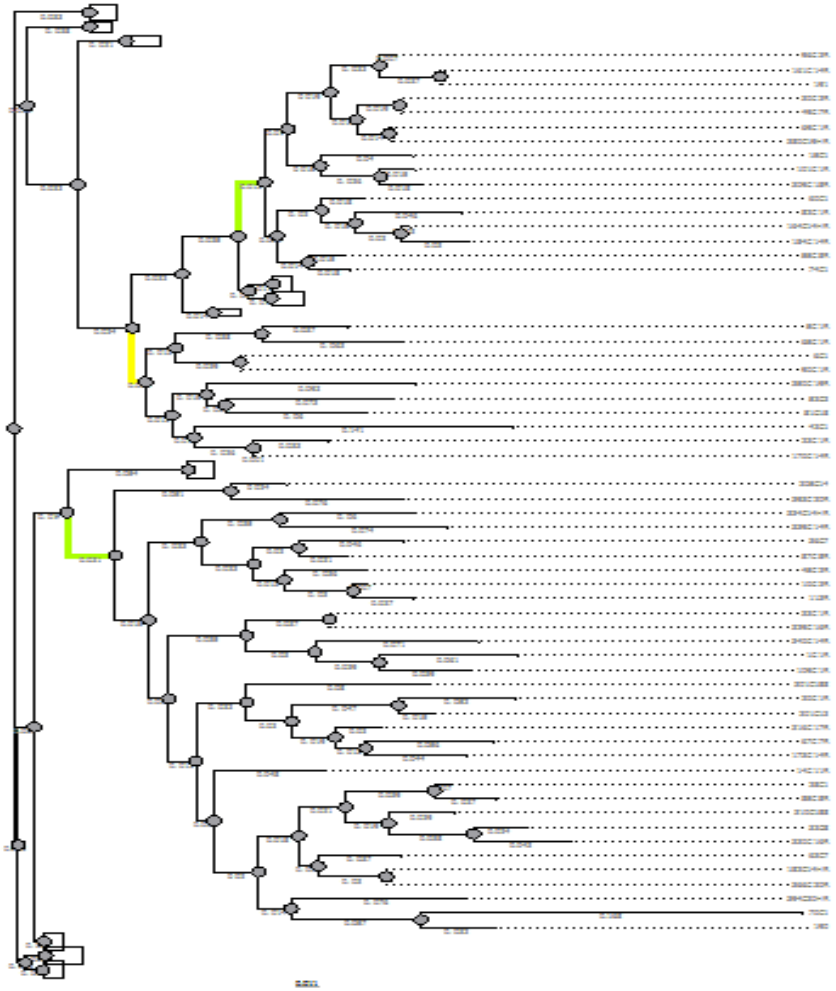


Figure 13 Phylogram showing clustering for F_1 population of Kokopo x Monyet with codominant markers (SSR) with green colour showing cluster with mostly resistant genotypes(R) and yellow colour showing a cluster with genotypes obtained from mostly the same cross (C)

The codominant markers (SSR) could cluster 12 genotypes as resistant and highly resistant from the F_1 hybrids of Kokopo x Monyet in one of the clusters (Figure 14) except for the few genotypes whose resistance status was not known (191, 15, 60 and 74) as they were not screened phenotypically for Foc race 1. Also, another cluster had 22/32 genotypes as resistant, 2/32 were susceptible where as 8 genotypes were not assigned a resistance group as they were not phenotyped for Foc race 1 (Figure 15). Also, dominant markers IRAP and ISSR clustere most resistant genotypes 18/32 and 1/32 genotype as susceptible where as 13 genotypes in this cluster were not assigned a resistance group as they were not phenotyped (Figure 16).

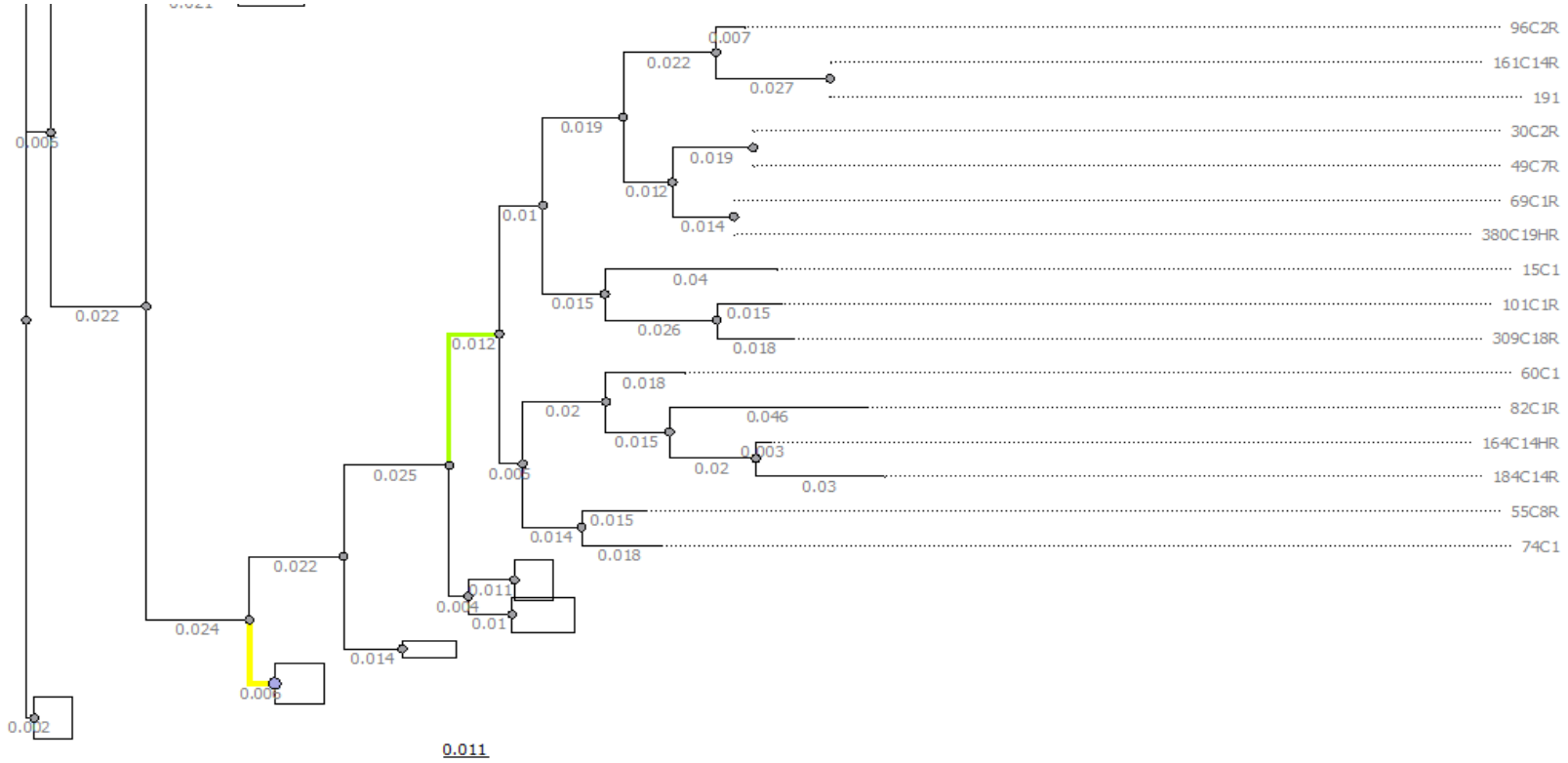


Figure 14 Phylogram for showing the F_1 genotypes mostly clustered as resistant (R) using codominant markers (SSR)

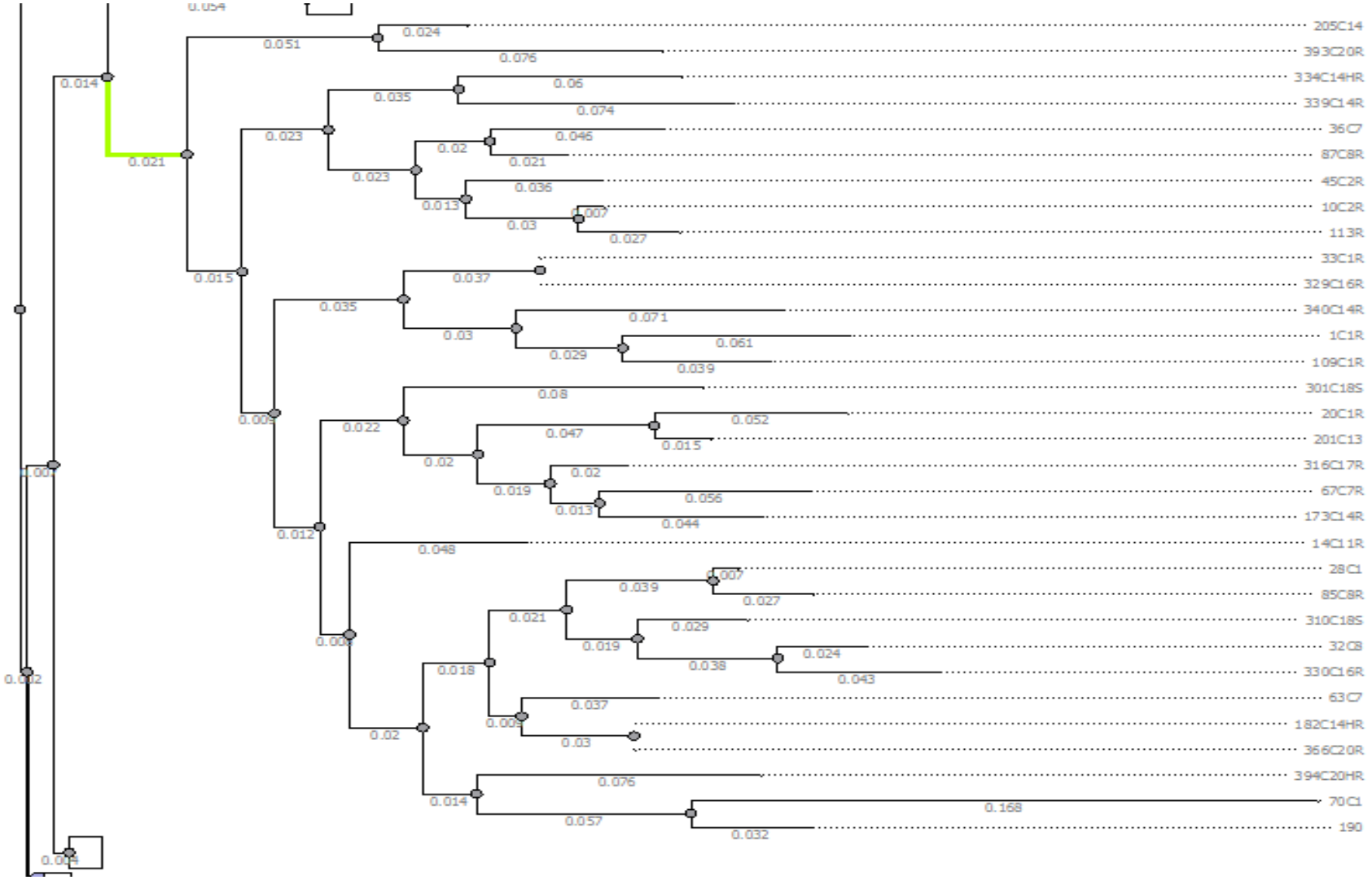


Figure 15 Phylogram for showing the F_1 genotypes mostly clustered as resistant (R) with codominant markers (SSR)

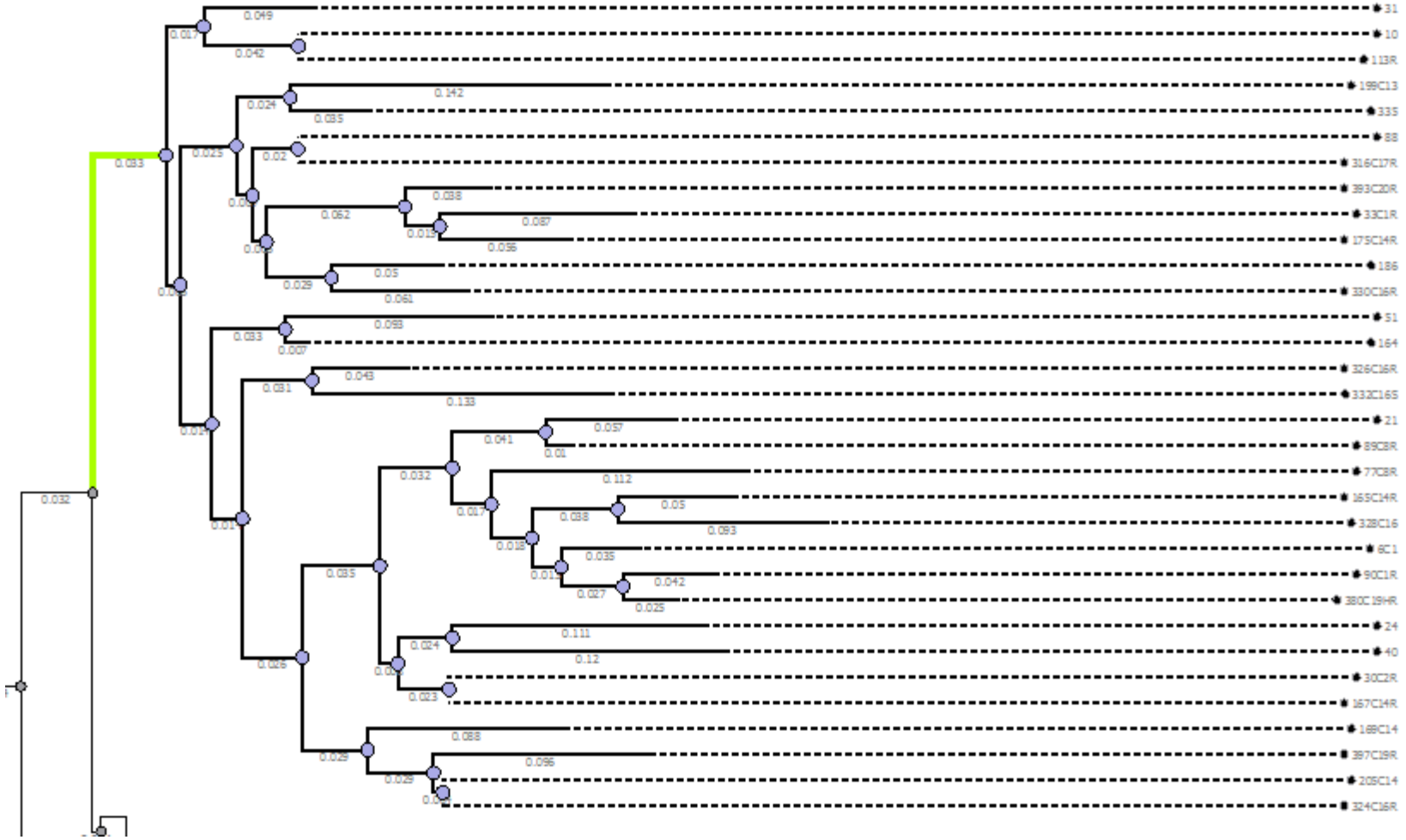


Figure 16 Phylogram for showing the F_1 genotypes mostly clustered as resistant (R) with dominant markers (ISSR and IRAP)

The cluster that contains the resistant F_1 hybrids by dominant markers showed that most genotypes had a band present as compared to genotypes without a band. Over all on average, 8.6 bands within this resistant cluster were present from the 15 markers as compared to an averaging of 6.4 absent bands across the 15 markers. only one genotype (332) was the only susceptible genotype clustered in this resistant cluster and it had bands 7 bands present less than the 8 absent bands across all the 15 markers (Table 17).

Table 17 Resistant genotypes clustered together using dominant markers (ISSR and IRAP) showing number of present and absent bands

Resistant cluster	6C1	10C2R	21C1	24C10	30C2R	31C1R	33C1R	40C10R	77C8R	88C1S	89C8R	90C1R	113R	164C14HR	165C14R	167C14R	169C14	175C14R	186C14R	199C13	206C13R	316C17R	324C16R	326C16R	328C16	330C16R	332C16S	335C14HR	380C19HR	393C20R	397C19R	**Total**
Present bands (1)	8	11	7	7	9	11	7	6	6	11	8	7	11	11	7	9	8	7	9	7	9	11	11	10	7	9	7	10	8	9	9	**8.6**
Absent bands(0)	7	4	8	8	6	4	8	9	9	4	7	8	4	4	8	6	7	8	6	8	6	4	4	5	8	6	8	5	7	6	6	**6.4**

The dominant markers (ISSR and IRAP) could cluster most genotypes as 7/13 susceptible and 5/13 genotypes as resistant from the F1 hybrids of Kokopo x Monyet in one of the clusters (Figure 17) and another cluster of 6/12 as susceptible genotypes and 3/12 genotypes as resistant (Figure 18) except for the genotypes (genotype 37,199,200 and 202) whose resistance status was not known as it was not screened phenotypically for Foc race 1.

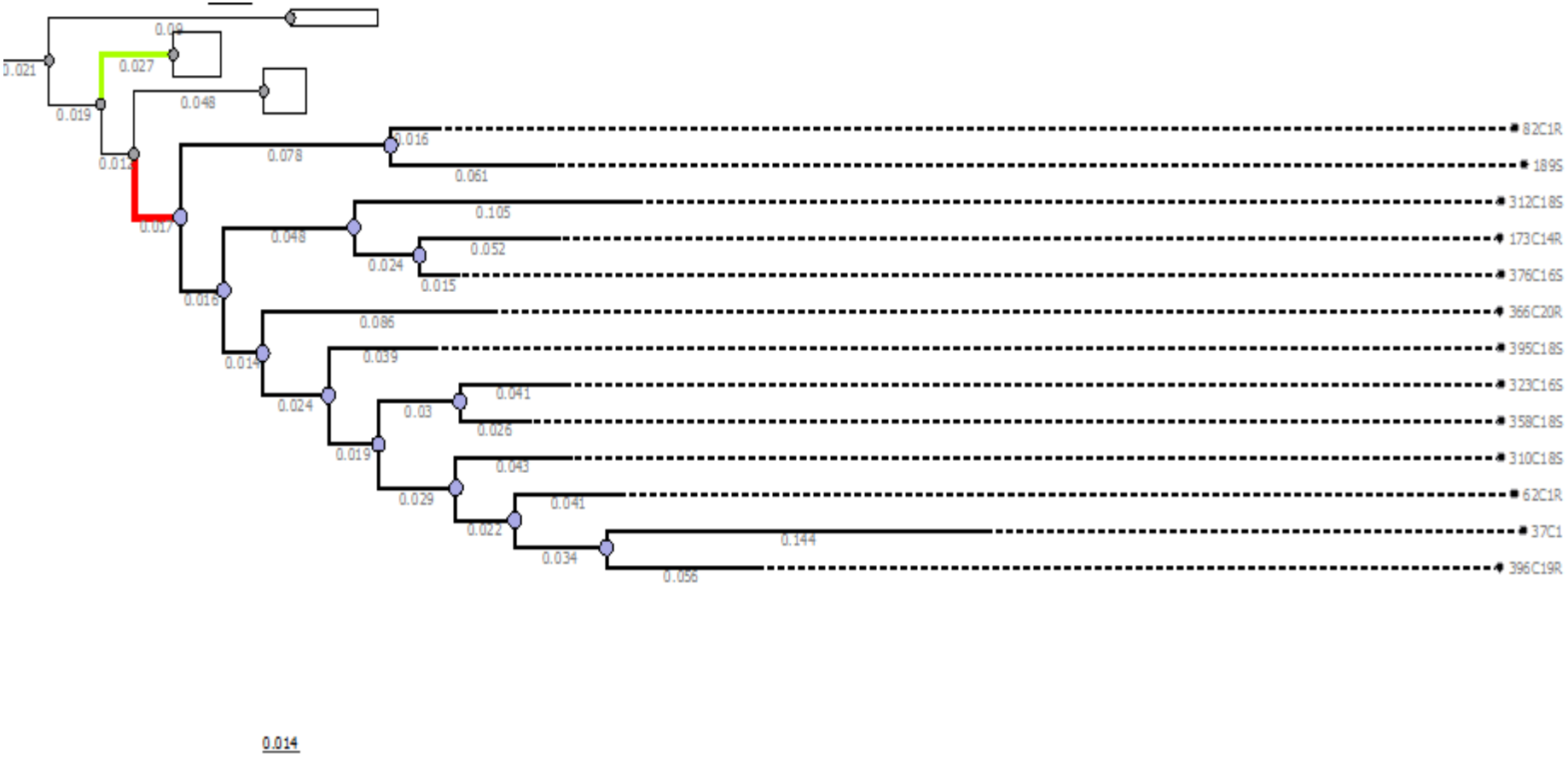


Figure 17 Phylogram for showing the F_1 genotypes mostly clustered as susceptible (S) using dominant markers (ISSR and IRAP)

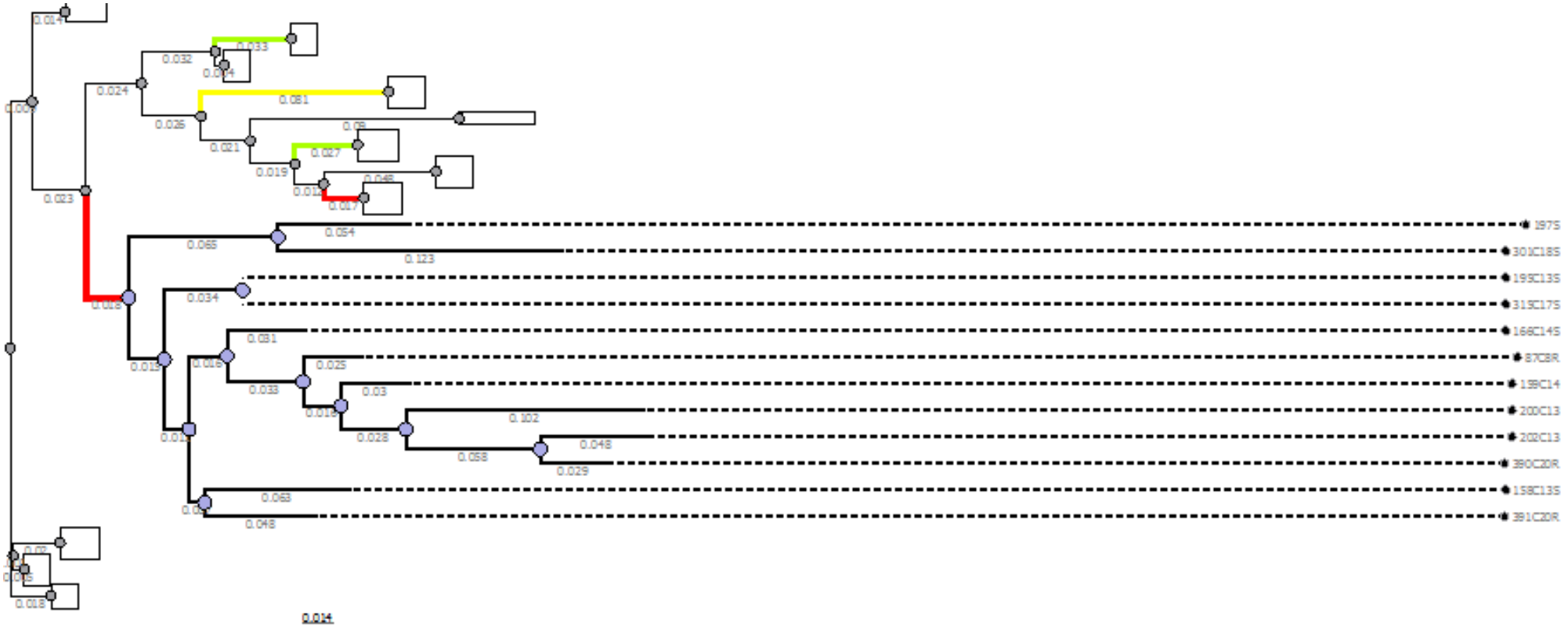


Figure 18 Phylogram for showing the F_1 genotypes mostly clustered as susceptible (S) using dominant markers (ISSR and IRAP)

Genotypes clustered together mostly as susceptible had almost same number of bands present as bands absent across all the 15 markers within both clusters (Table 24 and 25). On average in the first class, 7.2 bands were present and 7.8 absent across all the genotypes in the first cluster (Table 18) and in the second cluster, 7.9 bands were present and 7.1 absent bands across all the genotypes with the 15 markers (Table 19). The resistant genotypes that were clustered in these two susceptible clusters actually had a higher disease score of above 2.3 and cut off point for resistance is 2.9 (5/8 genotypes had score of above 2.3).

Table 18 Genotypes clustered mostly as susceptible by dominant markers in the first cluster

Susceptible cluster 1	37C1	62C1R	82C1R	173C14R	189S	310C18S	312C18S	323C16S	358C18S	366C20R	376C16S	395C18S	396C19R	Total
Present bands (1)	4	7	7	7	6	8	8	7	8	8	8	9	6	**7.2**
Absent bands(0)	11	8	8	8	9	7	7	8	7	7	7	6	9	**7.8**

Table 19 Genotypes clustered mostly as susceptible by dominant markers in the second cluster

Susceptible cluster 2	87C8R	158C13S	169C14	197S	199C13	200C13	202C13	301C18S	319C16S	390C20R	391C20R	Total
Present bands (1)	9	9	8	9	7	6	6	8	9	7	9	**7.9**
Absent bands(0)	6	6	7	6	8	9	9	7	6	8	6	**7.1**

The codominant markers (SSR) could cluster most genotypes 6/9 from same cross (cross 1) except for the three genotypes fthat came from a different crosses among the F_1 hybrids of Kokopo x Monyet (Figure 19).

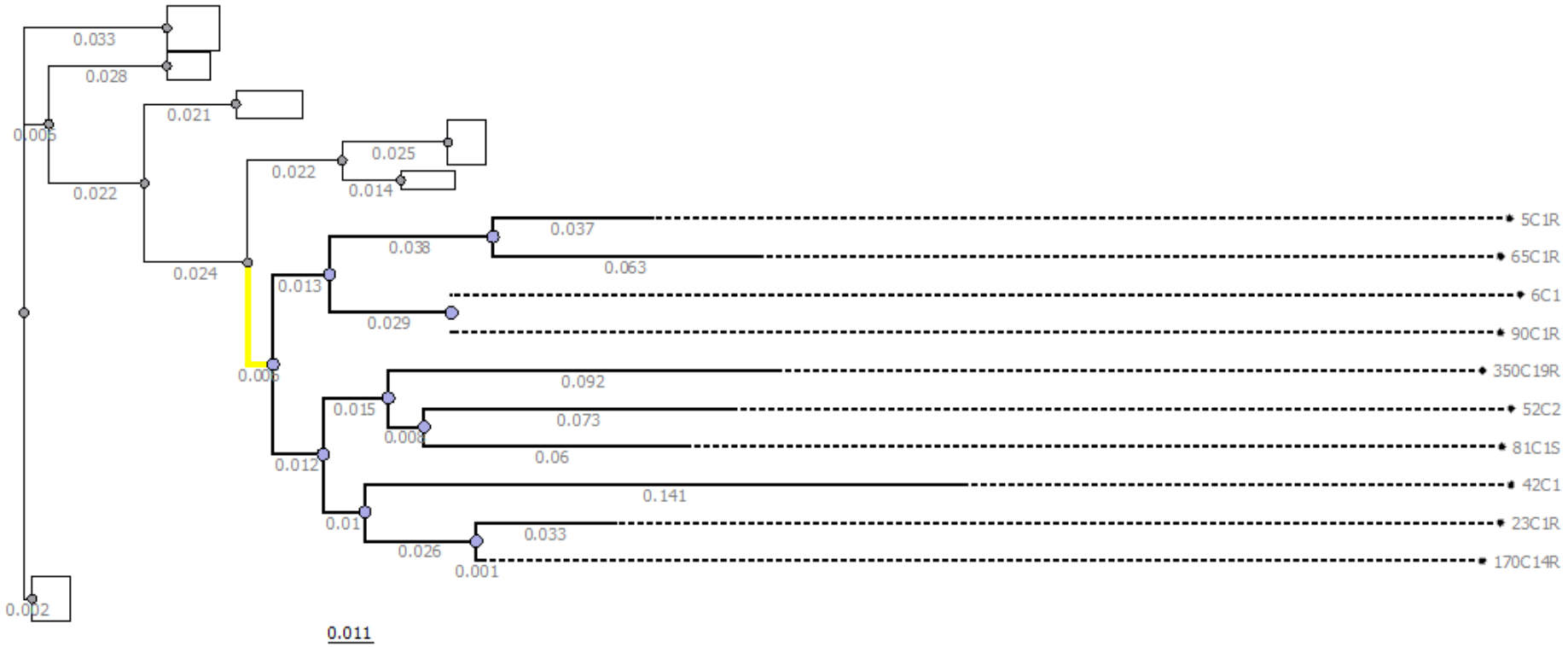


Figure 19 Phylogram for showing the F_1 genotypes mostly clustered with same cross (C1) with codominant markers (SSR)

Objective / Study 5. To perform a QTL analysis for *Foc* race 1 and race 4

1. For IRAP, ISSR and SSR, QTL analysis will be performed using: GACD (Zhang *et al*., 2016). GACD: Integrated Software for Genetic Analysis in Clonal F1 and Double Cross Populations.)

NB. 197 genotypes have been genotyped with IRAP, ISSR and SSR. Analysis of 140 genotypes that have been phenotyped is ongoing at UM.

A total of 35 markers segregating within Monyet x Kokopo F_1 hybrid population and 67 markers segregating within Mshale x Calcutta 4 F_1 population were identified (Table 20). The markers can be utilised for linkage map construction and location of QTL for *Foc* race 1.

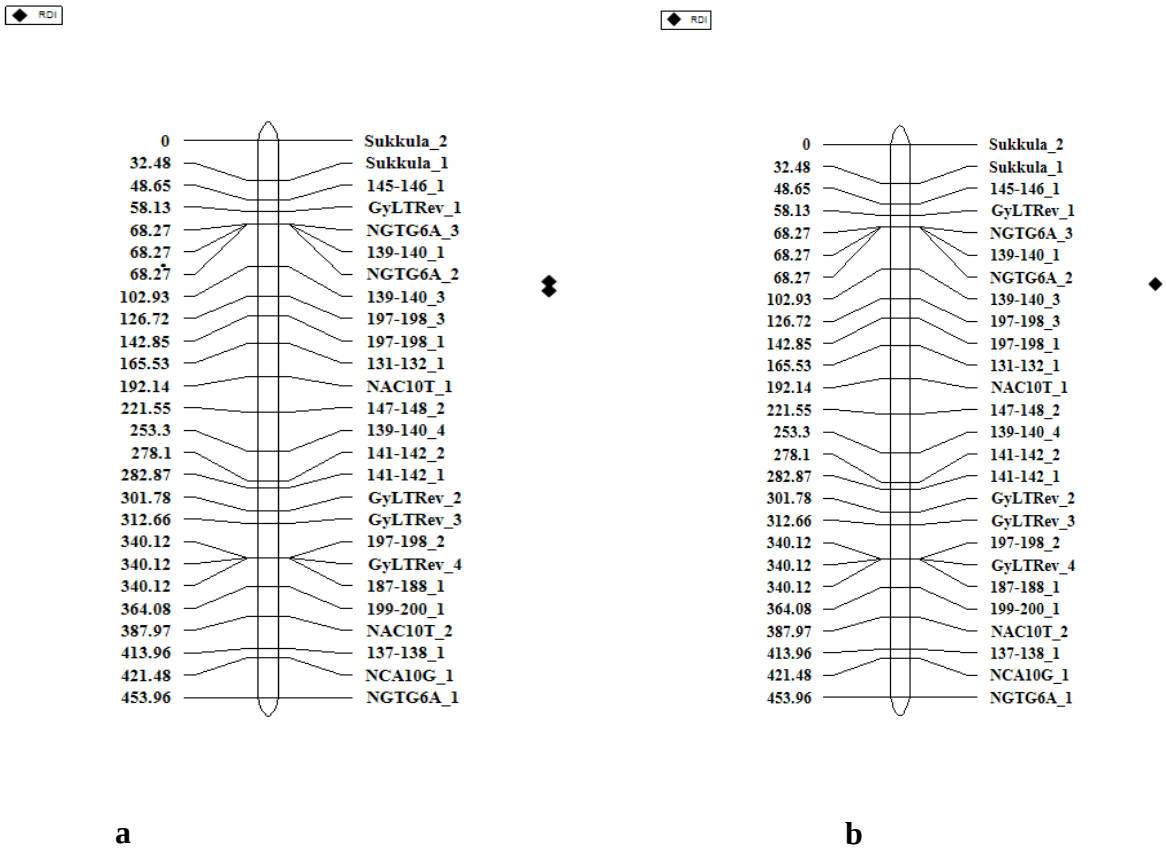
Table 20 Selected markers segregating in F_1 hybrids of Monyet x Kokopo and Mshale x Calcutta 4

Monyet X Kokopo F_1 population			Mshale x Calcutta 4 F_1 population		
IRAP Markers	ISSR Markers	SSR Markers	IRAP Markers	ISSR Markers	SSR Markers
GyLTRev_1	AC10T_1	131-132_1	GyLTRev_1	AC10G_1	131-132_1
GyLTRev_2	AC10T_2	131-132_2	GyLTRev_2	AC10G_2	131-132_2
GyLTRev_3	CA10G_1	137-138_1	GyLTRev_3	AC10G_3	131-132_3
GyLTRev_4	CTC6T_1	139-140_1	GyLTRev_4	AC10G_4	133-134_1
GyLTRev_8	GTG6A_1	139-140_2	GyLTRev_5	AC10G_5	133-134_2
GyLTRev_9	GTG6A_2	139-140_3	GyLTRev_6	AC10T_1	133-134_3
Sukkula_1	GTG6A_3	139-140_4	GyLTRev_7	AC10T_2	139-140_1
Sukkula_2	GTG6T_1	141-142_1	GyLTRev_8	AC10T_3	139-140_2
Sukkula_3		141-142_2	GyLTRev_9	ACC6G_1	139-140_3
		141-142_3	Sukkula_1	ACC6G_2	139-140_4
		145-146_1	Sukkula_2	ACC6G_3	139-140_5
		147-148_1	Sukkula_3	CAC6T_1	143_144_1
		147-148_2		CAC6T_2	145-146_1
		189-190_1		CAC6T_3	145-146_2
		197-198_1		GTG6A_1	147-148_1
		197-198_2		GTG6A_2	147-148_2
		197-198_3		GTG6A_3	147-148_3
		199-200_1		GTG6A_4	155-156_1
				TCG6G_1	155-156_2
				TCG6G_2	155-156_3
				TCG6G_3	155-156_4
					187-188_1
					187-188_2

187-188_3
201-202_1
201-202_2
203-204_1
203-204_2
203-204_3
BAG1-SSR1_1
BAG1-SSR2_1
BAG1-SSR2_2
BAG1-SSR2_3
BAG1-SSR2_4

QTL analysis in an F_1 population of Monyet x Kokopo

Marker trait association using GACD software revealed two QTLs on linkage group (LG) 1 (Figure 20a) at LOD score of 2.5. When a higher LOD score of 4.0 was used, one QTL at LG 1 remained (Figure 20b). The markers used were distributed on 7 LG within the whole genome (Figure 21).



a **b**

Figure 20 QTLs for *Foc* race 1 resistance on linkage group 1: **a** is LG 1 at LOD = 2.5 and **b** is LG 1 at LOD = 4.0

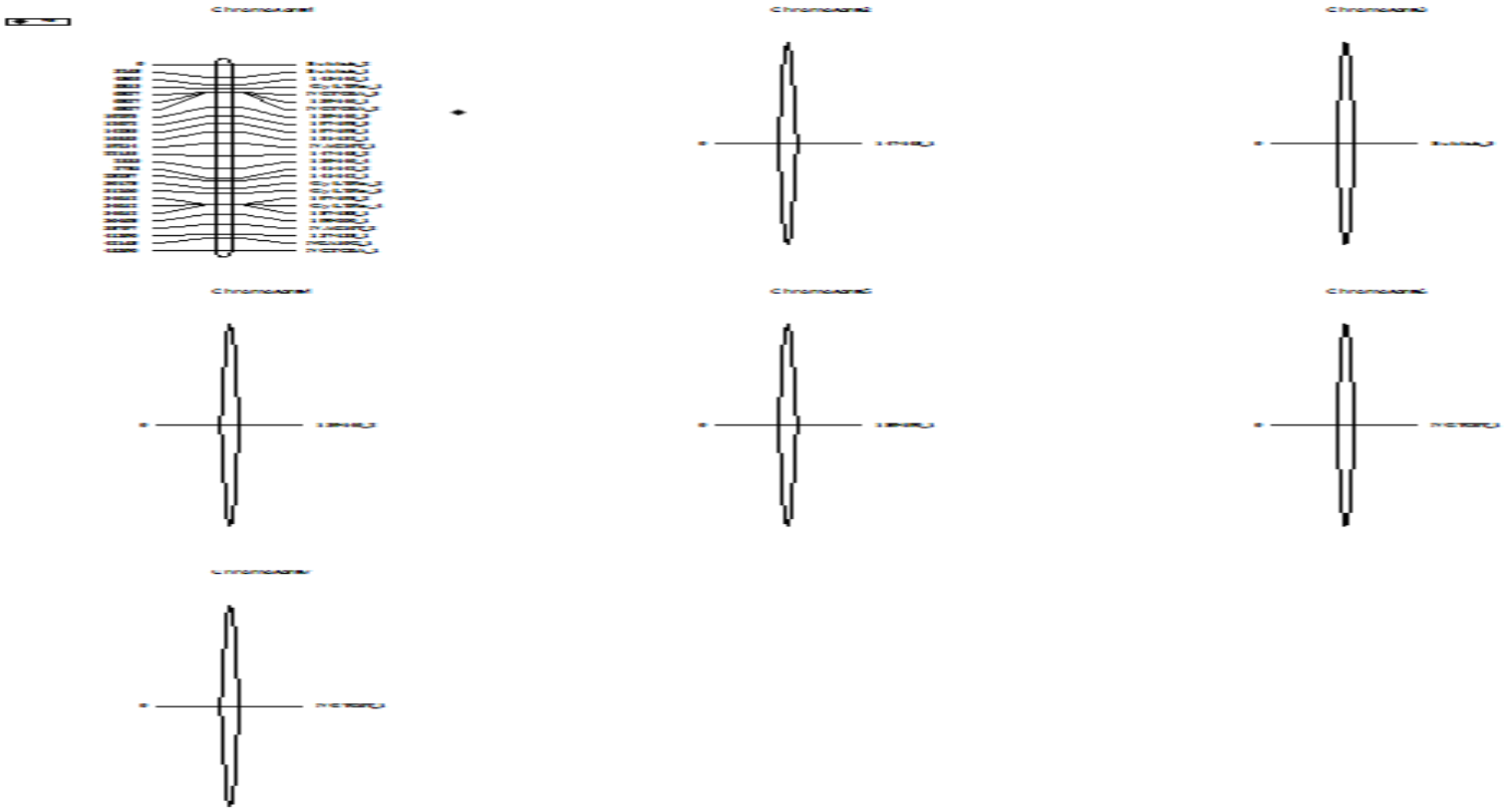


Figure 21 A whole genome showing alignment of markers on linkage groups and the location of a QTL

Genome wide association studies

Biallelic SNPs discovery

Starting from 50,898 chromosomal biallelic SNPs, filtering for no missing genotypes in the parental lines (Monyet and Kokopo) resulted in 37,436 biallelic SNPs. Out of the 37,436 total biallelic SNPs, 9,902 and 499 SNPs were not polymorphic between the two parents (yellow). Then, 3,458 and 5,568 SNPs were polymorphic between the two parents but not segregating in the mapping population (Red). The remaining 18,009 SNPs were segregating in the F_1 mapping population of Monyet by Kokopo cross (Table 4.20).

Table 21 Possible parent genotype combination using SNP markers

		Kokopo		
		0	1	11
Monyet	0	499	9635	3458
	1	451	189	48
	11	2035	390	206
	111	243	109	84
	1111	5568	4619	9902

Legend: 1 indicates the presence of the alternative SNP allele, Yellow indicates number of SNPs that are non-polymorphic, while red indicates polymorphic SNPs between parental lines and non-colored are the SNPs expected to segregate in the mapping population.

Genome trait association studies

Manhattan plots created in genome wide studies identified the most frequent SNP in the F_1 population of Monyet x Kokopo on chromosome 11 at a threshold of $-\log_{10} of\ 4$. Other more frequent SNPs were found to be located on chromosomes 1, 8, 9 10 and 11 at threshold of $-\log_{10} of\ 3$ (Figure 4.19). In total, nine genomic regions were considered to be significantly associated with *Foc* race 1 disease in this population located on chromosomes 1, 8, 9, 10 and 11.

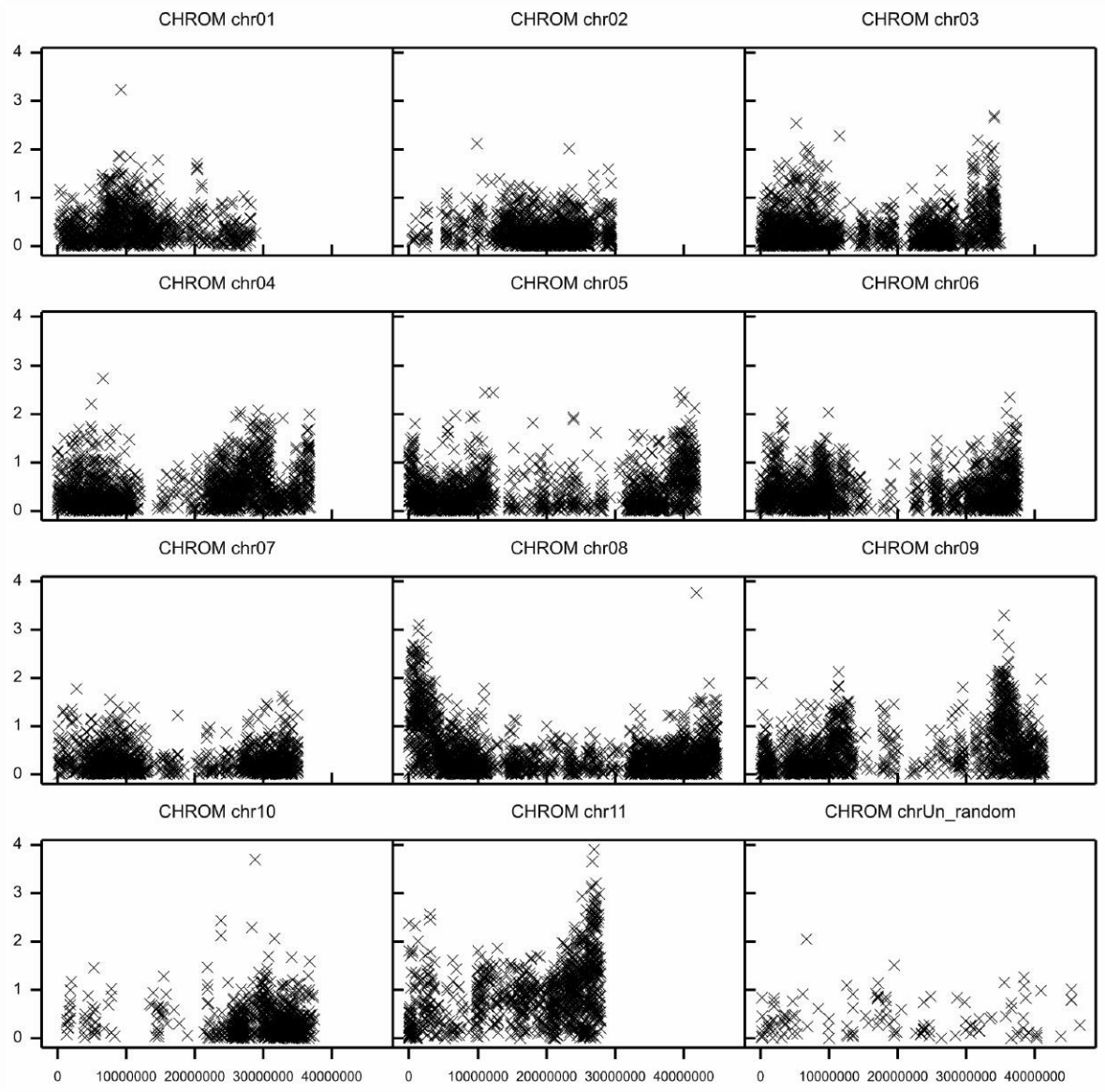


Figure 22 Manhattan plots for corm discoloration using only segregating markers indicating SNPs associated with *Foc* race 1

Linkage grouping

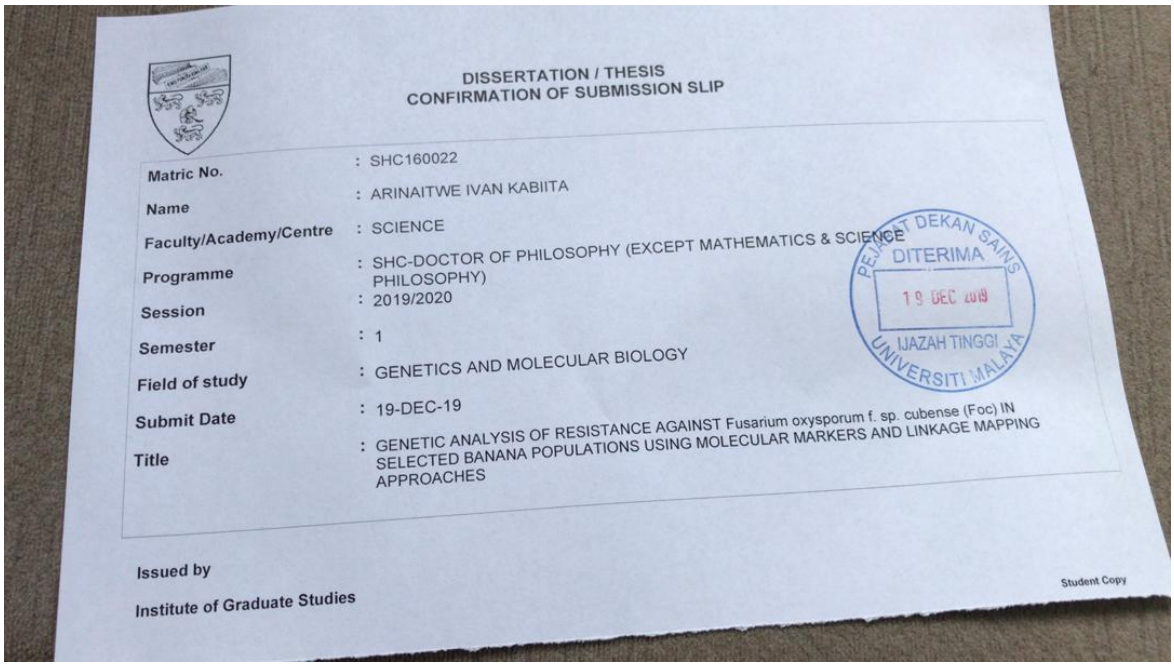
From the 4619 markers segregating AAAA x Aa (see Table 4.20), 1003 had less than 10% missing and/or wrong offspring genotypes (the expected offspring genotypes are AAAa and AAaa). Of these 1003 markers, 816 did not show segregation distortion (tested at a level = 0.001) and were used for linkage analysis. Visualisation of the recombination frequencies shows that markers on chromosome 3 and chromosome 8 had low recombination frequencies. Linkage grouping (recombination threshold = 0.20) was performed and resulted in the following numbers of markers in the formed linkage groups (Table 4.21).

Table 22 Numbers of markers on the linkage groups and their corresponding chromosomes

Linkage group	**Map length**	**#Markers**	**Corresponding chromosome**
1	140.6	40	1
11	368.3	107	6
12	227.3	88	7
13	24.8	12	9
14	64	14	9
17	24.8	10	10
18	61.9	15	10
19	162.4	60	11
2	130.4	42	2
4	808.8	193	3&8
5	35.2	13	3
6	183	71	4
7	124.9	32	4
9	304.2	90	5

Highlights (Progress)

Thesis writing is complete and submitted to UM for external examination



DISSERTATION / THESIS
CONFIRMATION OF SUBMISSION SLIP

Matric No.	: SHC160022
Name	: ARINAITWE IVAN KABIITA
Faculty/Academy/Centre	: SCIENCE
Programme	: SHC-DOCTOR OF PHILOSOPHY (EXCEPT MATHEMATICS & SCIENCE PHILOSOPHY)
Session	: 2019/2020
Semester	: 1
Field of study	: GENETICS AND MOLECULAR BIOLOGY
Submit Date	: 19-DEC-19
Title	: GENETIC ANALYSIS OF RESISTANCE AGAINST Fusarium oxysporum f. sp. cubense (Foc) IN SELECTED BANANA POPULATIONS USING MOLECULAR MARKERS AND LINKAGE MAPPING APPROACHES

PEJABAT DEKAN SAINS DITERIMA 19 DEC 2019 IJAZAH TINGGI UNIVERSITI MALAYA

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Two manuscripts published in peer review journals

1. **Arinaitwe, I.K.,** Teo, C.H., Kayat, F., Tumuhimbise, R., Uwimana, B., Kubiriba, J., Swennen, R., Harikrishna, J.A. and Othman, R.Y. (2019). Evaluation of banana germplasm and genetic analysis of an F 1 population for resistance to Fusarium oxysporum f. sp. cubense race 1. *Euphytica*, *215*(10), 175. doi:10.1007/s10681-019-2493-3

2. **Arinaitwe, I.K.,** Teo, C.H., Kayat, F., Tumuhimbise, R., Uwimana, B., Kubiriba, J., Harikrishna, J.A. and Othman, R.Y. (2019). MOLECULAR MARKERS AND THEIR APPLICATION IN FUSARIUM WILT STUDIES IN *Musa* spp. *Sains Malaysiana* 48 (9), 1841–1853. http://dx.doi.org/10.17576/jsm-2019-4809-05

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Evaluation of banana germplasm and genetic analysis of an F_1 population for resistance to *Fusarium oxysporum* f. sp. *cubense* race 1

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Abstract Fusarium wilt of bananas (*Musa* spp.), caused by *Fusarium oxysporum* f. sp. *cubense* (*Foc*) causes up to 100% yield loss in bananas. *Foc* race 1 in particular is very devastating to dessert bananas in Uganda. One of the effective control strategies for the disease is the development of resistant cultivars through breeding. The objectives of this study were to identify suitable banana germplasm for generating a segregating population for resistance to *Foc* race 1 and understand the mode of inheritance of resistance to *Foc* race 1. Twenty-two banana accessions sourced from the National Agricultural Research Organisation in Uganda were challenged with *Foc* race 1 in a screen house experiment. Monyet, resistant to *Foc* race 1 and Kokopo, susceptible, were selected and crossed to generate 142 F_1 genotypes. These F_1 genotypes were also challenged with *Foc* race 1 in a screen house experiment. Data were collected on rhizome discoloration index (RDI), leaf symptom index (LSI) and pseudo-stem splitting (PSS), and analysed for variability. The banana accessions evaluated showed varying degrees of resistance to *Foc* race 1. Segregation ratios for resistant versus susceptible progenies fitted 13:3 ($\chi^2 = 0.12$, $P = 0.73$) for RDI and 11:5 ($\chi^2 = 3.04$, $P = 0.08$) for PSS. Estimated broad sense heritability was 27.8% for RDI, 13.9% for LSI and 14.7% for PSS. The results suggest that resistance to *Foc* race 1 in banana is controlled by at least two dominant genes with epistatic interaction and that

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Molecular Markers and Their Application in Fusarium Wilt Studies in *Musa* spp.

(Penanda Molekul dan Aplikasinya dalam Kajian Fusarium Wilt pada *Musa* spp.)

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ABSTRACT

Bananas and plantains (Musa *spp.) are an important socio-economic fruit crop grown worldwide. Their production across the regions where they are grown is largely hampered by pests and diseases. Fusarium wilt is a disastrous diseases of bananas caused by the fungal pathogen* Fusarium oxysporum *f.sp.* cubense (Foc). *Managing it with chemicals, biological control agents and cultural methods is ineffective. Host plant resistance is the most effective and durable approach of managing most pest and disease epidemics in most plant species and could equally be effective in managing Fusarium wilt in bananas. Crossbreeding as one of the ways to introgress disease resistance genes and phenotyping for biotic and abiotic stresses currently used in banana breeding is apparently difficult to apply because of banana's low fertility, gigantic size, and long-life cycle which prolongs its breeding cycle. There is, therefore, a need to apply molecular markers in banana genetic improvement for Fusarium wilt resistance because of their accuracy, speed, robustness and effectiveness of operation. The objective of this article was to review and discuss molecular markers that have been successfully used in studying Fusarium wilt in bananas and some other important crops. Molecular markers discussed in this article include Random Amplified Polymorphic DNA, Sequence Characterized Amplified Region, Simple Sequence Repeat, Inter-Simple Sequence Repeat, and Single Nucleotide Polymorphism. The information discussed in this article informs future decisions to identify suitable marker systems for fine mapping of target regions and accelerated identification of quantitative trait loci for* Foc *resistance in bananas.*

Keywords: Banana; Fusarium oxysporum *f.sp.* Cubense*; molecular markers; quantitative trait loci; resistance*

ABSTRAK

Pisang dan plantain (Musa *spp.) adalah tanaman buah-buahan sosio-ekonomi penting yang berkembang di seluruh dunia. Pengeluarannya merentasi sempadan dengan sebahagian besar penanamannya dibantutkan oleh penyakit dan perosak. Fusarium wilt adalah sejenis penyakit pisang yang disebabkan oleh kulat patogen* Fusarium oxysporum *f.sp.* cubense (Foc). *Kaedah mengawal penyakit ini dengan menggunakan bahan kimia, agen kawalan biologi atau kaedah kultur tidak berkesan. Kerintangan tumbuhan perumah adalah pendekatan yang paling berkesan dan bertahan lama untuk mengawal wabak penyakit dan perosak dalam kebanyakan spesies tumbuhan dan berkesan dalam menguruskan Fusarium wilt pada pisang. Pembiakbakaan kacukan merupakan salah satu cara untuk introgres gen rintangan penyakit dan fenotip bagi tegasan biotik dan abiotiknya yang digunakan dalam penanaman pisang namun teknik ini sukar untuk diaplikasikan kerana kesuburan pisang yang rendah, saiz yang besar dan hayat kitaran yang panjang yang turut memanjangkan kitaran pembiakannya. Oleh itu, ada keperluan untuk menggunakan penanda molekul dalam meningkatkan genetik pisang untuk menghalang Fusarium wilt kerana ketepatan, kelajuan, keteguhan dan keberkesanan operasinya. Objektif kajian ini adalah untuk meneliti dan membincangkan penanda molekul yang telah berjaya digunakan dalam mengkaji Fusarium Wilt pisang dan sesetengah tanaman lain yang penting. Penanda molekul yang dibincangkan dalam kertas ini merangkumi DNA Polimorfik Rawak Teramplifikasi, Rantau Jujukan Pencirian Teramplifikasi, Pengulangan Jujukan Mudah, Pengulangan Jujukan Inter-Mudah dan Polimorfisme Nukleotida Tunggal. Maklumat yang dibincangkan dalam kajian ini menunjukkan keputusan pada masa depan dalam mengenal pasti sistem penanda sesuai bagi pemetaan kawasan sasaran lengkap dan pemecutan pengenalpastian lokus khi kuantitatif untuk rintangan* Foc *dalam pisang.*

Kata kunci: Fusarium oxysporum *f.sp.* Cubense*; lokus khi kuantitatif; penanda molekul; pisang; rintangan*

INTRODUCTION

Banana and plantains (*Musa* spp. hereafter referred to as banana) are among the most important fruits produced worldwide for food and commercial purposes (Brown et al. 2017; Looney 2016; Tourky et al. 2014). They are the second largest produced fruit after citrus (Tourky et al. 2014).

Banana is cultivated in more than 130 countries worldwide (FAOSTAT 2017). Its current global production is 161 million tonnes, of which close to 10% is deemed for export (FAOSTAT 2017). India is the largest producer of banana with 29.1 Million Metric Tons (MMT), followed by China (13.3 MMT) and the Philippines (8.9 MMT), all

References

Kumar, N. (2006). Problems and prospects of banana breeding in India. *Sphindia.Org*, *1*(2), 77–94. Retrieved from http://sphindia.org/index.php/jhs/article/view/358/0

Mustaffa, M. M., & Thangavelu, R. (2011). STATUS OF FUSARIUM WILT IN INDIA. In *Acta Horticulturae* (pp. 323–329). https://doi.org/10.17660/ActaHortic.2011.897.44

Sutanto Agus, Sudarsono, Dewi Sukma, H. C. (2011). The Study and Early Evaluation of Resistance of Banana Accessions for Wilt Disease Cause by Fusarium oxysporum f.sp. *Cabdirect.Org*. Retrieved from https://www.cabdirect.org/cabdirect/abstract/20133410438

van Bueren, E. T. L., Backes, G., de Vriend, H., & Østergård, H. (2010). The role of molecular markers and marker assisted selection in breeding for organic agriculture. *Euphytica*, *175*(1), 51–64. https://doi.org/10.1007/s10681-010-0169-0

Viljoen, A., Mahuku, G., Massawe, C., Ssali, R. T., Kimunye, J., Mostert, G., … Coyne, D. L. (2017). *Banana Diseases and Pests: Field Guide for Diagnostics and Data Collection*. Retrieved from http://breedingbetterbananas.org/wp-content/uploads/2017/02/Annex-12-BMGF-EABBP-2016.-Viljoen-et-al.Pests-and-Diseases.pdf

Zhang, L., Meng, L., & Wang, J. (2016). Users ’ Manual of GACD : Integrated Genetic Analysis Software for Clonal F 1 and Double Cross Populations. *Journal of Heredity*, *106*(6), 741–744. Retrieved from https://academic.oup.com/jhered/article-abstract/106/6/741/2622882